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Effectiveness of Empowerment Programme on Stress and Care Giving Burden among Care Givers of Chronically Ill Patients Admitted in MMIMS&R Hospital, Mullana Ambala, Haryana

Shalini Youssouf¹, Jyoti Sarin², Eenu³, Bindu Joseph³

¹Nursing Tutor, ²Director Principal, ³Assistant Professor, Maharishi Markandeshwar University, M.M. College of Nursing, Mullana, Ambala, Haryana, India

Abstract

Background- One of the most compassionate and self-sacrificing things a person can do is care for a sick loved one. Caring for a family member with impaired mental and behavioral functioning presents the most stressful of care giving situations. Keeping family caregivers healthy and able to provide care is crucial to maintaining our nation’s long-term healthcare system.

Aims – To assess the effectiveness of Empowerment programme regarding stress and care giving burden among care givers of chronically ill patients in experimental and comparison group.

Material and Methods - A Quasi Experimental non Equivalent control group pretest post test design. 95 care givers of chronically ill patients (50 in experimental and 45 in comparison group) selected from hospital by using convenience sampling technique. Empowerment programme was administered in experimental group. Selected demographic characteristics, standardized perceived stress scale and standardized care giver burden scale were used to collect data.

Results- The study showed that the mean post test 1 and post test 2 stress score was 12.44 ± 2.10 & 12.26±2.05 and 14.11 ± 3.11 & 14.40± 3.12 in experimental and comparison group respectively. There was no significant correlation between stress and care giving burden. There was no association of care giving burden score of the care givers in experimental and comparison group with their selected patient demographic characteristics and there was no significant association of care giving burden scores with their selected care giver demographic characteristics in experimental group and in comparison group except gender.

Conclusion- Empowerment programme was effective in reducing stress and care giving burden among care givers of chronically ill patients

Keywords- Effectiveness, Empowerment programme, Stress, Care giving burden

Introduction

A chronic condition “is a physical or mental health condition that lasts more than one year and causes functional restrictions or requires ongoing monitoring or treatment”. Chronic diseases includes cancer, diabetes, hypertension, stroke, heart disease, respiratory diseases, arthritis, obesity, and oral diseases which can lead to hospitalization, long-term disability, reduced quality of life, and death and requires care giving. The experience of caring for a sick family member can represent a great burden on the caregiver, leading to deprivation and changes in family dynamics.

Around 68 millions Indians and 95 lakhs people of Haryana caregivers provide extensive help to impaired relatives with chronic illness. This is also because they are unprepared to provide care, have inadequate
knowledge about care giving.

Caregivers’ tasks include both direct assistance (e.g., helping with activities of daily living, medication and lifestyle management) and less visible tasks such as assessing symptoms and follow health care systems.1,2 The demands of care giving often get additional stressors in caregivers’ lives, commonly referred to as caregiver burden.3,4,5

It has been shown that long-term chronic illnesses create an even greater burden on the family in comparison to acute illnesses.6 Caregivers of patients with chronic physical illnesses often experience feelings of isolation and fatigue related to their increased responsibilities and challenges.7 Caregivers experience high levels of burden due to immense physical, psychological, and economic strain, facing not only stresses of care giving but also the impending death of a loved one.8,9 Caregiver burden has been explained as a multidimensional response to the negative appraisal and perceived stress resulting from caring sick individuals. Keeping caregivers healthy and able to provide care is crucial to maintain our nation’s long-term healthcare system.

If caregivers remain healthy, the quality of life for care recipients will substantially improve.9 It is important to reduce the burden of the care giver by training them how to do personal care (bathing, feeding, toilet, dressing etc.) properly in order to save their time and care giving burden. So that they can do all these activities effectively and by following all steps of the daily care procedures. Empowerment programme can be helpful in reducing care giving burden of the care givers of the chronically ill admitted patients.10

There are few studies in the literature, however, that has investigated the effect of PMR exercises and skill training programme on the care giving related stress and care giving burden for care givers. Thus, this study aimed to assessing the effect of empowerment programme and PMR technique on reducing stress and care giving burden among caregivers of chronically ill patients.11

### Materials & Method

A Quasi - Experimental design (non-equivalent control group pre-test- post-test design) was used to achieve the objectives of this study. Power analysis was used to select the sample size. The sample included 95 care givers were selected using the convenience sampling method. (50 in experimental group and 45 in comparison group) for this study.

Formal administrative approval was obtained from the Medical Superintendent and ethical committee of MM (Deemed to be University), Mullana, Ambala (Letter Number IEC- 1511) to conduct the final study. Ninety care givers were selected using the convenience sampling method. (50 in experimental group and 45 in comparison group) for this study. Research participants were enrolled in the study after written informed consent and they were assured about the confidentiality of their response. Permission for pilot study was taken from the Medical Superintendent of MMIMS&R Hospital, Ambala. Permission for final study was taken from the Medical superintendent of MMIMS&R Hospital Consent was prepared and filled for the study subjects regarding their willingness to participate in the research study. Purpose of the study was explained to the sample subjects before data collection.

### Data Collection

For data collection, the research team initially developed a demographic characteristics in two parts after an extensive review of the relevant literature to achieve good content validity. It consist of 10 items related to demographic characteristics of patient and care giver i.e. age, gender,religion, marital status, education, occupation, income, type of family, area of residence, family history, duration of illness, use of any relaxation technique. Two another standardized tools were used to collect data. Perceived stress scale was a standardized tool used by researcher to assess the stress. There were total 10 items in the tool each item is having maximum marks 4 and minimum mark 0. Care giver burden scale was a standardized tool and it included 22 statements refer to how much burden a care giver feels while
taking care of their patient. Per statement care giver can choose one out of four option categories, varying from lowest score as 1 and highest to 4. Application of intervention was proceeded by administration of Empowerment programme for 4 days to each care giver. The empowerment programme included Progressive muscle relaxation technique session for 4 days daily along with administration of video of orientation to various important places of hospital, demonstration of oral care and bed bath procedure to the care givers and group discussion regarding management of stress, financial burden, diet management and personal hygiene of caregiver.

Intervention was pre evaluated by the experts before proceeding for administration.

To insure content validity, the tool was submitted to 9 experts: 3 from mental health nursing department, 6 from medical surgical department. The experts were requested to review and judge the items of adequacy and relevancy. The scale content validity was calculated by averaging item content validity index. The acceptable range of validity is 0.7 – 1. The tools were found to be valid for the study.

To assess the suitable sample size, a power analysis was performed. It was done with the help of formula Cohen’s d i.e. \( \mu_1 - \mu_2 / \text{SD} \) where \( \mu_1 \) was mean score of non-randomized control group, \( \mu_2 \) was mean score of non-randomized experimental group and S.D. was pooled standard deviation of both groups. The estimated effect size was 0.63 at the power of 0.80, the recommended sample for each group was 44. 10% extra sample was taken from the calculated sample size. Thus a total of 105 caregivers were included in the study. Due to attrition during data collection time the final sample size was 95 care givers (50 in experimental group and 45 in comparison group). Pre test was conducted in both the groups. Empowerment programme was administered in experimental group and routine care was carried out. In comparison group post test 1 was taken on day 4 and post test 2 was taken on day 13-17. In experimental group, post test 1 was taken on day 4 immediately after completion of intervention and post test 2 was taken on day 13-17.

**Data Analysis**

To analyze data, descriptive tests, including frequency, percentage, mean, and standard deviation (SD) and analytical tests, including the Kolmogorov–Smirnov test was conducted to indicate that the data were sampled from a population with a normal distribution. The correlation between stress and care giving burden mean score was examined by the Pearson correlation coefficient, and ANOVA for repeated measuring using the SPSS software (version 20). There was a significant difference at the level of \( P < 0.05 \).

**Results**

The results showed that less than half (44%) of the patients were in age group of 36-51 years in experimental group and one third (33.3%) of them were in age group of 52-67 years in comparison group. More than half of them (54%) in experimental group were females and 53.3% in comparison group were males. All the patients (100%) had surgical diagnosis in experimental group and medical diagnosis in comparison group. One third of the patients (32%) in experimental group and comparison group (33.3%) were having 8-11 months duration of illness. Most of the patients (72%) in experimental group and (73.3%) in comparison group were married. More than half (56%) in experimental group and 55.6% in comparison group of the patients visit to hospital in a month. Also the result showed that half of the care givers (50%) in experimental group and less than half (42.2%) in comparison group of the care givers were spouse. More than one third of the care givers (36%) were in 18-33 years of age in experimental group and less than half of the care givers (44.4%) were in 34-49 years of age in comparison group. More than half group (58%) of the care givers in experimental and more than half (55.6%) of the care givers were in comparison group were females.

Less than half of the caregivers (42%) in experimental group were having 5th pass education and
in comparison group one third of the caregivers (33.3%) were non literate. Less than one third of the caregivers (30%) in experimental group were having private job and 26.7% in comparison group were farmers. Less than half of the caregivers (44%) in experimental group had 7000-8000 rupees income per month and 40% in comparison group had 9001-10,000 rupees income per month. More than half of the caregivers (58%) in experimental group and (53.3%) in comparison group belonged to nuclear family. All caregivers (100%) in both the groups were not using any type of relaxation technique.

Frequency and Percentage Distribution of Experimental and Comparison group in terms of Level of Stress among Caregivers of Chronically ill Patients before administration of Empowerment Programme

![Figure 1 Bar diagram showing Level of Stress among Caregivers of Chronically ill patients before administration of Empowerment Programme](image1)

Frequency and Percentage distribution showing Comparison of Experimental and Comparison group in terms of level of Care Giving Burden among Caregivers of Chronically ill Patients before administration of Empowerment Programme

![Figure 2 Bar diagram showing level of care giving burden among Caregivers of Chronically ill Patients before administration of Empowerment Programme.](image2)
Research findings showed that the comparison of mean stress among care givers before the administration of empowerment programme in experimental and comparison group by using independent ‘t’ test. The calculated ‘t’ value was found to be statistically significant ( ‘t (93) =1.98; p=0.00*) which showed the difference between stress score was significant in both the groups and the comparison of mean score of care giving burden score among care givers before the administration of empowerment programme in experimental and comparison group by using independent ‘t’ test. In the experimental group, the mean pre-test care giving burden score of care givers was found to be 54.94 ± 4.683 and 52.73 ±8.097 in comparison group before the administration of empowerment programme with the mean difference of 2.206 and standard error of mean difference was 1.34. The calculated ‘t’ value was found to be (t(93) =1.98; p=.150NS) statistically non significant which showed that the difference between care giving burden score was non significant in both the groups.

Research results revealed that post test 1 and post test 2 stress score ranged from 8-18 & 8-16 in experimental and 9-21 &10-21 in comparison group. The mean post test 1 and post test 2 stress score was 12.44 ± 2.10 & 12.26±2.05 and 14.11 ± 3.11 & 14.40±3.12 in experimental and comparison group respectively and post test 1 and post test 2 care giving burden score ranged from 35-53 & 33-53 in experimental and 34-66 & 34-66 in comparison group. The mean post test 1 and post test 2 stress score was 41.34 ± 3.52 & 40.84 ± 3.62 and 51.98 ± 5.28 & 51.56 ± 5.31 in experimental and comparison group respectively. [Table 1, Table 2]. Results of ANOVA with repeated measurement uncovered that in experimental and comparison group there was a significant difference in the mean stress score in pre test (25.96) (19.96), post test 1 (12.44) (14.11) and post test 2 (12.26) (14.40) and computed F value was 706.7 and 42.23, p= 0.00 which was statistically significant at 0.05 level of significance. Also, in experimental and comparison group there was a significant difference in the mean stress score in pre test (54.94) (52.73), post test 1 (41.34) (51.98) and post test 2 (40.84) (51.56) and computed F value was 28.94 and .885, p= 0.00 and p=.354NS in experimental and comparison group respectively, which was statistically significant at 0.05 level of significance in experimental group [Table 3, Table 4]. Pearson correlation coefficient showed that there is no significant relationship between stress and care giving burden. In addition, research results also demonstrated that there was no significant association of association of pre-test stress score of the care givers in experimental and comparison group with their selected patient demographic characteristics.

**Table 1 showing Mean, Range, Median and Standard Deviation of Stress among Caregivers of Chronically ill Patients after Administration of Empowerment Programme in Experimental and Comparison group**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Range</th>
<th>Mean ±SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Post 1</td>
<td>Post 2</td>
<td>Post 1</td>
</tr>
<tr>
<td>Stress</td>
<td>Experimental group n=(50 )</td>
<td>8-18</td>
<td>8-16</td>
<td>12.44±2.10</td>
</tr>
<tr>
<td></td>
<td>Comparison group n=(45)</td>
<td>9-21</td>
<td>10-21</td>
<td>14.11±3.11</td>
</tr>
</tbody>
</table>

Maximum score=40 Minimum score=0
TABLE 2 showing Mean, Range, Median and Standard Deviation of Care Giving Burden among Caregivers of Chronically ill Patients after Administration of Empowerment Program in Experimental and Comparison group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Range</th>
<th>Mean ±SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Post 1</td>
<td>Post 2</td>
<td>Post 1</td>
</tr>
<tr>
<td>Care giving</td>
<td>Experimental group n=(50)</td>
<td>35-53</td>
<td>34-66</td>
<td>41.34 ±3.52</td>
</tr>
<tr>
<td>burden</td>
<td>Comparison group n=(45)</td>
<td>33-53</td>
<td>34-66</td>
<td>51.98 ±5.28</td>
</tr>
</tbody>
</table>

Maximum score=88
Minimum score=1

TABLE 3 showing Repeated measure ANOVA showing that significant difference within group in terms of Stress among Experimental and Comparison Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Test</th>
<th>Mean</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group n=50</td>
<td>Pre test</td>
<td>25.96</td>
<td>70.67</td>
<td>0.00*</td>
</tr>
<tr>
<td></td>
<td>Post test 1</td>
<td>12.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post test 2</td>
<td>12.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparison group n=45</td>
<td>Pre test</td>
<td>19.96</td>
<td>42.23</td>
<td>0.00*</td>
</tr>
<tr>
<td></td>
<td>Post test 1</td>
<td>14.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post test 2</td>
<td>14.40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant p≤0.05
NS Non Significant p≥0.05

TABLE 4 showing Repeated measures ANOVA showing the significant difference within group in terms of Care Giving Burden in Experimental and Comparison group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Test</th>
<th>Mean</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group n=50</td>
<td>Pre test</td>
<td>54.94</td>
<td>28.94</td>
<td>0.00*</td>
</tr>
<tr>
<td></td>
<td>Post test 1</td>
<td>41.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post test 2</td>
<td>40.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparison group n=45</td>
<td>Pre test</td>
<td>52.73</td>
<td>0.88</td>
<td>0.35NS</td>
</tr>
<tr>
<td></td>
<td>Post test 1</td>
<td>51.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post test 2</td>
<td>51.56</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant p≤0.05
NS Non Significant p≥0.05
Discussion

In this study, more than half of the care givers (58%) were females and most were married (68%). This findings was consistent with the results of study conducted by Nimisha K. Parekha, Shamita Shahb et al which showed that most number of care givers were females (66.9%) and majority of them were married (87.7%). Another study conducted by Sharon L. Lewis, Denise Miner-Williams et al which showed that majority of the care givers (81.3%) were married.

In this study half of the care givers (50%) were spouse of patients. The finding was contradictory with the result of study conducted by Sharon L. Lewis, Denise Miner-Williams et al which showed that majority of the care givers (74.5%) relationship with the patient was spouse.

In the present study less than one third of the care givers (30%) were having private job. The finding was contradictory to the study conducted by Sharon L. Lewis, Denise Miner-Williams et al which showed that maximum (75.5%) of the care givers were unemployed.

In the present study none of the care givers were using any type of relaxation technique. This finding was contradictory with the result of study conducted by Nimisha K. Parekha, Shamita Shahb et al which showed that more than half (53%) of the care givers were not using any relaxation techniques.

In the present study, the mean stress scores of care givers in experimental group before and after the administration of empowerment programme was 25.96 and 12.44 in post test 1 respectively and the p value was statistically significant p=0.00*. The result was consistent with the study conducted by Palak Patel which showed that mean stress score before and after administration of the intervention was 64.67 and 36.67 respectively and the p value was statistically significant p=0.00*.

Recommendations

1. The study can be replicated on a large scale to investigate whether the significant findings can be sustained among a larger group.
2. A similar study can be conducted in different settings
3. The experimental study can be conducted to find out the effectiveness of Progressive Muscle Relaxation Therapy on stress among caregivers of mentally ill patients.
4. A comparative study can be conducted to find out the level of stress and care giving burden among care givers of patients admitted in the selected government and private hospitals.
5. A Qualitative study can be done to assess stress and care giving burden among care givers of chronically ill patients.

Conclusion

Empowerment programme was effective in reducing stress and care giving burden among care givers of chronically ill patients.

Acknowledgment: This paper is based on the findings of an approved research project with a registration number CTRI/2020/04/024895. Also, we would like to thank all the people who helped us to perform this study, especially care givers.

Financial support and sponsorship Nil.

Conflicts of interest There are no conflicts of interest.

References

3. Pearlin LI, Mullan JT, Semple SJ, Skaff MM.


Study of Various Patterns of Azygos Venous System and its Clinical Significance

Aarti Rohilla¹, Monika Rathee², Kamal Singh¹, Suresh Kanta Rathee³
¹Professor, ²Demonstrator, ³Sr. Prof. & Head, Department of Anatomy, Pt. B. D. Sharma PGIMS, Rohtak

Abstract

Background: The azygos system includes those veins which are straight in course, paravertebral in position and not accompanied with the corresponding arteries. The azygos vein may arise as lumbar azygos vein from the back of inferior vena cava or by the union of right subcostal and right ascending lumbar veins. It enters the thorax, receives lower 8 right posterior intercostal veins, superior intercostal vein, hemiazygos and accessory hemiazygos veins and ends by joining the superior vena cava. The hemiazygos vein is formed on the left in a manner similar to that of azygos vein. The aim of the present study was to investigate the various patterns of the azygos venous system.

Methods: A total number of 30 cadavers were studied and were evaluated based on the study of Anson BJ and Mcvay CB.

Conclusion: Out of 30 specimens studied, 17 (56%) specimens showed normal pattern, 5 (16%) specimens showed caterpillar pattern and rest 8 (26%) specimens showed ladder pattern.

Azygos system of veins serve as an alternative drainage channel between superior vena cava and inferior vena cava in case of obstruction. The knowledge of various patterns could be useful for radiologists and surgeons to prevent intra-operative hazards.

Key Words: Azygos venous system, Caterpillar, Hemiazygos vein, Ladder.

Introduction

The azygos system includes those veins which are straight in course, paravertebral in position and not accompanied with the corresponding arteries. This system communicates with the vena caval system in front and with the vertebral venous plexus behind and can provide an alternative pathway for blood to the right atrium when either of the venae cavae is blocked. The terminal veins of this system are the azygos, the hemiazygos and the accessory hemiazygos veins.

The azygos vein is unpaired and mostly lies on the right side of the thoracic vertebral column draining itself into the superior vena cava. While the hemiazygos vein and the accessory hemiazygos vein present on the left side of the body, they are considered tributaries of azygos vein rather than its left side equivalent¹, hence the vein is so named.

Variations can be found by various researchers in origin and branches of different arteries like external carotid artery² and brachial artery³, unilateral or bilateral absence of nerves⁴ and also in venous system⁵. The azygos system of veins is also subjected to wide range of variations in relation to origin, course, pattern, tributaries and anastomoses⁶.
Anomalies of azygos veins are not very rare. Several deviations in pattern of this venous system are reported in the literature and these variations result predominately due to its complex embryological development. The development of this system is controversial but the azygos vein is considered to derive from the upper right supracardinal vein, the azygos arch from an upper segment of the right posterior cardinal vein and the hemiazygos and accessory hemiazygos veins from the left supracardinal vein and the terminal part of the left posterior cardinal vein.

The persistence of the embryological subcentral anastomosis between the right and left supracardinal veins form the transverse anastomosis between azygos and hemiazygos vein that gives the appearance of a ladder.

The various patterns of this system do exist and are physically normal. So, knowledge of these patterns is important during magnetic resonance imaging reports and mediastinal computed tomography and also important for cardiothoracic surgeons to prevent inadvertent haemorrhagic complications during mediastinal surgeries.

Results

In the present study, out of 30 specimens studied, 17 (56%) specimens showed normal pattern of azygos venous system, while the 5 (16%) specimens showed the (caterpillar pattern) single azygos vein towards midline and rest 8 (26%) specimens showed ladder pattern (as shown in Table 1). A ladder is a vertical or inclined set of rungs or steps.

Various patterns are as follows:

Type 1. Normal pattern of azygos venous system (Atypical).

Type 2. Caterpillar Pattern (Single Azygos Vein): Shifting of azygos vein towards midline along with absence of hemiazygos vein and accessory hemiazygos vein. The right and left posterior intercostal veins directly drain into the azygos vein and the pattern resembles to a caterpillar (Fig.1a, 1b, 1c).

Type 3. Ladder Pattern: There is transverse connection between the hemiazygos and accessory hemiazygos veins with the azygos vein across the vertebral column like a ladder (Fig.2a, 2b, 2c).

<table>
<thead>
<tr>
<th>Pattern of azygos venous system</th>
<th>No. of specimens</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (atypical)</td>
<td>17</td>
<td>56%</td>
</tr>
<tr>
<td>Caterpillar (single azygos vein)</td>
<td>5</td>
<td>16%</td>
</tr>
<tr>
<td>Ladder (transverse connection)</td>
<td>8</td>
<td>26%</td>
</tr>
</tbody>
</table>
Discussion

According to Anson’s system of classification:

Type-I: This primitive and embryological form consists of two separate veins lying in parallel to each other in the posterior mediastinum, being anterior and lateral to the vertebral column with no connections between them. It is seen in 1% cases.

Type-II: 98% of all cases are in this form and it is known as the transition type. There are multiple retroaortic anastomoses between the azygos and hemiazygos venous systems.

Type-III: It consists of a single azygos vein lying at the midline, on the anterior surface of the vertebral column.

In the present study, 5 of our cases (caterpillar pattern) show shifting of azygos vein towards midline with the absence of hemiazygos vein and accessory hemiazygos vein comes under Anson’s type-III.

The other 8 cases (ladder pattern) show horizontal or retroaortic anastomosis between the hemiazygos and accessory hemiazygos veins with the azygos vein. This is according to type-II Anson’s classification. It is due to the persistence of venous channels between the azygos lines of two sides.

Bergmann et al have reported variations of azygos vein. The azygos vein receives the hemiazygos vein at T_8 or T_9 and accessory hemiazygos vein at T_6 or T_7 vertebra. When hemiazygos and the accessory hemiazygos veins are not completely formed, the posterior intercostal veins on the left side of thorax may drain into azygos vein independently and it lies in the midline.

Dharan N & Soames R reported a single vein which is formed by union of azygos and hemiazygos veins which is located in midline draining the right and left posterior intercostal veins into the superior vena cava and 26 cases of retroaortic communication in their study.

T. Kutoglu et al in his study observed 1 case of single azygos vein and 44 cases of multiple retroaortic anastomosis.

Shivanal U et al found shifting of azygos vein towards midline along with absence of accessory hemiazygos vein and similar type of transverse connections between azygos and hemiazygos venous system.

Satheesha Nayak et al observed the midline position of azygos vein which was possibly due to the old age and also reported 6 horizontal channels across the vertebral column connecting the azygos vein with the accessory hemiazygos and hemiazygos veins.

Saito A et al also reported the shifting of azygos vein towards the midline or left side due to aging.

Rao Y S & Banerjee A found the similar observation of three transverse retroaortic anastomosis between azygos and hemiazygos veins.

Kumar et al in his case report found a ladder pattern of azygos venous system.

Table 2. The following studies have classified the azygos venous system into three main types. Each of which have different sub-grouping.

<table>
<thead>
<tr>
<th>Research Study</th>
<th>Parallel Pattern (Type I)</th>
<th>Ladder Pattern (Type II)</th>
<th>Caterpillar Pattern (Type III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anson and McVay</td>
<td>1</td>
<td>98</td>
<td>1</td>
</tr>
<tr>
<td>Bergmann et al</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Dahran &amp; Soames</td>
<td>1</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>Kutoglu et al</td>
<td>1</td>
<td>44</td>
<td>1</td>
</tr>
</tbody>
</table>
Knowledge of these different variations is important in CT and MRI scans because some variations of azygos venous system can be confused with aneurysm, lymphadenopathy and tumours of posterior mediastinum.

Fig.1: Showing Azygos Venous System Caterpillar Pattern (1a, 1b, 1c)

Fig.(1a,1b,1c)- Presenting the caterpillar pattern of azygos venous system with reverse curving of azygos vein in fig.1(a); SAz- single azygos vein; RPIV- right posterior intercostal vein; LPIV- left posterior intercostal vein.

Fig.2: Showing Azygos Venous System Ladder Pattern (2a, 2b, 2c)

Fig.(2a,2b,2c)- Showing azygos venous system ladder pattern; Az- azygos vein; AHz- accessory hemiazygos vein; Hz- hemiazygos vein; CC- cross communication
Conclusion

Azygos system of veins serve as an alternative drainage channel between inferior vena cava and superior vena cava in case of obstruction. Anomalies of this system are often detected only during surgery which cause intra-operative hazards mainly haemorrhage. This knowledge of various patterns could be useful for surgeons to prevent such hazards.

Ethical Clearance- Taken from Biomedical Research Ethics Committee, Pt. B. D. Sharma Post Graduate Institute of Medical Sciences, UHS, Rohtak.

Source of Funding- Self

Conflict of Interest - Nil

References

Fetal Ilium as a Tool For Sex Determination: Discriminant Functional Analysis

Aarti¹, Luv Sharma², Kamal Singh¹

¹Professor, Department of Anatomy, ²Professor, Department of Forensic Medicine, Pt. B. D. Sharma PGIMS, Rohtak, Haryana, India

Abstract

Background: Sex determination has been the most intriguing puzzle for forensic pathologists and anthropologists for which efforts are being done since long. Sexual dimorphism is well established in the adult pelvis and it is known to provide the highest level of information about sexual dimorphism. This study was conducted to know whether this dimorphism exists in fetal bones?

Method: A total of 34 pairs of fetal pelvis bones (22 Males + 12 Females), age ranging from 4 months to full term were collected from unidentified dead fetuses brought in the Department of Forensic Medicine for the routine medicolegal autopsies, to study for sexual dimorphism in the Department of Anatomy, Pt. B. D. Sharma PGIMS, Rohtak. Samples were divided in 2 age groups and various metric parameters were recorded with the help of digital vernier caliper. Data obtained was subjected to descriptive & discriminant functional analysis.

Conclusion: Results of Descriptive and Discriminant Functional Analysis showed that sex determination can be done with 100% of accuracy by using different combinations of parameters of fetal ilium. This study illustrates that sexual dimorphism exists from early fetal life & after mid pregnancy; it can be clearly established by discriminant functional analysis.

This research was limited in its analysis due to less availability of specimens as this represents only the results in north Indian population. Future studies should be done to explore different populations to better understand the sexual dimorphism in fetal bones of different geographic contexts.

Key Words: Fetal Ilium; Sexual Dimorphism; Iliac Length.

Introduction

Sex determination is crucial for both archaeological inference and identification in forensic contexts. Forensic pathologists and anthropologists are trying to resolve the mystery of sex determination since long.

Although sexual dimorphism is well established with the adult pelvis and it is considered to supply the best level of data about sexual dimorphism.¹ However, studies on fetal specimens have reported contradictory results.²⁻⁴ Sexually distinctive features of fetal and neonatal pelvis have been reported.⁵ After that, there are many attempts to develop both metrical and morphological techniques for estimating sex in juvenile skeletons. The most consistent results so far have been obtained from morphological analysis of the ilium.⁶ Some researchers are of opinion that secondary sexual characteristics did not appear until puberty, so are the chances of estimating sex of foetal remains, while some have reported the
sexual differences in foetal ilium. The purpose of this research is to establish whether fetal specimens can be sexed accurately by discriminant analysis on ilium bone.

Material And Methods

The study was conducted in Department of Anatomy in collaboration with Department of Forensic Medicine, Pt. B. D. Sharma PGIMS Rohtak. A total of 34 pairs of fetal pelvis bones (22 Males + 12 Females), age ranging from 4 months to full term were collected unidentified dead fetuses brought in the Department of Forensic Medicine for the routine medicolegal autopsies during a period of three years. Only intact fetal pelvic bone sample from known and recognizable sex were collected. Fragmentary and unidentifiable fetal remains were not included in the study. Any gross malformation, congenital anomalies and fragmented bones were excluded from the study.

MEASUREMENTS FOR THE STUDY

Collected samples were divided in the following groups as shown in table 1.

<table>
<thead>
<tr>
<th>Specimen Sex/Side</th>
<th>Fetal Ilium Bone Specimen (Male)</th>
<th>Fetal Ilium Bone Specimen (Female)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;6 Months</td>
<td>&gt;6 Months</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>1a</td>
<td>2a</td>
</tr>
<tr>
<td></td>
<td>1b</td>
<td>2b</td>
</tr>
</tbody>
</table>

Each sample was measured separately and the following measurements were noted after careful examination. (Fig.1).

Fig.1: Showing Metric Parameters of Fetal Ilium: Iliac Length (A-B), Sciatic Notch Angle (D), Sciatic Notch Width (C-E), Sciatic Notch Depth (Perpendicular line at D), Anterior Sciatic Notch Length (E-D), Posterior Sciatic Notch Length (C-D)
Metric Parameters of Fetal Ilium

1. Iliac Length (mm): Maximum Length from anterior to posterior iliac spine (A to B).\(^1\)

2. Sciatic Notch Angle (\(0\)): Using the apex of the notch (D) as the angle point of origin, the maximum angle was formed by the anterior and posterior edges of the sciatic notch (Angle C-D-E).

3. Sciatic Notch Width (mm): Maximum Breadth from anterior and posterior sciatic notch edges (C to E).

4. Sciatic Notch Depth (mm): Maximum length of line drawn perpendicular from notch apex (D) to horizontal axis created by sciatic notch width (C to E).

5. Anterior Sciatic Notch Length (mm): Maximum Length from Anterior sciatic notch edge to Apex (E to D).

6. Posterior Sciatic Notch Length (mm): Maximum length from Posterior sciatic notch edge to Apex (C to D).

These were measured by the digital vernier caliper with the least count of 0.01 mm. All the measurements were taken by the same investigator in the same setting. Each reading was taken thrice at the interval of 1 month, and statistically analyzed to rule out intra-observer error. The results of the study were computed and analyzed with SPSS software 16. Normal Descriptive Statistics i.e., Mean, standard deviation & P-Value as well as Canonical discriminant function coefficients in Discriminant Function Analyses were performed. The discriminant function formula is as follows:

\[ F(X) = A_1X_1 + A_2X_2 + \ldots + A_NX_N + C \]

Where \(F(X)\) represents the discriminant function score, \(X_1\) to \(X_N\) are the measured variables, \(A_1\) to \(A_N\) are the unstandardized coefficients of each variable and \(C\) is the function’s constant.

Results

Descriptive statistics of both the sexes for Left & Right sides of Fetal Ilium Bone were analyzed and compared respectively (Table 2 & 3).

Parameters which showed the statistically significant difference on comparison among left sides of the same age group are: posterior sciatic notch length on comparison between Group 1a & Group 3a, iliac length & sciatic notch width on comparison between Group 2a & Group 4a (Table 2).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Morphometric Parameter</th>
<th>&lt; 6 Months (Mean ± SD)</th>
<th>&gt; 6 Months (Mean ± SD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (Group 1a)</td>
<td>Female (Group 3a)</td>
<td>Male (Group 2a)</td>
<td>Female (Group 4a)</td>
</tr>
<tr>
<td>1</td>
<td>Iliac Length (mm)</td>
<td>15.1±2.24</td>
<td>16.6±1.19</td>
<td>0.138</td>
</tr>
<tr>
<td>2</td>
<td>Sciatic Notch Angle (0)</td>
<td>123±9.91</td>
<td>121±5.46</td>
<td>0.569</td>
</tr>
<tr>
<td>3</td>
<td>Sciatic Notch Width (mm)</td>
<td>5.04±0.94</td>
<td>5.17±0.36</td>
<td>0.756</td>
</tr>
<tr>
<td>4</td>
<td>Sciatic Notch Depth (mm)</td>
<td>1.13±0.31</td>
<td>1.55±0.09</td>
<td>0.090</td>
</tr>
<tr>
<td>5</td>
<td>Anterior Sciatic Notch Length (mm)</td>
<td>3.07±0.63</td>
<td>2.73±0.21</td>
<td>0.230</td>
</tr>
<tr>
<td>6</td>
<td>Posterior Sciatic Notch Length (mm)</td>
<td>2.55±0.38</td>
<td>3.47±0.44</td>
<td>0.000</td>
</tr>
</tbody>
</table>
On comparison of right sides of both the sexes in the same age group a statistically significant difference was observed in the following parameters: anterior sciatic notch length on comparison between Group 1b & Group 3b; and in iliac length, sciatic notch width, anterior sciatic notch length & posterior sciatic notch length on comparison between Group 2b & Group 4b (Table 3).

**TABLE 3: DESCRIPTIVE STATISTICS OF THE RIGHT ILIAC BONE MEASUREMENTS**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Morphometric Parameter</th>
<th>&lt; 6 Months (Mean ± SD)</th>
<th>&gt; 6 Months (Mean ± SD)</th>
<th>P Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male (Group 1b)</td>
<td>Female (Group 3b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Iliac Length (mm)</td>
<td>15.26±2.24</td>
<td>16.62±1.06</td>
<td>0.178</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>Sciatic Notch Angle (0)</td>
<td>122±8.53</td>
<td>127.3±5.39</td>
<td>0.213</td>
<td>0.552</td>
</tr>
<tr>
<td>3</td>
<td>Sciatic Notch Width (mm)</td>
<td>5.19±0.81</td>
<td>5.48±0.72</td>
<td>0.469</td>
<td>0.012</td>
</tr>
<tr>
<td>4</td>
<td>Sciatic Notch Depth (mm)</td>
<td>1.39±0.36</td>
<td>1.46±0.30</td>
<td>0.703</td>
<td>0.141</td>
</tr>
<tr>
<td>5</td>
<td>Anterior Sciatic Notch Length (mm)</td>
<td>2.69±0.37</td>
<td>3.15±0.56</td>
<td>0.047</td>
<td>0.037</td>
</tr>
<tr>
<td>6</td>
<td>Posterior Sciatic Notch Length (mm)</td>
<td>2.92±0.46</td>
<td>2.99±0.35</td>
<td>0.734</td>
<td>0.041</td>
</tr>
</tbody>
</table>

On applying discriminant function analysis on the measurements of fetal ilium more astonishing results came out which showed that these parameters provide us a good criterion in estimating significance of percentage accuracy in determination of sex (Table 4 & 5).
TABLE 4: DISCRIMINANT FUNCTION ANALYSIS SHOWING % ACCURACY FOR SEX DETERMINATION BY TAKING INDIVIDUAL PARAMETERS OF LEFT & RIGHT SIDES OF FETAL ILIUM BONE IN LESS THAN 6MTHS AGE GROUP.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Age Group: Less than 6 Months</th>
<th>Correctly Classified (%) BY DFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Left Side</td>
<td>Right Side</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1a (Male)</td>
<td>Group 3a (Female)</td>
</tr>
<tr>
<td>A</td>
<td>Iliac Length (mm)</td>
<td>71.4</td>
<td>66.7</td>
</tr>
<tr>
<td>B</td>
<td>Sciatic Notch Angle (0)</td>
<td>57.1</td>
<td>66.7</td>
</tr>
<tr>
<td>C</td>
<td>Sciatic Notch Width (mm)</td>
<td>42.9</td>
<td>66.7</td>
</tr>
<tr>
<td>D</td>
<td>Sciatic Notch Depth (mm)</td>
<td>71.4</td>
<td>100</td>
</tr>
<tr>
<td>E</td>
<td>Anterior Sciatic Notch Length (mm)</td>
<td>57.1</td>
<td>66.7</td>
</tr>
<tr>
<td>F</td>
<td>Posterior Sciatic Notch Length (mm)</td>
<td>85.7</td>
<td>66.7</td>
</tr>
</tbody>
</table>

On applying DFA on the individual parameters in age group of less than six month (Group 1a & 3a) has produced a sex determination accuracy of 85.7% in males with posterior sciatic notch length; 100% in females with sciatic notch depth; and 80% accuracy with sciatic notch depth & posterior sciatic notch length in both on left side. Whereas, a sex determination accuracy of 71.4% in males with iliac length & sciatic notch depth; 66.7% in females with all the parameters except sciatic notch depth; and 70% accuracy with iliac length in both on right side in Group 1b & 3b (Table 4).
### TABLE 5: DISCRIMINANT FUNCTION ANALYSIS SHOWING % ACCURACY FOR SEX DETERMINATION BY TAKING INDIVIDUAL PARAMETERS OF LEFT & RIGHT SIDES OF FETAL ILIUM BONE IN MORE THAN 6MTHS AGE GROUP

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Age Group: More than 6 Months to Full Term</th>
<th>Correctly Classified (%) BY DFA</th>
<th>Left Side</th>
<th>Right Side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Group 2a</td>
<td>Group 4a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Male)</td>
<td>(Female)</td>
</tr>
<tr>
<td>A</td>
<td>Iliac Length (mm)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>B</td>
<td>Sciatic Notch Angle (0)</td>
<td>25.3</td>
<td>66.7</td>
<td>42.9</td>
<td>75</td>
</tr>
<tr>
<td>C</td>
<td>Sciatic Notch Width (mm)</td>
<td>50</td>
<td>66.7</td>
<td>57.1</td>
<td>100</td>
</tr>
<tr>
<td>D</td>
<td>Sciatic Notch Depth (mm)</td>
<td>75</td>
<td>33.3</td>
<td>57.1</td>
<td>75</td>
</tr>
<tr>
<td>E</td>
<td>Anterior Sciatic Notch Length (mm)</td>
<td>50</td>
<td>33.3</td>
<td>42.9</td>
<td>75</td>
</tr>
<tr>
<td>F</td>
<td>Posterior Sciatic Notch Length (mm)</td>
<td>50</td>
<td>33.3</td>
<td>42.9</td>
<td>75</td>
</tr>
</tbody>
</table>

On applying DFA on the individual parameters in age group of more than six month (Group 2 & 4) has produced a sex determination accuracy of 100% in Males with Sciatic Notch Width; and 100% in males, females and both with Iliac Length on both right and left sides. A 100% of accuracy for sex determination has been observed with different combinations of parameters of fetal ilium (Table 5).

**Discussion**

Determination of sex had been the subject of interest since the eighteenth century. The difficulty in recognizing & applying morphological traits to the fetal bones has already been established & the methods prove unreliable when applied to different samples varying in the expression of morphological traits. Adult traits established for sexual dimorphism can’t be replicated & projected to fetal remains. In the adult pelvis, the primary focused area is the sciatic notch for sex determination. The same has been tried to project on fetal remains but with contrasting results.

Descriptive analysis of the present study showed that sciatic notch is wider in males than females in both the age groups (Group 1 & 2) and deeper in females in age group <6 months) and deeper in males in age groups >6 months. Thomson observed that the sciatic notch is wider and shallower in females than in males, the reason being the examined intact pelvis and measured the width...
of the sciatic notch as the length from the anterior greater sciatic notch to the margin of the sacrum that met the posterior inferior iliac spine posteriorly & it does not correspond to the standardized measurement currently applied. While Fazekas & Kosa (1978) stated that males show more significant notch depth and females display larger notch lengths.14

Schutkowski examined the Spitalfields London Juvenile Skeletal Collection, which contained individuals aged from birth to 11 years. The greater sciatic notch depth and angle, iliac crest curvature, and arch criterion were studied and it was concluded that the sciatic notch was mainly useful for the determination of sex. The visual assessment of sciatic notch angle determined angles more than 900 as females and ~ 900 as males. But the present study showed that sciatic notch angle was more than 1200 in all the age groups in both males and females.3 So, the sciatic notch has not been proved to be a good indicator of sex in fetal remains. Schutkowski used the fetal iliac bone outlines, especially the sciatic notch & proposed discriminant analysis, to determine the sex of fetus and infants based on the ilium, but did not consider age as criteria.4 Discriminant analysis is a versatile statistical method used to classify observations into two or more groups/categories or assign subjects to one group among several known groups. It has been used over descriptive analysis by researchers on various bone morphometric analyses to correctly classify the amongst the known groups.15-17 The discriminant analysis of fetal ilium by a 3D geometric morphometric approach with multi-slice computed tomography and results did not show any difference between male and female shapes in all age groups.18

The results of this study show that by discriminant analysis classification of sex can be done with 100% accuracy by taking fetal iliac length as criteria in late fetal age & early fetal age sex can be classified with 80% accuracy by taking Posterior Sciatic Notch as criteria and with 100% accuracy by the combination of parameters.

Embryological Basis

Ilium is the first bone to appear in both the cartilaginous template and the ossification centres. The sacroiliac joint both a synovial and synarthrosis joint is clearly formed by 10 weeks of intrauterine life.19 The three fetal pelvic bones are visible by ultrasound early in development; specifically, the iliac wings are evident by the end of the first trimester.20 Differences found in male and female pelvis reflect influences of hormones, particularly testosterone, on the development of the embryo as it progresses from an unsexed to sexed individual approximately the 8th week of intrauterine life.21, 22 At this time, testosterone is secreted at high levels and this continues until roughly the 20th week of intrauterine life. Male and female pelvic dimorphism has been clearly evident by 24th to 25th weeks of intrauterine life9, 23 with specific differences found in the angle and breadth of the sciatic notch, sub-pubic angle, and ischial length.8, 9, 14, 24

Conclusion

This study illustrates that sexual dimorphism exists from early fetal life & after mid-pregnancy; it can be clearly established by discriminant functional analysis. The findings in our study showed that discriminant functional analysis of fetal ilium bone determination of sex can be done with nearly 100% of accuracy and this can be used as a potential tool for forensic investigation for evaluation of sex in severely mutilated and decomposed bodies during post-mortem examination. Although a smaller number of samples and the only north-Indian population is a limitation to the study, future studies should explore different populations to better understand the sexual dimorphism in fetal bones of different geographic contexts.

Ethical Clearance: The samples were postmortem samples taken from unidentified bodies during routine medicolegal autopsies done by one of the authors himself. Thus, ethical clearance is not required.

Source of Funding: Self.

Conflict of Interest: Nil.
References

Review on Current Trends in Hypertension

Aarti Thakur¹, Ruchi Kumari², Sakshi Tomar¹

¹Nursing Tutor, Department of Obstetrics and Gynaecology Nursing, ²Nursing Tutor, Department of Medical Surgical Nursing, Chitkara University College of Nursing, Chitkara University, Himachal Pradesh, India

Abstract

Hypertension is one of the serious medical condition that has risk factor for developing cardiovascular diseases. Around 33% of individuals having hypertension are undiagnosed and those who are diagnosed are not taking proper treatment. One of the significant reason for hypertension is premature death. In this review, we focused on modern directions of hypertension management with an attention on screening, diagnosis and drug selection. In urban areas of India hypertension incidence is estimated upto 20 to 40% and in rural area it is upto 12 to 17%. Hypertension shows no symptoms and during routine check-ups we can diagnose it. For management of hypertension the guidelines were developed by ministry of health. Various threats were identified regarding management of hypertension.

Keywords - Hypertension, Screening, Pre-hypertensive Stage, Malignant Hypertension

Introduction

World Health Organization has defined Hypertension as increase in systolic and diastolic B.P above 140/90mm Hg or both. When blood Pressure is more than 140/90 mm Hg we define it as Hypertension according to AHA.¹

In world everyone is facing common problem known as HTN. About 1 billion of individuals are suffering from HTN and it is expected that this figure may increase up to 2025. HTN is known as quiet executioner because it shows no symptoms. Sometimes during routine check-ups most of individuals are diagnosed as Hypertensive.² Systolic blood pressure is defined as contraction of pressure ventricles whereas when the ventricles are filling with the blood reflects is known as diastolic blood pressure. One of the most important determinant of cardiovascular, myocardial and coronary heart disease is systolic blood pressure.³

According to national committee on prevention, evaluation, detection and treatment of increased blood pressure classification of blood pressure is taken on 3 clinical visits.⁴

Corresponding Author
Aarti Thakur,
Nursing Tutor, Department of Obstetrics and Gynaecology Nursing, Chitkara University College of Nursing, Chitkara University, Himachal Pradesh, India
Email- aarti.thakur@chitkarauniversity.edu.in
Phone Number- 91-8587041553
Table 1 Classification of adult blood pressure

<table>
<thead>
<tr>
<th>Definition</th>
<th>BP(Diastolic)</th>
<th>BP(Systolic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;85</td>
<td>100-120</td>
</tr>
<tr>
<td>Borderline</td>
<td>90-94</td>
<td>141-160</td>
</tr>
<tr>
<td>Mild</td>
<td>95-104</td>
<td>161-180</td>
</tr>
<tr>
<td>Moderate</td>
<td>105-114</td>
<td>&gt;180</td>
</tr>
<tr>
<td>Isolate Systolic HTN</td>
<td>&lt;90</td>
<td>≥140</td>
</tr>
<tr>
<td>Severe</td>
<td>&gt;114</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>&gt;140</td>
<td></td>
</tr>
</tbody>
</table>

Pre-hypertensive stage is defined as High normal hypertension. Stage 1 is defined as when patients B.P is mild or borderline. Hypertension Stage 2 is defined as when patients diastolic B.P is 100 & systolic is >140. When patient’s diastolic B.P is more than 130 months & systolic B.P is more than 200 than it is termed as malignant HTN. This stage of hypertension can lead to renal failure as well as severe retinopathy. Some common Symptoms which may be seen in malignant HTN are visual disturbance and headache.5

On basis of etiology hypertension is of two types i.e. primary and secondary HTN. About 95% of cases are seen of primary hypertension whereas 5% of cases accounts for secondary HTN. The exact cause of primary hypertension is unknown but there are some specific causes of secondary HTN which are listed below in table.6

Table 2: Causes of Secondary Hypertension

<table>
<thead>
<tr>
<th>Causes</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diseases of Renal system</td>
<td>Glomerulonephritis, chronic pyelonephritis, Renal parenchymal disease</td>
</tr>
<tr>
<td>Diseases of Endocrine system</td>
<td>Hyperaldosteronism, Cushing’s syndrome, acromegaly, hyperparathyroidism, hyperthyroidism</td>
</tr>
<tr>
<td>Drugs</td>
<td>Cyclosporine, Adrenocorticosteroids, Phenothiazine, Alcohol</td>
</tr>
<tr>
<td></td>
<td>Oral contraceptives, tricyclic antidepressants</td>
</tr>
</tbody>
</table>

Pharmacological Management of HTN

The initial step for the treatment of HTN is assessment. Pharmacotherapy & non pharmacotherapy are also used for the treatment of hypertension. Guidelines which are recommended by joint national committee include 3 blood pressure measurements of both arms at alternate health check-up visits.7 Assessment should be done before starting the treatment of HTN. Assessment mainly includes client’s history, physical examination & causes through which basic investigation can be done for determining the first line agent for treatment of HTN. We must be aware of condition like DM, thyrotoxicosis& pregnancy. Assessment and diagnosis of hypertension should be done appropriately in clients.8 For clients having DM Blood Pressure should be done appropriately in clients. For clients having DM blood pressure should be managed till readings of 130/80 mm Hg and for other clients of BP should be managed at the readings of <140/85 mm Hg. Rapid Reduction of blood pressure can
be fatal because it may cause risk for developing stroke. Clients who are in HTN stage 1 are recommended for lifestyle changes, pharmacology & non pharmacologic therapy. Blood pressure should be reassessed for about 3 to 6 months prior to prescribing drugs. If there is no reduction in B.P after reassessment then it is suggested to start drug therapy.9

Non –Pharmacological approaches for Hypertension10:

- Bland Diet
- Exercise (Aerobic)
- Dieting Reduction
- Stress Buster
- Decrease alcohol
- Smoking Cessation
- Decrease exacerbate factors example- pain
- Controlling factors of arteriosclerosis

Drug selection Principle -

Pharmacological treatment should be given to clients with HTN or sustained pressure. If readings of HTN is more than 140/90 mm of Hg than decision for prescribing drugs depends upon risk of presence of diabetes, coronary events or end organ damage.11

Thiazides and blockers are agents for initiating treatment. Angiotensin receptor blockers are the agents which are less studied but are effective.12

If client condition is not getting improving from one drug it’s better to switch to another drug. The various factors such as Sex, age and race are considered as the most appropriate for first line treatment.13

ACE-I may retard progression of nephropathy in diabetes mellitus condition while beta blockers may retard progression of palpitations associated increased level of thyroid hormones.14

Thiazides and beta blockers are the first line agents for treating HTN having less side effects. Beta blockers drugs should be avoided in conditions such as DM because it may create hyperglycaemic reactions. Atenolol, metoprolol and bisoprolol are the drugs which must be used by considering appropriate dose in cardiovascular conditions.14

ACE-I agents are the first line agents or clients having left-ventricular dysfunction. Methyldopa or labetalol are the drugs which are used as first line agents during pregnancy because these drugs do not cause any harmful effect to foetus.15

In some of clients pulmonary edema is seen for that loop diuretics are prescribed because of their short duration .The effective antihypertensive drugs are ACE inhibitors. Drugs such as Diltiazen and Verampril cause adverse effects such as heart blockage, sinus tachycardia. The drugs that should be avoided are short acting CCBs because of large variations in tachycardia & B.P.16

<table>
<thead>
<tr>
<th>Patient Assessment</th>
<th>Target BP</th>
<th>Initial Drug Choice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AHA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary prevention</td>
<td>Framingham risk score&lt;10%</td>
<td>≤ 140/90</td>
</tr>
<tr>
<td>Framingham risk score&lt;10%</td>
<td>≤ 130/80</td>
<td>1st – ARB 2nd – Thiazide diuretic 3rd- BB</td>
</tr>
</tbody>
</table>

ACEI – Angiotensin converting enzyme inhibitor, BB- beta blocker, CCB – calcium channel blocker, ARB – Angiotensin Receptor Blocker
Table 5: Classes of Antihypertensive Agents

<table>
<thead>
<tr>
<th>α Blockers</th>
<th>ACE-Inhibitors</th>
<th>ARBs</th>
<th>Beta-blockers</th>
<th>CCBs</th>
<th>CAAs</th>
<th>Diuretics</th>
<th>Vasodilators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxazocin</td>
<td>Captopril</td>
<td>Irbesartan</td>
<td>Non-Selective</td>
<td>Nandolol</td>
<td>Diltiazem</td>
<td>Clonidine</td>
<td>Thiazide</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>Enalapril</td>
<td>Losartan</td>
<td>Oxprenol</td>
<td>Isradipine</td>
<td>Methyldopa</td>
<td>Loop</td>
<td>Bendrofluzide</td>
</tr>
<tr>
<td>Prazosin</td>
<td>Fosinopril</td>
<td>Telmisartan</td>
<td>Pindolol</td>
<td>Nicardipine</td>
<td>Moxonidine</td>
<td>Torsemide</td>
<td></td>
</tr>
<tr>
<td>Lisinopril</td>
<td>Valsartan</td>
<td>Propranolol</td>
<td>Nifedipine</td>
<td>Reserpine</td>
<td>Potassium sparing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ramipril</td>
<td>Sotalol</td>
<td>-</td>
<td>Nimodipine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Timolol</td>
<td>Cardio selective</td>
<td>Atenolol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bisoprolol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Metoprolol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β- Blockers</td>
<td>Carvedilol</td>
<td>-</td>
<td>Labetalol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ISA</td>
<td>Acebutolol</td>
<td>-</td>
<td>Oxprenol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Pindolol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CCA- centrally acting anti-adrenergic, HCTZ- hydrochlorothiazide, ISA- intrinsic sympathomimetic activity

Hypertensive crises

The blood pressure in accelerated hypertension is equal to or greater than systolic 18mmhg or diastolic 110mmhg. Some patients may be asymptomatic but some of them may report headache, breathing difficulty, visual failure, altered level of consciousness. The goal for management of hypertensive crisis is prompt which leads to sudden decrease in blood pressure. Hypertensive crisis does not have specific treatment, only furosemide plus hydralazine is advised every 15 minutes intravenously till the normal range of blood pressure is achieved.19

Major side-effects of antihypertensive drugs

The major side effects of thiazides are postural hypotension, hyperlipidaemia, impaired glucose tolerance, hypokalaemia, neonatal thrombocytopenia and hyperuricemia. Beta blockers have some adverse effects such as heart failure, cold peripheries, bradycardia, asthma, fatigue and bronchospasm.20

Low doses of ACE inhibitors are well tolerated and may cause side effects such as hyperkalaemia, renal failure, skin rashes and dry cough. Hydralazine causes systemic lupus erythematosus if given to slow acetylators but is reversible on omission of drug. Hydralazine
causes fever, vascular headache, hepatitis and peripheral neuropathy. CCBs causes adverse effects such as gum hyperplasia, headache, flushing, ankle edema and fatigue.20

**Ethical Consideration:** There was no involvement of subjects hence ethical permission is not required.

**Funding:** No external funding was provided for this review

**Conflicts of Interest:** None of the authors disclose any conflicts of interest, either real or perceived.

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15. Ministry of Health-GOK clinical guidelines for diagnosis and treatment of common conditions in Kenya (October 2002); 47-51.


17. American Diabetes Association. Treatment of


Effectiveness of Lockdown in Reducing the Spread of COVID-19

Adyati Satya Puspita, Judya Sukmana, Lestari Dewi, Erina Yatmasari

1 Student at Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia, 2 Lecturers and Master of Patobiologi at Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia, 3 Lecturers at Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia

Abstract

At the end of 2019, a mysterious outbreak appeared, forming an atypical pneumonia suspected of originating from an animal market in Wuhan China. The outbreak is a new type of coronavirus which is named as COVID-19 disease (2019 – nCoV, Novel Coronavirus). COVID 19 disease is a viral infection caused by SARS-CoV-2, namely the acute respiratory syndrome coronavirus 2 which emerged in Wuhan, China. Vaccines and antiviral drugs have not been found, meanwhile COVID-19 are prevented by using non-pharmacological interventions, one of the actions taken by several countries is to create policy protocols such as lockdowns.

The results of this study indicate that lockdown is effective in reducing the spread of COVID-19, it can be seen from the significant decrease in R0 and Rt, <1 in several countries after lockdown such as UK 0.99 (0.96–1.02), Italy 0.89 (0.87–0.91), French 0.76 (0.72–0.82) and Spain 0.74 (0.71–0.78) which means that someone who is infected cannot infect other people and the disease will die (disappear). The value of Rt also shows a consistent decline Rt to <1 (0.88) after 2 weeks of lockdown in Italy.

The conclusion from this literature show that lockdowns can reduce the spread of COVID-19 which is shown by the number of incidents before and after the lockdown which has decreased to zero cases in China. The decline in cases also occurred in Europe although at the beginning of the lockdown it was not significant but it was increasingly effective and continued to be significant after the lockdown was imposed.

Keywords: Effectiveness, COVID-19, Lockdown, Spread, R0, Rt

Introduction

At the end of 2019, a mysterious outbreak appeared in the form of atypical pneumonia suspected to have originated in an animal market in Wuhan China. The outbreak is a new type of coronavirus named Novel Coronavirus 2019 (2019-nCoV / COVID 19) by the World Health Organization (WHO)1.

Novel Coronavirus disease 19 (2019 - nCoV) is a viral infection caused by SARS-CoV-2, namely the acute respiratory syndrome coronavirus 2 which emerged in Wuhan, China2.

The rapid spread of the virus has led to implementation of preventive measures in the COVID-19 outbreak, such as reducing interactions between infected, uninfected and unidentified individuals. Recent research shows preventive measures begin with quarantine of infected and possibly infected individuals and prohibiting travel to restricted areas. Previous research has shown travel restrictions or interaction restrictions have had a positive effect on past SARS, Ebola, and Pes outbreaks. The lockdown in Wuhan is strictly limiting various activities and form of home quarantine. Social distancing, namely limiting community activities but not as strict as

Corresponding Author:
Judya Sukmana
e-mail: judya.sukmana@hangtuah.ac.id
lockdown because residents are still allowed to leave the house for a maximum of 30 minutes³.

*Lockdown* is a limitation of physical contact between individuals and form of instructions not to leave the house in order to avoid the outside environment including with infected people without symptoms, in this condition only urgent matters or emergencies are allowed to do general activities. Lockdowns are increasingly being applied to countries affected by the COVID-19 outbreak such as Italy, China, UK, India, France and Spain but in contrast to the strict lockdown in China, the Italian government allows its people to continue to work, leave the house within hours and must maintain minimum distance of 1 meter per person. The State of Indonesia in the context of controlling the implementation of health quarantine based on Law Number 6 of 2018, one of the measures of health quarantine is large-scale social restrictions (PSBB) instead of lockdown ³–⁵.

**Methodology**

This study uses a literature study method with data from international journals (Scimago, Scopus) or national indexed (SINTA) which can be accessed completely. Articles were collected from the year 2015-2020.

**Lockdown**

A total of 214 countries have confirmed cases of COVID-19. With the increasing number of cases and deaths in this outbreak, many countries are taking strict restrictive measures such as school holidays, quarantine at home, working from home to imposing lockdowns (full restrictions on international and domestic travel) to slow the spread of the COVID-19 outbreak “Flatten the curve”. Lockdowns in each country is different depending on the effect it produces, apart from functioning to reduce the spread of the outbreak, lockdowns also have a good impact on the environment ⁶,⁷.

**Result**

The cases in Italy before the lockdown were 9,172 cases a day later to 10,149 cases. The number of cases in India before the lockdown was 492 which then continued to increase. The country of Paris before the lockdown numbered 2,281 cases while the UK country had 2,630 cases before the lockdown⁸. China on February 29, 2020 there were 79,394 confirmed cases and 2,838 deaths from COVID-19, 48,557 cases came from Wuhan ⁹. WHO data shows that as of May 12, 2020, more than 4 million confirmed cases, around 280,000 deaths and around 215 countries, regions or areas were confirmed infected. Europe and North America starting in May, were the continents worst affected by COVID-19, namely 1,755,790 and 1,743,717 cases as of 12 May 2020, Spain with 224,000 confirmed cases, followed by Russia 221,334 cases, England 219,187 cases, Italy 219,070 and Germany 169,575 which continues to grow rapidly⁸,¹⁰.

European countries report the number of cases up to 6.6 million cases (1.46% of the population), 1.2 million cases in Africa (0.10%) and 90,000 cases in China (0.01% of the population)¹¹.

**Table 1. R0 Value Before and After Lockdown in Several Countries**

<table>
<thead>
<tr>
<th>Country</th>
<th>R0 Before</th>
<th>R0 After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wuhan China</td>
<td>2.71</td>
<td>0.06</td>
</tr>
<tr>
<td>Italy</td>
<td>2.6</td>
<td>0.88</td>
</tr>
<tr>
<td>UK</td>
<td>2.1</td>
<td>0.99</td>
</tr>
<tr>
<td>Spain</td>
<td>5.89</td>
<td>0.48</td>
</tr>
<tr>
<td>French</td>
<td>2.9</td>
<td>0.67</td>
</tr>
</tbody>
</table>
Table 1 shows the estimation of R0 according to Lonergan and Chalmers that lockdown can reduce R0 below the number 1, even if only in some countries. In USA, the number of cases (n) 1,551,853 had R0 before 3.6 (3.4–3.8) and R0 after 0.97 (0.94–1.00), UK n 248,818 with R0 before 2.1 (1.8–2.3) and R0 after 0.99 (0.96–1.02), Italy with 227,364 cases and having R0 before 2.2 (2.0–2.4) and R0 after 0.89 (0.87–0.91), France with n 143,845 and R0 before 2.0 (1.8–2.1) and R0 after 0.76 (0.72–0.82), Spain with 233,286 cases with R0 before 2.2 (2.1–2.4) and R0 after 0.74 (0.71–0.78)\(^12\).

Table 2. R0 Before and After Lockdown in Wuhan, Hubei Province, China

Y. Li and friends’ research in China before Wuhan lockdown (before 23 January 2020) R0: 5.6015 in mainland China, after Wuhan lockdown and before the Hubei Province lockdown (23-26 January) R0: 6, 6037. Hubei Province in lockdown and without large-scale case screening, R0 in Hubei: 3.7732 and R0 outside Hubei Province: 0.9943, but after lockdown and large-scale case screening, R0 in Hubei becomes: 0.2020 and R0 outside Hubei province becomes: 0.0472 so that the average R0 in Hubei Province is 3.4094 until February 25, 2020\(^13\). Fang, Wang and Yang’s research shows that the lockdown in Wuhan shows a decrease in inflow into Wuhan by 76.64% and outflow from Wuhan by 56.35%, besides that with counterfactual simulations show that lockdown in the city of Wuhan has contributed to reducing the total infected cases outside of Wuhan, where if it is not carried out the lockdown shows the case rate was 64.81% higher in 347 cities in China outside Hubei Province and 52.64% higher in 16 cities outside of Wuhan that you are in Hubei Province \(^14\).
Figure 1. shows the curve of R0 after control measures in Wuhan on January 23, 2020. Lockdown shown by a blue vertical line, R0 begins to decline. The epidemic is slowly dying, seen from R0 starting at 2.71 on 23 January then dropping to below 1 on 8 February 2020 and dropping to 0.06 on 6 March 202015.

Another study showed complete lockdowns in Kensington and Chelsea without movement would reduce infections by 97%. Lockdown starts from March 9, 2020 in London. Based on several scenarios and baseline cases, it shows when the lockdown is eliminated on May 8, 2020 without any additional intervention R0: 2.56, while when weekly checks under lockdown conditions R0 shows 0.50 16.

COVID 19 resurfaced in the UK starting in late summer after most restrictions were lifted. In October, a large population-based study found 50,000-100,000 new infections each day. The British government on 12 October 2020 announced a social distancing program that is differentiated by each region which consists of 3 levels. The least restrictive grade 1 areas change to grade 2 or 3 as the incidence of infection increases. Tier 1 regions have a 10pm curfew and restrictions on the number of individuals when meeting. Region 2 is restrictions on individuals from neighbors and it is advisable not to travel if it is not important, while in region level 3 there are restrictions on closing places such as hotels and recreation areas such as pubs and restaurants. Incidence continues to increase in all regions of the UK despite these steps17.

The national lockdown will be carried out starting 5 November 2020 for 4 weeks. The 2nd lockdown is similar to the first one but schools and universities can be opened. Northern Ireland and Wales went into lockdown in mid-October. Lockdown in Northern Ireland allows retail can open and its citizens can gather with neighbors of up to 2 neighbors while in the country of Wales retail non-essential retail cannot open and residents are expected to always be at home and not make contact with neighbors.
Table 3. Types of Preventive Measures in the UK

<table>
<thead>
<tr>
<th>Type of intervention</th>
<th>Decrease of Rt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tier 2</td>
<td>2% (0-4)</td>
</tr>
<tr>
<td>tier 3</td>
<td>10% (6-14)</td>
</tr>
</tbody>
</table>

**LOCKDOWN IN NORTH IRELAND**

<table>
<thead>
<tr>
<th></th>
<th>Decrease of Rt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>School closed</td>
<td>35% (30-41)</td>
</tr>
<tr>
<td>School opened</td>
<td>22% (15-27)</td>
</tr>
</tbody>
</table>

**LOCKDOWN IN WALES**

<table>
<thead>
<tr>
<th></th>
<th>Decrease of Rt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>School closed</td>
<td>44% (37-49)</td>
</tr>
<tr>
<td>School opened</td>
<td>32% (25-39)</td>
</tr>
</tbody>
</table>

**LOCKDOWN IN LONDON**

<table>
<thead>
<tr>
<th></th>
<th>Decrease of Rt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>School closed</td>
<td>36% (29-42)</td>
</tr>
<tr>
<td>School opened</td>
<td>22% (15-29)</td>
</tr>
</tbody>
</table>

Research by Davies and colleagues showed that the Rt value decreased after various interventions such as: level 2 areas decreased by 2% (0 - 4), level 3 decreased by 10% (6 - 14), the type of lockdown in Northern Ireland, when schools were closed: 35% (30 - 41), schools opened: 22% (15 - 27), lockdown in Wales when schools closed: 44% (37 - 49), schools opened: 32% (25 - 39) and lockdowns in England decreased when the school was closed 36% (29 - 42), when the school was opened 22% (15 - 29).18

Evaluating the progressive result of transmission reduction through Rt which are divided into: 1) before lockdown, 2) the first week and 3) the second week after lockdown (18 and 25 March). Average R0 2.83 – 3.10 in 8 region in Italy. March 10, 2020 Rt 1.79 – 3.36, then 1 week after the lockdown (March 18) Rt fell consistently (Rt 1.28) and in the second week (March 25) Rt <1 (0.88) consecutively for 3 weeks in almost all regions.19

Lockdown show high effectiveness in reducing spread and mortality, but has a negative effect if it continues to be prolonged in the form of economic and social instability of the country.16 Journals about lockdown show when a country is late in lockdown the number of infected cases will be more and increase rapidly than countries that lockdown earlier. Countries that apply lockdowns with additional interventions or variables have better lockdown results besides that individual compliance with lockdowns shows a decrease in the spread of COVID-19 which is in accordance with the objectives of the lockdown itself.
Other studies showed no significant decrease in the number of daily cases after the lockdown instead an increase in the number of daily cases after 3 weeks during the lockdown. Cases are increasing rapidly possibly due to a lot of migration in various regions, besides that the failure of the lockdown in India during the lockdown period could be the result of several groups of individuals who did not follow the restrictions in India or because the number of tests carried out during a certain period has increased significantly which causes some studies have different results especially in some Indian country studies\textsuperscript{20}.

**Conclusion**

Lockdown is effective in reducing the spread of COVID-19 in several countries. Incidence of the spread of COVID-19 from several journals shows that after lockdown the case is lower than before the implementation of the lockdown, especially in China and European. Lockdown has the effectiveness in reducing the spread of COVID-19.

**Ethical Clearance** – Not required since it is a literature review

**Source of Funding** – nil

**Conflict of Interest** – nil

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A Comparative Analysis of Misery Index and Its Impact on Health Indicators Across The Globe

Aishwarya¹, Rajmohan² Suganya.P³, Prabu.D⁴, Bharathwaj⁵, M.R.Prashanthy³
¹Undergraduate Student, ²Reader, ³Postgraduate Student, ⁴Professor and Head of the Department, ⁵Lecturer, SRM Dental College, Ramapuram

Abstract

Aim: This study aims to analyze the impact/relationship of the health indicators and the human development index over the globe’s misery index.

Materials and Method: In this paper, a comparative data analysis of the misery index, health indicators like mortality rate, disability rate, Quality of life index, and human development index, along with the ranking based on the human development index, were done by tabulating the data collected from the electronic sources.

Results: There was a strong correlation between the misery index, human development index, and health indicators.

Conclusion: This paper clearly shows that the misery index of a country was highly influenced by the health indicators, such as the mortality rate, disability rate, and Quality of life. Further, the human development index also had a comparative impact on the misery index of a country. Hence, this study depicted a descriptive statistical relationship among the misery index, the health indicators (mortality rate, disability rate, Quality of life) and the Human Development Index (HDI).

Keywords: Misery index, health indicators, mortality rate, Quality of life, Human Development Index (HDI).

Introduction

The misery index is an economic indicator, created by economist Arthur Okun. The index determines the performance of an average citizen economically in the macroeconomic states of a country. The misery index is calculated by adding up the inflation rate and unemployment rate of a nation.

The economic growth of a nation depends on the employment, price stability, and economic development of the citizen. These parameters are challenging to be calculated due to reasons like variables in the same units, etc. Hence, researchers have tried to use one figure that would help to represent the macroeconomic condition [1].

Okun’s Misery Index (economic discomfort index, 1970) was the first single variable index summarizing various macroeconomic indicators to deliver the economic state of the country [2]. Okun’s index is the most widely accepted and commonly used index attributable to its simplicity and competence to measure absolute values. This index is derived by adding up the unemployment rate and the inflation rates by catering equal weight to both the grades. An increase in the poor economic performance, unemployment, or rise in inflation rates will sequentially induce an increase in misery index. The misery index modified by Robert Baro (1999), Hanke (2009), but still Okun’s index is widely
used by the politicians to know whether the condition is getting worse or better [1,2].

Health is most important than anything in the world. The health of a population plays an influential role in the development of the country. Hence, specific indicators, such as the health indicators [3, 4], can measure the nation’s health status.

The main aim of health indicators is to keep the nation’s health score for the betterment [3]. There are ten leading health indicators to assess health status. Of those ten health indicators, the mortality rate, disability rate, and the Quality of life were the traditionally used indicators as death is a well-recorded event.

The mortality rate is a measure of the number of deaths in a particular population, per unit of time. The mortality rate expressed as the number of deaths per 1000 individuals per year. Quality of life is the general well-being of individuals in a society outlining the negative and positive features of life. Quality of life includes various contexts such as education, politics, religious belief, environment and many more country indicators.

Disability rate is a cumulative index of all types of disabilities, such as the visual disability, physical and mental disability seen per 1000 people of a country/community [3, 4]. The Human development index (HDI) is another similar index used commonly for the measurement of development. This index sums up the measure of life expectancy, education, and living standard, sequentially giving a clear quantifying idea for an individual [5].

This paper analyze the misery index all over the globe in the year 2019 and its relation with health indicators such as the mortality rate, disability rate, Quality of life and the human development index.

**Materials and Method**

In this paper, Misery index and its relationship with health indicators were evaluated. The Misery index for the year 2019 was retrieved from the electronic database [6]. Based on the misery index, the most ten miserable countries, the moderate ten countries, and the least ten miserable countries were only considered for ease and generalization of the analysis.

Simultaneously, the data regarding the health indicators among top ten, middle ten and least ten miserable countries for the year 2019 were also collected [7]. The health indicators such as disability rate, mortality rate and the Quality of life of an individual in a country were obtained from the electronic database [8].

Then the data regarding the Human Development Index (HDI) of these countries were also obtained from the online sources given by the United Nations development program for the year 2019 [9]. The collected data were analyzed and tabulated accordingly in the Microsoft excel using descriptive analysis.

**Results**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>COUNTRY</th>
<th>MISERY INDEX (SCORE)</th>
<th>MORTALITY RATE</th>
<th>DISABILITY RATE</th>
<th>QUALITY OF LIFE (SCORE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>VENEZUELA</td>
<td>1746439.1</td>
<td>5.3</td>
<td>5.4</td>
<td>157.91</td>
</tr>
<tr>
<td>2.</td>
<td>ARGENTINA</td>
<td>105.6</td>
<td>7.5</td>
<td>12.9</td>
<td>121.02</td>
</tr>
<tr>
<td>3.</td>
<td>IRAN</td>
<td>75.7</td>
<td>5.3</td>
<td>13</td>
<td>75.22</td>
</tr>
<tr>
<td>4.</td>
<td>BRAZIL</td>
<td>53.6</td>
<td>6.7</td>
<td>1.9</td>
<td>103.87</td>
</tr>
</tbody>
</table>
Table 1 depicts the most miserable countries of the world and the health indicators like the mortality rate, disability rate, and the Quality of life. Further, the table shows that higher the misery index of the country will sequentially have a higher mortality rate along with the disability rate but lower Quality of life and HDI.

### TABLE 2: RANKING OF MODERATE MISERABLE COUNTRIES AND ITS RELATION WITH HEALTH INDICATORS

<table>
<thead>
<tr>
<th>S.NO</th>
<th>RANKING OF MODERATE MISERABLE COUNTRIES</th>
<th>MISERY INDEX (SCORE)</th>
<th>MORTALITY RATE</th>
<th>DISABILITY RATE</th>
<th>QUALITY OF LIFE (SCORE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INDIA</td>
<td>13.2</td>
<td>7.3</td>
<td>2.1</td>
<td>115.41</td>
</tr>
<tr>
<td>2</td>
<td>BANGLADESH</td>
<td>12.6</td>
<td>5.4</td>
<td>10</td>
<td>69.3</td>
</tr>
<tr>
<td>3</td>
<td>ALBANIA</td>
<td>12.2</td>
<td>6.9</td>
<td>6.2</td>
<td>99.01</td>
</tr>
<tr>
<td>4</td>
<td>ECUADOR</td>
<td>12.2</td>
<td>5.1</td>
<td>2</td>
<td>128.06</td>
</tr>
<tr>
<td>5</td>
<td>EL SALVADOR</td>
<td>12</td>
<td>5.8</td>
<td>10</td>
<td>83.01</td>
</tr>
<tr>
<td>6</td>
<td>PHILIPPINES</td>
<td>11.8</td>
<td>6.1</td>
<td>1.5</td>
<td>88.23</td>
</tr>
<tr>
<td>7</td>
<td>CYPRUS</td>
<td>11.7</td>
<td>6.8</td>
<td>2</td>
<td>152.72</td>
</tr>
<tr>
<td>8</td>
<td>CROATIA</td>
<td>10.9</td>
<td>12.4</td>
<td>3</td>
<td>164.69</td>
</tr>
<tr>
<td>9</td>
<td>BOLIVIA</td>
<td>10.8</td>
<td>6.3</td>
<td>10</td>
<td>134.54</td>
</tr>
<tr>
<td>10</td>
<td>CANADA</td>
<td>10.8</td>
<td>8.8</td>
<td>13.7</td>
<td>169.42</td>
</tr>
</tbody>
</table>
Table 2 depicts the relationship of health indicators moderate miserable countries and the health indicators. The mortality rate, disability rate and Quality of life of the moderate miserable countries remain at a moderate level.

**TABLE 3: RANKING OF LEAST MISERABLE COUNTRIES AND ITS RELATION WITH HEALTH INDICATORS**

<table>
<thead>
<tr>
<th>S.no</th>
<th>Ranking Of Least Miserable Countries</th>
<th>Misery Index (Score)</th>
<th>Mortality Rate</th>
<th>Disability Rate</th>
<th>Quality of Life (Score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MALAYSIA</td>
<td>5.1</td>
<td>5.2</td>
<td>1.3</td>
<td>120.39</td>
</tr>
<tr>
<td>2</td>
<td>CZECH REPUBLIC</td>
<td>5</td>
<td>10.5</td>
<td>10.3</td>
<td>160.37</td>
</tr>
<tr>
<td>3</td>
<td>NETHERLAND</td>
<td>4.7</td>
<td>9</td>
<td>15</td>
<td>186.41</td>
</tr>
<tr>
<td>4</td>
<td>TAIWAN</td>
<td>4.4</td>
<td>7.6</td>
<td>14.7</td>
<td>146.59</td>
</tr>
<tr>
<td>5</td>
<td>SWITZERLAND</td>
<td>4.2</td>
<td>8.4</td>
<td>14.5</td>
<td>196.08</td>
</tr>
<tr>
<td>6</td>
<td>CHINA</td>
<td>4.2</td>
<td>8</td>
<td>15</td>
<td>99.87</td>
</tr>
<tr>
<td>7</td>
<td>AUSTRALIA</td>
<td>3.9</td>
<td>7.3</td>
<td>17.5</td>
<td>189.73</td>
</tr>
<tr>
<td>8</td>
<td>JAPAN</td>
<td>3.3</td>
<td>9.9</td>
<td>5.9</td>
<td>176.46</td>
</tr>
<tr>
<td>9</td>
<td>HUNGARY</td>
<td>2.6</td>
<td>12.8</td>
<td>6.1</td>
<td>133.06</td>
</tr>
<tr>
<td>10</td>
<td>THAILAND</td>
<td>1.7</td>
<td>8.1</td>
<td>2.2</td>
<td>104.54</td>
</tr>
</tbody>
</table>

Table 3 depicts the least miserable countries and its relation with health indicators. The mortality rate and the disability rate were considerably low while the quality of life index remains high.

**Discussion**

In this study, the misery index and its association with health indicators were evaluated. Table 1 shows about the most miserable countries and its relation with the mortality rate, disability rate, and Quality of life of the corresponding countries. Ukraine had the second highest mortality rate globally, with the highest disability rate sequentially being in the most miseries countries. On the contrary, Ukraine had an average quality of life index due to the best political and administrative structure [10].

Venezuela stands the top most miserable country in the world in the year 2019. This country had an average...
mortality and disability rate along with a good quality of life index on analysis. The main reason of this country to be among the top miseries countries was due to the fall in oil production, which made up to 95% of the country export, leading to fall of the inflation rate. Further, this country had high female empowerment along with specific educational transitions that aided the improvement of the country economic and social status. Additionally, table 1 shows that the countries with high misery index scores also had a high mortality and disability rate, whereas a comparatively low quality of life index.

In table 2, the countries had an average mortality rate, disability rate, and good Quality of life index without much variation. Further, these countries remain with the same misery index or mild variation for the past two years. This was attributed to the country’s political administration, workforce, and the environment. Except Bangladesh, Philippines, and El Salvador had a less quality of life index due to the insufficient remittances which affects the economic status of these countries. Further, table 2 shows India with an average misery index and an average mortality rate, disability rate, and good Quality of life value. The urbanization in India from traditional rural economies to a modern industrial country had been a problematic transformation as there were many barriers and obstacles leads the country to be miserable.

In table 3, all the countries had a high quality of life index, indicating that they can provide a sophisticated, comfortable, and safe environment for the people. Further, the other indicators like mortality rate and disability rate also appear to be below average, except Australia having a high disability rate due to indigenous Australians. Furthermore, studies conducted showed that countries like Australia, Switzerland, and Japan had a gradual increase in economic development as well as their expenditure and scheme implements to improve health sectors in the country.

On analyzing fig 2, 4 and 6, it can be noted that countries with high and average Misery index score have a low Human development index score and those countries with low misery index score had high Human development index score. Hence it can be concluded that the countries with lowest misery index have a good human development index.

On the overall analysis, the countries with high misery index scores had comparatively higher mortality and disability rate and simultaneously had a low quality of life and human development index. Similarly, the country with low misery index scores had a higher quality of life index and the human development index, along with a low mortality and disability rate.

Hence, the misery index is directly proportional to the mortality rate and disability rate of the country, i.e., higher the misery index, the higher will be the mortality and disability rate and vice versa. The misery index is inversely proportional to the Quality of life index and human development index, i.e., higher the misery index, lower the Quality of life and human development index, and vice versa.

Limitations:

This study only included available data till the year 2020. Only countries based on most, moderate and least miserable countries were considered, and the respective data about these countries was specifically collected for the year 2019. Further inclusion of all other countries will give more relevant results and a better understanding of this perspective.

Conclusion

The overall analysis of this study caters to a very strong point that the misery index of a country has a direct or indirect influence on the health indicators and the Human Development Index (HDI). Thus, a country with a high or low misery index scores will surely have a reflection on health indicators and HDI. Hence, the health indicators, the HDI, and the misery index of a country influence each other either directly or indirectly. This paper helps to identify the appropriate drawback of a particular nation which on rectification aids in the betterment of the same.
Ethical Clearance: Taken from institution review board of Public Health Dentistry Department, SRM Dental College, Ramapuram

Source of Funding: Self

Conflict of Interest: Nil

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The Relationship of Perceived Benefit, Perceived Barrier, and Knowledge with Vaccine Hesitancy among Anti- and Pro-Vaccine Community

Aisyah Nur Izzati¹, Retno Indarwati², Makhfudli²
¹Student at Master of Nursing, Faculty of Nursing, ²Lecture, Department of Mental and Community, Faculty of Nursing, Universitas Airlangga, Surabaya, Indonesia

Abstract

Introduction: The phenomenon of basic vaccine hesitancy in parents is the biggest challenge faced by health workers in the global era currently. This study aimed to analyze the relationship between perceived benefits, perceived barriers, and knowledge about vaccines towards vaccine hesitancy among anti and pro-vaccine communities on social media Facebook.

Methods: This study used a cross-sectional design. The sample was 150 members of the anti-vaccine community and 234 members of the pro-vaccine community on social media Facebook using a purposive sampling technique. Data were collected using questionnaire namely; HBM (health belief model) questionnaire which was translated and modified, and Vaccine Hesitancy Scale (VHS) questionnaire. The independent variables in this study were perceived benefits of vaccines, perceived barriers to vaccines, knowledge about vaccines. The dependent variable was vaccine hesitancy. Spearman rho analysis was used to measure the relationship between the dependent and independent variables.

Results: The results of the Spearman rho analysis showed that the perceived benefits of vaccines (p=0.363 of anti-vaccine, p=0.702 of pro-vaccine), the perceived barriers to vaccines (p=0.410 of anti-vaccine, p=0.341 of pro-vaccine), and knowledge about vaccines (p=0.413 of anti-vaccine, p=0.192 of pro-vaccine) was related to vaccine hesitancy in parents.

Discussion: This study results indicated that the perceived benefits of vaccines, perceived barriers to vaccines, and knowledge about vaccines are related to vaccine hesitancy in parents. The implications of the study are essential information for health workers to determine the right and wise strategy in responding to the increasing vaccine hesitancy in public so that basic vaccine coverage will increase.

Keywords: anti-vaccine, vaccine hesitancy, health belief model

Introduction

The number of countries reporting vaccine hesitancy in parents has continued to increase since 2017.¹ This controversial issue regarding vaccines is the biggest challenge for the implementation of vaccines in Indonesia.² It influences the stagnation of complete basic vaccine coverage. Basic Health Research of the Indonesian Ministry of Health showed that status of complete basic vaccination coverage in children aged 12-23 months had decreased from 59.2% in 2013 to 57.9% in 2018. Vaccination coverage must be maintained high and evenly distributed throughout the region to avoid the occurrence of extraordinary epidemic. Therefore, the government and health workers need to solve this...
problem immediately.³

World Health Organization stated that the anti-vaccine community was one of the ten most significant threats to global health in 2019 since this phenomenon could potentially increase the risk of an epidemic of a disease.⁴ The determinant of vaccine hesitancy among parents needs to be investigated further. A study conducted by Brunson stated that vaccine hesitancy and acceptance is a complex and multi-faceted issue so that the way health workers understand them also cannot be universally equalized.⁵

Pro-contra debate of vaccines in Indonesia has spread up again following the emergence of diverse arguments from society ranging from influencers, artists, and even health workers who are broadly spread through social media.⁶ Pro-vaccine community consider vaccines are essential to prevent infectious diseases for their children. On the contrary, anti-vaccine community consider vaccines contain dangerous substances that can harm their children. Community members on social media not only share textual context but also images to emphasize the message to other members.⁵ The debate between the anti- and pro-vaccine communities on the digital platform about vaccine controversy may decrease public trust in the government and healthcare providers in vaccination program.⁷

Vaccine hesitancy is one of the major challenges in achieving complete basic vaccine coverage. Some parents who accept the administration of vaccines are still worried.⁸ Schalkwyk⁹ in his study stated that social media is now used as a media to spread dangerous information about vaccines to strengthen the hesitancy of parents in giving vaccines to their children. Social media was chosen because of the increase people’s dependency online media to obtain accurate health information.¹⁰ The type of social media most used to spread anti-vaccine propaganda is the Facebook Group.¹¹

Indonesia is the fourth highest user of social media Facebook in the world with 130 million active users per month (Hootsuite and We Are Social, 2018).¹² Study results of the Ipsos-Center for International Governance Innovation (CIGI) showed that 65 percent of internet and social media users in Indonesia are susceptible to misinformation provided in cyberspace without prior confirmation. Thus, negative content including hoaxes about vaccines are easy to widespread in community. The current problems in the community obviously cause for concern for all health workers. Various studies on the pros and cons of vaccination have been broadly studied in several other countries, but still very limited in Indonesia. This study aimed to analyze basic vaccine hesitancy in the anti- and pro-vaccine communities on social media Facebook.

Material and Methods

This study used a cross-sectional design. The study was conducted in June-August 2020 by distributing questionnaires to respondents through Google form. The population in this study was mothers of the members of the anti-vaccine and pro-vaccine communities on social media Facebook. The number of the anti-vaccine community members on Facebook was 2,900 people, while the pro-vaccine community members on social media Facebook was 119,000 people. A total of 384 respondents (150 members of the anti-vaccine and 234 members of the pro-vaccine communities) were taken using purposive sampling technique. The inclusion criteria were mothers aged 18-40 years, are active real accounts, and are willing to be respondent. The independent variables in this study were perceived benefits of vaccines, perceived barriers to vaccines, and knowledge about vaccines. The dependent variable in this study was the basic vaccine hesitancy. The research instruments used in this study were adapted from the HBM questionnaire from Hwang et al. 2017, which was translated and modified. The vaccine hesitancy questionnaire was adapted from the Vaccine Hesitancy Scale (VHS) questionnaire by Saphiro G.K. et al. 2018

Quantitative data analysis in this study was performed using the Spearman Rho statistical test.

Results

Most of the anti- and pro-vaccine respondents
were 26-35 years old. Almost all of the religion of the respondents was Muslim, while only a few people were Christians and Catholics on the anti-vaccine group. The religions of pro-vaccine respondents were more diverse, ranging from Islam, Christianity, Catholicism, Hinduism, and Buddhism. The ethnicity of most of the respondents was Javanese, while the rest were Madurese, Batak, Chinese, Osing, and Balinese. Most respondents lived in urban areas, were university graduated, and almost half were high school graduated. More than 50% of the respondents were housewives, while the rest were self-employed, civil servants, and traders with most of them having income of more than 2,500,000 (Table 1).

Table 2 shows that all independent variables showed a significant relationship with basic vaccine hesitancy in the anti- and pro-vaccine communities.

<table>
<thead>
<tr>
<th>Demographic Characteristic</th>
<th>Category</th>
<th>Anti-vaccine</th>
<th>Pro-vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Percentage (%)</td>
<td>N</td>
</tr>
<tr>
<td>Age (year)</td>
<td>18-25</td>
<td>21 14</td>
<td>50 21.4</td>
</tr>
<tr>
<td></td>
<td>26-30</td>
<td>51 34</td>
<td>92 39.3</td>
</tr>
<tr>
<td></td>
<td>31-35</td>
<td>47 31.3</td>
<td>76 32.5</td>
</tr>
<tr>
<td></td>
<td>36-40</td>
<td>31 20.7</td>
<td>16 6.8</td>
</tr>
<tr>
<td>Religion</td>
<td>Islam</td>
<td>146 97.3</td>
<td>192 82.1</td>
</tr>
<tr>
<td></td>
<td>Christian</td>
<td>3 2</td>
<td>27 11.5</td>
</tr>
<tr>
<td></td>
<td>Catholic</td>
<td>1 0.7</td>
<td>11 4.7</td>
</tr>
<tr>
<td></td>
<td>Hindu</td>
<td>0 0</td>
<td>3 1.3</td>
</tr>
<tr>
<td></td>
<td>Buddha</td>
<td>0 0</td>
<td>1 0.4</td>
</tr>
<tr>
<td>Ethnic</td>
<td>Javanese</td>
<td>133 88.7</td>
<td>177 75.6</td>
</tr>
<tr>
<td></td>
<td>Madura</td>
<td>4 2.7</td>
<td>3 1.3</td>
</tr>
<tr>
<td></td>
<td>Batak</td>
<td>6 4</td>
<td>28 11.9</td>
</tr>
<tr>
<td></td>
<td>Chinese</td>
<td>3 2</td>
<td>13 5.6</td>
</tr>
<tr>
<td></td>
<td>Osing</td>
<td>2 1.3</td>
<td>10 4.3</td>
</tr>
<tr>
<td></td>
<td>Balinese</td>
<td>2 1.3</td>
<td>3 1.3</td>
</tr>
<tr>
<td>Area of residence</td>
<td>Urban</td>
<td>80 53.3</td>
<td>139 59.4</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>70 46.7</td>
<td>95 40.6</td>
</tr>
</tbody>
</table>
Cont. Table 1. Demographic Characteristics of the Respondents (n = 384)

<table>
<thead>
<tr>
<th>Level of Education</th>
<th>Elementary school</th>
<th>Junior high school</th>
<th>Senior high school</th>
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<td>53</td>
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<td>2</td>
<td>35,4</td>
<td>61,3</td>
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<td>82</td>
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<td>0,4</td>
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<th>Housewife</th>
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<tbody>
<tr>
<td></td>
<td>84</td>
<td>36</td>
<td>21</td>
<td>9</td>
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<td></td>
<td>56</td>
<td>24</td>
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<td>6</td>
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<tr>
<td></td>
<td>170</td>
<td>41</td>
<td>20</td>
<td>3</td>
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<td>72,7</td>
<td>17,5</td>
<td>8,5</td>
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<th>&gt;2.500.000</th>
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<td></td>
<td>70</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>46,7</td>
<td>53,3</td>
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<td></td>
<td>108</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>46,2</td>
<td>53,8</td>
</tr>
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</table>

Table 2. Test of univariate analysis in the anti and pro-vaccine communities

<table>
<thead>
<tr>
<th>Variables</th>
<th>Category</th>
<th>Anti-vaccine</th>
<th>Pro-vaccine</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Perceived benefit</td>
<td>Low</td>
<td>16</td>
<td>10,7</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>80</td>
<td>53,3</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>54</td>
<td>36,0</td>
</tr>
<tr>
<td></td>
<td>Spearman Rho</td>
<td>p = 0,000</td>
<td>r = 0,363</td>
</tr>
<tr>
<td>Percieved barrier</td>
<td>Low</td>
<td>15</td>
<td>10,0</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>99</td>
<td>66,0</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>36</td>
<td>24,0</td>
</tr>
<tr>
<td></td>
<td>Spearman Rho</td>
<td>p = 0,000</td>
<td>r = 0,410</td>
</tr>
<tr>
<td>Vaccine’s knowledge</td>
<td>Poor</td>
<td>18</td>
<td>12,0</td>
</tr>
<tr>
<td></td>
<td>Enough</td>
<td>43</td>
<td>28,7</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>89</td>
<td>59,3</td>
</tr>
<tr>
<td></td>
<td>Spearman Rho</td>
<td>p = 0,000</td>
<td>r = 0,413</td>
</tr>
</tbody>
</table>
Almost all pro-vaccine respondents had a high level of perceived benefits (95.7%). Meanwhile, for the anti-vaccine group, more than half of the respondents had a moderate level of perceived benefits (53.3%), 36% had a high level of perceived benefits, and 10.7% of respondents had a low level of perceived benefits. More than half of the anti-vaccine respondents had a moderate level of perceived barriers (66%), 24% were at a high level, and only 10% were at a low level. Meanwhile, in the pro-vaccine group, more than half of the respondents had a low level of perceived barriers (56.4%), while only 2 respondents had a high level of perceived barriers. More than half of the anti-vaccine respondents had good knowledge (59.3%), 28.7% was sufficient, and 12% had a lack of knowledge. Meanwhile, most of the pro-vaccine respondents had good knowledge about vaccines (85.9%) and only 4 respondents had a poor knowledge (1.7%). The results of statistical tests using Spearman Rho showed that perceived benefits, perceived barriers, and knowledge about vaccines had significant relationships with basic vaccine hesitancy.

**Discussion**

The results of this study indicated that the perceived benefits of vaccines are related to basic vaccine hesitancy in parents. Gowda\(^\text{13}\) stated that parents’ perceived benefits of vaccination are a specific factor that affects them in considering decisions about vaccination.

The results of a systematic review conducted by Forster\(^\text{14}\) found that there are different perspectives about the benefits of vaccination between people who refuse and accept vaccines. Anti-vaccines community doubt the benefit of vaccines and worry over their children safety. This study results were in line with the previous study, which showed that most of the anti-vaccine respondents in this study confirmed that they disapproved that vaccination was the same as maintaining the health of the surrounding community. They also disagreed with the arguments that all basic vaccines were given by the government is effective and beneficial when there are still incidents that may sacrifice a handful of children.

Karafillakis\(^\text{15}\) emphasized that as of today, scientists tended to discuss the importance of maintaining Vaccine-Preventable Disease (VPD) risks in a population, although most people are more interested in vaccines risks and side effects that might occur to their children. Analyzing the perceived benefits of vaccines felt by each parent seems tricky because it is easier for a person to overestimate risk by comparing its underestimated benefits. Parents are more interested in reading other people’s narratives or stories related to the negative effects of vaccines rather than considering the vaccines benefits at the population level.

The health workers must deliver information accurately regarding the risks and benefits of vaccines to the community.\(^\text{16}\) Health workers must be honest in explaining the possible side effects of vaccines because providing information focusing on the benefits of vaccination only is ineffective in influencing parents’ intention to vaccination program.\(^\text{17}\) Health workers should be encouraged to perform effective and open communication, such as by holding discussions while respecting the thoughts and perspectives of parents refusing vaccines.

This study also showed that perceived barriers to vaccination were related to basic vaccine hesitancy in parents. This result is in line with the previous research conducted by William\(^\text{18}\), which stated that parents who hesitate to vaccination had high perceived barriers to vaccines, one of which was a sense of concern about the safety of vaccines. The skepticism regarding to the vaccines safety is a complex issue that is being globalized today.\(^\text{19}\) The belief that giving vaccines into the children’s body will lead to various long-term dangerous side effects and even death is the essential reason for parents to refuse to vaccinate their children.\(^\text{20}\)

Anderson\(^\text{21}\) added that other barriers that can trigger vaccine hesitancy include confusing vaccine schedules, long queues, and inconvenience in the vaccine process. These various barriers indirectly contribute to the low rate of basic vaccine coverage and delays in the schedule for providing vaccination to children. The previous
study conducted in four countries by Olorunsaiye et al.\textsuperscript{22} showed that the waiting time period in health facilities becomes a barrier for parents to give vaccines. Some parents prefer to stay at home and carry out other essential work rather than have to queue for a long time at health services. This issue may often be underestimated but needs to be addressed immediately to shorten the waiting time for parents who intend to give vaccines in health services.

Another barrier experienced by almost all anti- and pro-vaccine participants was related to the depletion of vaccine stocks. Several participants complained that they had to go back home since the health services run out of vaccine stocks. Although health care worker had arranged the new scheduled, but the parents considered it was not effective. According to the statement of Panting\textsuperscript{23} that the lack of vaccine stocks and the poor organized schedule of vaccination in health services become one of the reasons for vaccine hesitancy in parents. Overcoming vaccine hesitancy needs a particular process of detection, diagnosis, and intervention since there is no simple strategy that can overcome all the barriers felt by parents in accepting vaccines.\textsuperscript{24} Health workers are required to collaborate with policymakers to minimize a number of barriers for parents to accept basic vaccines.\textsuperscript{19}

The results of this study indicated that knowledge about vaccines influences basic vaccine hesitancy. It is in accordance with the previous study conducted by Facciola\textsuperscript{25} which found that parents with low knowledge about vaccines tend to feel hesitant about vaccination. Consideration of parents’ decisions regarding vaccines is context-specific and does not rely on knowledge about vaccines and information obtained only but also on attitudes, values, experiences, and emotions.\textsuperscript{26} A study conducted by Ugezu\textsuperscript{27} stated that the knowledge and attitudes of health workers regarding vaccines influence the decision of parents to give vaccines. Therefore, health workers need to have the good knowledge and attitude about vaccination program since they are most trusted by the parents and community in the process of making decisions about vaccines (Dybsand, Hall and Carson, 2019).\textsuperscript{28}

Knowledge about vaccines is essential but not sufficient to change one’s perception of vaccine hesitancy.\textsuperscript{29} Providing information constantly and firmly to parents who are hesitant about vaccines can be contradictory because if someone is given continuous information that contradicts their values, they will react defensively and lead to resistance.\textsuperscript{30} Therefore, health workers need to identify the most appropriate communication strategies to reduce these unwanted impacts. According to Shapiro\textsuperscript{31} effective communication that is performed gradually is the main tool for health workers to disseminate the information about the vaccines needed, the timing of administration, and side effects to increase parents’ acceptance of vaccines.

**Conclusion**

The results of this study indicated that the perceived benefits of vaccines, perceived barriers to vaccines, and knowledge of vaccines are related to basic vaccine hesitancy in parents. The implications of the results of this study are essential information for health workers to determine the right and wise strategy to respond to the increasing vaccine hesitancy of the public so that basic vaccine coverage will increase. This study was conducted to analyze the background of vaccine hesitancy in mothers who are members of the anti and pro-vaccine communities on social media. Further research is needed to explore various other factors that cause vaccine hesitancy in parents in real life.

**Conflict of Interest** : None

**Funding** This study was funded by Indonesian Ministry of Research and Technology

**Ethical Clearance** : This study had received research ethics approval by the Ethics Committee of the Faculty of Nursing of Airlangga University number 1837-KEPK.

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Knowledge and Attitude Regarding Medication Error among Nursing Students in a Selected College at Mangaluru

Ajanya Shaju1, Aleesha Babu1, Dona Sebastian1, Mary Joseph1, Pavithra K2
1IVth Year B.Sc Nursing Student, 2Lecturer, Department of Medical Surgical Nursing, Yenepoya Nursing College, Yenepoya (Deemed to be Univesity), Mangaluru, Karnataka

Abstract

Background & Aim: Errors are an integral part of human life. In the human healthcare system, advancements are all time high. The system of treatment is becoming more hi-tech and more sophisticated which is vulnerable to the errors at the same time. Effective medication error reporting is a necessary tool to prevent and reduce its occurrence. This study aimed to assess the knowledge and attitude regarding medication error among nursing students.

Methods: A Descriptive approach was adopted for the study. By Stratified Random sampling 125 Nursing Students were selected. The data was collected using Demographic Performa, Structured knowledge questionnaire and Attitude scale. The data obtained were analyzed by descriptive and inferential statistics using SPSS version 16.0.

Results: Results showed that majority 67.2% of the Nursing students had average knowledge and 88% had positive attitude towards the reporting of medication error. There was no significant correlation between knowledge and attitude among nursing student regarding medication error.

Conclusion: The Study concluded that despite sufficient knowledge and favourable attitudes towards medication error reporting, there is still an under-reporting of medication errors when it comes to practice. It was clear that the nurses need specific information about what constitutes medication error.

Key words: Attitude, Knowledge, Medication error, Nursing Students

Introduction

Medicines cure, but they can also kill or cause severe adverse reactions if a wrong medicine or wrong dose is administered. Medication error represents the largest single cause of error in the hospital settings. Medication treatment is a basic primary treatment for any type of illness. It is a multi-disciplinary approach that requires coordinated efforts and involvement of healthcare professionals to carefully distribute medicines to the patients without causing any error.

Patient safety is a common goal for every healthcare provider. One of the major issues for safety is medication errors. It is important indicator of health care delivery system because potential injury to patients, Over 2 million serious ADRs requiring hospitalization, causing death occur each year.

Mostly everybody in the world takes medication at one time or another. In India, the medication errors and medication related problems are mainly due to irrational use of medications. The number of errors and distribution of errors might vary in different settings. It has been reported that experts believe at least one medication error occurs per hospital patient every day.

A study conducted on the medication error in a general hospital of Bangalore; Karnataka which showed that the overall incidence of medication error was 38.12%.
Medication error is caused by the health care professionals, who involve in the direct patient care such as Nurses, Doctors and the Pharmacists which lead to the harmful effects on the patients which can end up in death sometimes. However, it can be prevented largely if an extra caution and concern is taken by the Nurses.6

Nurses play a major role in preventing, identifying and reporting the medication error. Inspite of the medication error, some nurses fail to report the error because of their perceived barrier. In order to prevent the medication error completely, nurse’s perception should be changed by enhancing their knowledge regarding the safe practices of medication administration.7 Hence the awareness regarding the various causes, management and reporting the medication error plays a major role in reducing the medication error incidents and its consequences.

Materials and Methods

The Descriptive correlational research is being conducted in Yenepoya Nursing College, Karnataka, India after obtaining the ethical clearance (Protocol no 2019/029) from Institutional Ethics Committee, Yenepoya (Deemed to be University). Nursing students of 2nd yr, 3rd yr, 4th yr B.Sc. (N) course, 1st and 2nd yr P B B.Sc (N) course were included in the study. 125 Nursing students were selected by Stratified Random sampling. Informed consent was obtained and confidentiality of information were assured. Students of 1st yr B.Sc (N) not learnt about medication administration, Students of M.Sc Nursing course and Students who are not available during the period of data collection were excluded from the study. Data was collected using self prepared knowledge questionnaire and attitude scale, which was validated by 7 experts. The reliability coefficient of the tool was 0.795.

The data were analyzed by descriptive and inferential statistics using SPSS Version 16.0. Demographic variable, knowledge questionnaire, attitude scale were analyzed using the descriptive Statistics such as Frequency, Percentage, Mean and Standard Deviation, Karl Pearson co-relation co-efficient was used to correlate the knowledge and attitude score of nursing students and Chi-square test was used to find the association between the Knowledge and attitude score with selected demographic variable.

Results

Description of sample characteristics

Frequency and Percentage distribution was computed to describe the sample characteristics. The baseline sample characteristics of the participants showed that majority (93.6%) of the subjects was females and majority 46.4% belongs to the age group 18-20 years. With regard to education. Majority (72.8%) of the subjects were studying B.Sc Nursing. About 62.4% of the subjects had previous information about medication error through curriculum. About 84.8% of the subjects had medical professionals in the family of which 10.4% were doctors, 68.8% were nurses, 4.8% were pharmacists and 0.8% were dentist. Majority (76.2%) of the subjects have not witnessed medication error. 5 of the subjects have committed medication error. Majority 74.4% of the subjects have not attended any conference or workshops on medication error.

Knowledge on Medication error

Table 1: Frequency and percentage distribution of nursing students according to the grading of their knowledge score

<table>
<thead>
<tr>
<th>Classification</th>
<th>Frequency (f)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good Knowledge</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Average Knowledge</td>
<td>84</td>
<td>67.2</td>
</tr>
<tr>
<td>Poor Knowledge</td>
<td>41</td>
<td>32.8</td>
</tr>
</tbody>
</table>
Table 1 shows that Majority (67.2%) subjects had average knowledge where as 32.8% had poor knowledge regarding medication error.

The Mean knowledge score of nursing students regarding medication error is 11.95 ±3.007.

Attitude of nursing students towards reporting of medication error

Fig no 1 depicts that Majority 88% of the nursing students had positive attitude whereas 12% had negative attitude towards reporting of medication error.

Fig no 1: Bar graph showing the distribution of attitude of nursing students towards reporting of medication error

Table 2: Mean and Mean percentage of Attitude Components  n=125

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Attitude aspects</th>
<th>Mean</th>
<th>Mean%</th>
</tr>
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<tr>
<td>1.</td>
<td>Fear of Being recognized as incompetent</td>
<td>1.51</td>
<td>12.58</td>
</tr>
<tr>
<td>2.</td>
<td>Fear of Patient or families negative attitude</td>
<td>1.68</td>
<td>14</td>
</tr>
<tr>
<td>3.</td>
<td>Fear of Physicians reprimand</td>
<td>1.59</td>
<td>13.25</td>
</tr>
<tr>
<td>4.</td>
<td>Fear of nursing staffs reprimand</td>
<td>1.54</td>
<td>12.83</td>
</tr>
<tr>
<td>5.</td>
<td>Fear of teachers reprimand</td>
<td>1.75</td>
<td>14.58</td>
</tr>
<tr>
<td>6.</td>
<td>Fear of Decreasing evaluation score</td>
<td>1.90</td>
<td>15.83</td>
</tr>
<tr>
<td>7.</td>
<td>Fear of Being blamed of MAEs results</td>
<td>1.76</td>
<td>14.66</td>
</tr>
<tr>
<td>8.</td>
<td>Fear of Negative feed back</td>
<td>1.79</td>
<td>14.91</td>
</tr>
<tr>
<td>9.</td>
<td>Fear of reaction from peers groups or friends</td>
<td>2.09</td>
<td>17.41</td>
</tr>
<tr>
<td>10.</td>
<td>Think MAEs not important to report</td>
<td>2.14</td>
<td>17.83</td>
</tr>
<tr>
<td>11.</td>
<td>Too much time for reporting the error</td>
<td>2.14</td>
<td>17.83</td>
</tr>
<tr>
<td>12.</td>
<td>Lengthy procedure to report the error</td>
<td>2.18</td>
<td>18.16</td>
</tr>
</tbody>
</table>
Table no 2 depicts that The 5 most common barrier for reporting medication error was lengthy procedure to report medication error (18.16%), Too much time for reporting the error (17.83%), Think Medication administration errors not important to report (17.83%), Fear of reaction from peers groups or friends (17.41%) and Fear of decreasing evaluation score (15.83%).

**Correlation between knowledge and attitude score regarding Medication error**

Karl Pearson co- relation co-efficient was used to correlate the knowledge and attitude score of nursing students. There was a no significant correlation between knowledge and attitude score of nursing student regarding medication error (p=0.057).

Association between knowledge score with selected demographic variables

Chi square association with knowledge score indicated that Age ($\chi^2=4.967, p<0.05$), Year of study ($\chi^2=5.437, p <0.05$) and Attended any conference or workshop ($\chi^2= 11.129, p<0.05$) were statistically significant at 0.05 level.

The findings also revealed that gender, previous information, health professional in the family, witnessed medication error, committed medication error was statistically non significant at 0.05 level.

Association between Attitude score with selected demographic variables

Chi square association with Attitude score indicated that year of the study ($\chi^2 = 3.519, p < 0.05$), Previous information ($\chi^2=1.909, p <0.05$) were statistically significant at 0.05 level.

The findings also revealed that age, gender, health professional in the family, witnessed medication error, committed medication error, attended conferences was statistically non significant at 0.05 level.

**Discussion**

Medication error is a big threat to the patient’s safety. It is the commonest error occurring in the health care set up due to the negligence or malpractice. This study was intended to assess the knowledge and attitude of nursing students regarding medication error.

The demographics of this study reflected that majority (93.6%) of the participants were females. This observation was common in a study conducted by Remya. E (2016) which reveal that the maximum number of subjects was females (96%).

The present study showed that showed that 67.2% samples had average knowledge whereas 32.8% have poor knowledge regarding medication error. This finding is supported by a study conducted by A.Samundeeswari and G Muthamil Selvi (2018) which revealed that majority (34%) of them have average knowledge and 30% had poor knowledge regarding medication error.

According to the findings of the study most of the participants (88%) had positive attitude towards reporting of medication error. This finding was consistent with the study conducted by Alsulami SL et al (2019) which revealed that majority (90%) of them have positive attitude towards medication error reporting. Another study conducted by Remya. E (2016) majority (62%) of the nurses had favorable attitude.

This study finding revealed that there was no significant correlation between knowledge and attitude score of nursing students regarding medication error. This finding was consistent with the study conducted by Asem N (2019) which showed that there was no correlation between knowledge and attitude of the physicians.

The present study revealed that there was a significant association between knowledge score and selected demographic variable such as age ($\chi^2=4.967, p<0.05$). This finding was congruent with the study conducted by Carandang RR (2015) which showed that there was a significant association between knowledge score and selected demographic variable such as age ($\chi^2=7.370, p<0.05$).
As per the findings there was a significant association between attitude score and selected demographic variable such as education ($\chi^2=3.519$, $p<0.05$), this findings is supported by the study conducted by Ramya E (2016) which showed that there was no association between attitude score and selected demographic variable such as education status ($\chi^2=8.09$, $p<0.05$).

**Conclusion**

Medication error is one of the preventable problem which is leading to serious complications. By virtue of the direct patient-care activities, nurses are in an excellent position to detect and to report the medication errors. The quality of care delivered can be improved, if the errors is identified, reported and necessary actions are taken to minimize the errors. Through this study it is concluded that majority of the nursing students had average knowledge and favourable attitude towards reporting medication error. Nurses need to be updated and trained regarding the safe medication administration. This will increase the competence of the nurses and also to maintains the standards of the Quality health care.

**Acknowledgement:** We thank Yenepoya Nursing college, Yenepoya (Deemed to be University) for providing the opportunity to conduct the study and for their constant support for the completion of the work.

**Conflict of Intrest:** All the authors declare that they have no conflict of interest

**Informed Consent:** Informed consent was obtained from all the study participants.

**Ethical Approval:** obtained the ethical clearance from Institutional Ethics Committee, Yenepoya (Deemed to be University) (Protocol no 2019/029).

**Funding Sources:** Self Funded.

**References**

from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6468206/


An Assessment of Oral Health Status among Lead Battery Factory Workers in Ghaziabad UP a Cross Sectional Study

Akanksha Monga¹, Thanveer K², Bhuvandeep Gupta³, Aparna Aggarwal⁴, Nisha Yadav⁵, Meena Jain⁶, Ankur Sharma⁷

¹Sr. Lecturer, Department of Public Health Dentistry and Research & Innovation Catalyst, Manav Rachna Dental College, FDS, MRIIRS, Faridabad, ²Professor, Department of Public Health Dentistry, Teerthankar Mahavir University, Moradabad, ³Professor, Department of Public Health Dentistry, ITS Dental College, Greater Noida, ⁴Associate Professor, Department of Oral Medicine & Radiology, Vitaldent Advanced Dental Clinic, Faridabad, ⁵Sr. Lecturer, ⁶Associate Professor and Head, ⁷Lecturer, Department of Public Health Dentistry, Manav Rachna Dental College and Research & Innovation Catalyst, FDS, MRIIRS, Faridabad

Abstract

Introduction: Industrial workers are a well-defined group of a population. They have frequent shifts, low socio-economic status and neglected oral hygiene as they are at risk for health and dental problems. Industrial revolution has provided a lot of scope in employment worldwide for many which has improved the standard of living of many individuals. Health at workplace is considered essential now a days in various countries due to this rapid economic growth and industrial progress. In various industries like acid battery industry, chemical and textile industry, are exposed to hazardous substances and radiations. Studies have also reported a positive association between battery exposures to strong acids and periodontal pockets or gingival bleeding among exposed workers. Therefore, the purpose of the present study is to assess the oral health status of lead battery factory workers in Ghaziabad Uttar Pradesh.

Material and Method: The “World Health Organization (WHO) oral health assessment form -2013” was used to record the clinical findings. For the diagnosis of dental caries, WHO type III examination was carried out using mouth mirrors and explorers while using adequate illumination. The components of the form used were - General information, Dentition status (crown, root), periodontal status, loss of attachment, dental erosion, and oral mucosal lesions. Results: The mean DMFT scores were (5.72 3.17) females had more number of teeth with gingival bleeding compared to males. It was also found 72.3% of battery factory workers had pocket depth more than 6mm. 51.5% workers in the study had LOA of score 3 (as per WHO proforma) Mean number of teeth affected with erosion was more in males (4.76 4.02)

Conclusion: Oral health statuses of factory workers are highly affected due to lead acid used in industries.

Keywords – Oral health, Lead workers, dental erosion, oral lesions

Introduction

Human health is determined by many factors and environment is one among them which is very important.

Corresponding author:
Dr. Nisha Yadav
Address - BDS, MDS, Sr. Lecturer, Department of Public Health Dentistry and Research & Innovation catalyst, Manav Rachna Dental College, FDS, MRIIRS, Faridabad, Email- nisha.mrdc@mrei.ac.in
Mob- +91 9911227035

The study of any disease means studying man and his environment so, key to man’s health depend mostly on his environment. Initially, disease was thought to be due to cosmological and magical effect of God. Hippocrates was the first person who related the environment and the disease¹.

The latest concept of the environment is not restricted to physical environment only, but it also means social, economic and occupational condition. Occupational environment is related to work place conditions which
Industrial workers are a well-defined group of a population. They have frequent shifts, low socioeconomic status and neglected oral hygiene as they are at risk for health and dental problems. Industrial revolution has provided a lot of scope in employment worldwide for many which has improved the standard of living of many individuals. Health at workplace is considered essential now a days in various countries due to this rapid economic growth and industrial progress. In various industries like acid battery industry, chemical and textile industry, are exposed to hazardous substances and radiations. This results in deterioration of the health of workers of these industries. Some studies have proven that there is an association between occupational exposure and increased incidence of diseases.

It has been already known that the teeth of industrial workers are affected who are exposed to inorganic acids at workplace. These acids have high corrosive effect on tooth structure which causes inflammatory reactions. Further, these inflammatory reactions cause a decrease in salivary Ph and compromised immunity, thus making the person more prone to various infections in oral cavity. Soft tissues also gets irritated due to chronic exposure to these acids which results in other oral diseases such as periodontal disease or oral mucosal lesions. Exposure to these acidic contaminants can occur in factories of sulfuric acid, lead acid, petrochemical, metals and semiconductors etc.

Sometimes, pathologic changes in oral cavity may be the first sign that indicates absorption or toxicity related to certain toxic agents. Due to the substantial increase in the use of chemical substances that have adverse effects on oral health, industrial dentistry has become a subject of major consideration and constituted a new branch in the field of dentistry. Many adverse oral health conditions were reported among workers exposed to lead and the most common reported adverse effects were the Dental erosion cases with decay, missed and filled teeth, gingivitis, pockets and other periodontal conditions including periodontal attachment loss (PAL). It is mainly in the battery industry, where vast quantities of lead acid are used as an essential part of the battery making process. Other than lead, nicotine and alcohol are also responsible for occurrence of severe forms of periodontal destruction, such as PAL.

Exposure to acid mists causes periodontal diseases. This occurs due to changes in intracellular and extracellular pH which affects cell growth and differentiation. The prevalence of dental erosions is higher in workers in battery and galvanizing occupations. Battery workers have the highest prevalence 60% of erosion. Acidic mist exposure can erode the enamel and dentin of teeth and makes teeth vulnerable to acidic de-calcification. Mainly the anterior teeth exposed to the atmosphere are affected and completely dissolved. Some studies have also reported a positive association between battery exposures to strong acids and periodontal pockets or gingival bleeding among exposed workers.

There is very little information on the effects of occupational hazards on oral health status of individuals in developing countries. Therefore, the purpose of the present study is to assess the oral health status of lead battery factory workers in Ghaziabad Uttar Pradesh.

**Material and Methods**

A cross-sectional study was conducted in district Ghaziabad lead battery factory workers from April 2019 to August 2019. Ethical clearance was obtained from institutional ethical committee (IEC). All information regarding battery factory workers was obtained from Ghaziabad Battery Udyog association. Permission to conduct the study was obtained from Ghaziabad Battery Udyog association. A group of 50 subjects were selected from a factory to conduct pilot study to check the feasibility. The examiner was calibrated and trained in department of public health dentistry before the main study. The entire sampling frame that is, all the battery factory workers in Ghaziabad city who had given consent, willing to participate and who will be present on the day of study will constitute the final sample of this study. There were 25 factories and 1 factory was included in pilot study the total population came among
them was 1111. Informed written consent was taken from the workers of the factory before their participation in the study in order to prevent any inconvenience and to ensure full cooperation. Out of 1111, 998 were present on the days of data collection from them 850 gave consent for participation in the study. Therefore, the response rate came out to be 85%.

The “World Health Organization (WHO) oral health assessment form 2013” was used to record the clinical findings. For the diagnosis of dental caries, WHO type III examination was carried out using mouth mirrors and explorers while using adequate illumination. Factory workers were allowed to sit on a chair or stool as per availability. A table to place the instruments was placed within easy reach of examiner. The recording assistant was allowed to sit close to the examiner. Periodontal assessments were done by community periodontal index (CPI)-probes. The diagnosis of oral lesions was carried out using WHO criteria.

The components of the form used were General information, Dentition status (crown, root), and periodontal status, loss of attachment, dental erosion and oral mucosal lesions. The data was analyzed using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 23.0.

Results

This study was conducted on 850 battery factory workers of Ghaziabad to assess their oral health status. The mean age of the study population was (38.95 ± 13.22) with the youngest age being 18 and the oldest being 78. Among the 850 battery factory workers studied, 647 (76.1%) were males and 203 (23.9%) were females.

Mean number of teeth present in the study population was (28.79 ± 3.82) which was found to decrease as age increases Mean DMFT score for the study population was 5.72 ± 3.17 with highest mean DMFT found in the >64 years age group and the least mean DMFT score of 4.82 ± 2.22 seen among 35-44 year olds. Males and females had similar mean DMFT scores.

Root Caries

Mean number of teeth affected with root caries increased with age. Mean number of teeth with root caries was 0.38 ± 0.78 with male workers having comparatively higher mean root caries (0.47 ± 0.81) when compared to females (0.13 ± 0.62).

Gingival Bleeding

Female subjects were seen to be having more mean number of teeth with gingival bleeding (9.67 ± 6.16) compared to males (8.23 ± 4.68).

Periodontal Pocket

On examination for periodontal pockets, it was found that 615 (72.3%) of the battery workers had deep pockets (pocket depth 6 mm or more), 130 (15.2%) had shallow pockets (4 - 5 mm pocket depth) and 105 (12.3%) were free from any periodontal pockets.

Loss of Attachment

A loss of attachment score of 3 (Cemento-enamel junction is between 6 to mm and between the upper limit of black band and 8.55 ring of CPI probe) was seen in 435 (51.1%) of the subjects.

Dental Erosion

Mean number of eroded teeth was 4.48 ± 3.82 with the least number of teeth with erosion seen in <35 years old (2.81 ± 2.99) and the highest mean number of teeth with erosion was seen among 45-54 year old (7.4 ± 3.33). Teeth of male workers had higher mean number of affected teeth (4.76 ± 4.02) in comparison to female workers who had 3.59 ± 2.95 mean number of teeth with erosion.

Oral Mucosal Lesions

On examination for oral mucosal lesions, 195 (23%) of the battery workers did not have any kind of lesions. 128 (15%) of the workers had aphthous, herpetic or traumatic ulceration on lips, buccal mucosa and tongue. Lichen planus was seen in 14 (1.6%) of workers on their buccal mucosa. 17 (2%) workers were having candidiasis
on their tongue and 21 (2.47%) had ANUG (acute necrotizing gingivitis)

Table 1 Age wise distribution of study subjects according to the No. of teeth with gingival Bleeding

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of teeth with gingival bleeding (Mean ± S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>7.95 ± 4.77</td>
</tr>
<tr>
<td>35 – 44</td>
<td>9.77 ± 6.0</td>
</tr>
<tr>
<td>45-54</td>
<td>8.60 ± 3.96</td>
</tr>
<tr>
<td>55-64</td>
<td>9.12 ± 4.28</td>
</tr>
<tr>
<td>&lt;64</td>
<td>7.13 ± 4.96</td>
</tr>
<tr>
<td>Total</td>
<td>8.58 ± 5.1</td>
</tr>
</tbody>
</table>

Table 2 Distribution of study subjects according to severity of dental erosion

<table>
<thead>
<tr>
<th>Severity of Dental erosion</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sign of erosion</td>
<td>256</td>
<td>30.1%</td>
</tr>
<tr>
<td>Enamel lesion</td>
<td>233</td>
<td>27.41%</td>
</tr>
<tr>
<td>Dentinal lesion</td>
<td>266</td>
<td>31.2%</td>
</tr>
<tr>
<td>Pulpal Involvement</td>
<td>95</td>
<td>11.1%</td>
</tr>
</tbody>
</table>

Table 3 Age wise distribution of study subjects according to the No. of teeth with shallow pocket

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of teeth with shallow pocket (Mean ± S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>2.44 ± 2.48</td>
</tr>
<tr>
<td>35 – 44</td>
<td>1.97 ± 2.12</td>
</tr>
<tr>
<td>45-54</td>
<td>2.79 ± 2.38</td>
</tr>
<tr>
<td>55-64</td>
<td>1.77 ± 1.88</td>
</tr>
<tr>
<td>&gt;64</td>
<td>0.94 ± 1.15</td>
</tr>
<tr>
<td>Total</td>
<td>2.2 ± 2.3</td>
</tr>
</tbody>
</table>
Table 4 Distribution of study subjects according to highest score of loss of attachment

<table>
<thead>
<tr>
<th>Loss of attachment</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>112</td>
<td>13.1%</td>
</tr>
<tr>
<td>1</td>
<td>83</td>
<td>9.7%</td>
</tr>
<tr>
<td>2</td>
<td>220</td>
<td>25.8%</td>
</tr>
<tr>
<td>3</td>
<td>435</td>
<td>51.1%</td>
</tr>
</tbody>
</table>

Table 5

<table>
<thead>
<tr>
<th></th>
<th>MALE</th>
<th>FEMALE</th>
<th>X2 value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decayed</td>
<td>554</td>
<td>198</td>
<td>182.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Missing</td>
<td>355</td>
<td>85</td>
<td>97.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Filled</td>
<td>59</td>
<td>10</td>
<td>5.004</td>
<td>0.172</td>
</tr>
<tr>
<td>Root caries</td>
<td>177</td>
<td>9</td>
<td>65.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gingival Bleeding</td>
<td>571</td>
<td>184</td>
<td>0.887</td>
<td>0.210</td>
</tr>
<tr>
<td>LOA</td>
<td>545</td>
<td>193</td>
<td>28.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Erosion</td>
<td>462</td>
<td>193</td>
<td>13.24</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Discussion

It has been observed that oral injuries are common with direct contact in occupational exposure. In battery industries, sulfuric acid has been used in large quantity. This sulfuric acid contains >20% of sulfur dioxide dissolved in the acid, which has a sharp penetrating odor. Acid mist is frequently detected in the lead factory area as it continuously discharges from open containers and leakage from pipes. Exposure is detectable to human beings at a level of 0.5 to 0.7 mg/m$^3$, is irritating at 1.0 to 2.0 mg/m$^3$ and causes coughing at 5.0 to 6.0 mg/m$^3$.\(^2\)

In different study settings acid mist concentration varied from .08 to 5mg/m\(^2\).\(^{26,4,5}\) High concentration of acid fumes in the working environment is related to the higher prevalence of teeth erosion.\(^{27,28}\)

In our study it was found that dental caries experience is 95.8% with maximum caries in subjects of age below 35 years whereas the caries prevalence was 71.1% for age above 35 years in the study done by Rushabh J Dagli et al\(^{29}\) and the dental caries experience came out to be 62.5% in the study done by P. Basavraj et al\(^{30}\)

Results of this study showed the prevalence of periodontal disease in battery factory workers to be 88.9%. Bleeding was observed for all age groups. Similar results were observed by Wang et al (2002)\(^{31}\) and Baelum et al (2003)\(^{32}\). Periodontitis was less frequently seen in other studies (Garcia and Cutress, 1986)\(^{33}\)(Oliver et al, 1989)\(^{34}\) (El-Quaderi and QueteishTaani, 2004)\(^{35}\).
The results of this study are in accordance with the findings of the WHO Global Oral Data Bank, as most of the patients show some type of bleeding when different sextants are evaluated (over 90%).

In the present study, 69.8% showed some degree of dental erosion, this may be attributed to the working environment. The reason for this could be the Lead and sulfuric acid being used as one of the ingredients in the production work of factory. Whereas, Zabińska O et al (1982)36, Lie T et al (1988)37 found most of the workers with dental erosion (90%) 23.7% of the workers with more than 10 years of working experience showed 3rd degree of Dental erosion compared to 0% from 1-10 years and the difference is statistically significant. This is in agreement with the study done by Amin WM et al (2001)38. They studied dental erosion on workers of battery industries of Jordan and found significant increase in the erosion among the study. In our study we found highest mean number of eroded teeth was 7.40 among 45 – 54 years of age group.

Limitations

1. Grouping the participants into study and comparison group was not done based on the working site of the study participants.

2. Work experience was not counted as more no. of subjects were below 35 years of age.

3. The exposure level of the participants to the acid fumes was not measured quantitatively.

Conclusion

Industrial sector plays a key role in national development. The factory employees who form the lifeline of any factory area, working in a confined factory environment in par with machines are at high health risks. However, many a times they are not given due importance. Hence, continuous professional research and development is essential to improve overall health and development of such population, so as to have a healthy productive labor population for development of any nation.

As a revitalization of existing oral health service which is mainly of treatment aspect, it should be oriented more towards preventive health care. So we conclude that in such population continuous research and interventions are needed to improve the overall health status of these specific occupational subjects.

Recommendations

In the light of the present alarming findings associated with excessive exposure to sulphuric acid fumes in the workplace, occupational health authorities are invited to implement effective safety measures, including:

- Efficient surveillance and routine monitoring of acid fumes in workplace air
- Installation of efficient ventilation and exhaust systems of the work sites
- Automation of manufacturing processes
- To reduce the threshold limits below the level that is safe for teeth might be the measure of choice to decrease the risk of dental erosion
- Government must take suitable measures and a strict law for the rights of workers regarding health should be formulated along with regular inspections and follow up.

References


29. Duraiswamy P, Kumar TS, Dagli RJ, Chandrakant, Kulkarni S. Dental caries experience and treatment


Cytotoxic Effect of Silver Nanoparticles Prepared by Biosurfactant Produced from Pathogenic Bacteria

Aleaaabdulhusseinjameel, Nadhimhussanhayder2, Amnah Rami Abdullah3, Laithahmedyaaqoob4
1Research Scholar, Department of Biology, College of Al-Rasheed, Baghdad, Iraq. 2Research Scholar, Department of Biotechnology, College of Science, Baghdad University, Iraq. 3Research Scholar, Department of Biology, College of Al-Rasheed, Baghdad, Iraq. 4Research Scholar, Department of Biotechnology, College of Science, Baghdad university, Baghdad

Abstract

This study was aimed to biosynthesis of silver nanoparticles by using biosurfactant (lipopeptides) produced from local isolate L. plantarum isolated from clinical samples of Iraqi healthy women which previous identify grown in natural media (BCDFTM), then studied it cytotoxic effect against MCF-7 cell line. The cytotoxic effect of biosurfactant against MCF-7 cell line using 400 μg/ml reached 61.49 % of growth inhibition which is acceptable from cytotoxicity viewpoint. Silver nanoparticles application in vitro as cytotoxic activity against MCF-7 cell line using 400 μg/ml reached 55.67 % in growth inhibition, while when nanoparticles combined with lipopeptides obtained increase the growth inhibition MCF-7 cell and reached 68.44% at the same concentration of AgNPs.

Keywords: Biosurfactant, lipopeptide, nanoparticles, XRD, AFM, TEM

Introduction

Microbes occupy a strong population in the living world. They possess extra and intracellular vital products such as antibiotics, enzymes, toxins, biopolymers and pigments. More than 10,000 active broad-spectrum metabolites with medicinal properties have been isolated from these microbes (1). However, most of the microbial worlds remain unexplored owing to its vastness. Recent studies confirmed that only <0.1% of microbial world has been investigated till date (2). When listing microbial bioactive compounds, biosurfactants (BSs) are such metabolites with many interesting properties due to their multiple diversities in both structures and functions also with their pronounced usage in industries. BSs are basically amphiphilic surface active agents in bacteria, fungi, and classes of actinomycetes. They belong to classes of glycolipid, glycolipoproteins, glycopeptides, or lipoproteins, lipopeptides or derivatives of fatty acids (3), and less likely glycoglycerolipids too (4).

Biosurfactant are now mediating new developments in the field of Nanotechnology. It observed that biosurfactant produced by microorganisms could play a very important role in aggregation and stabilization process (5). Biosurfactant use has therefore now emerged as a green alternative for enhancing both nanoparticles synthesis (reducing agent) and stabilization (stabilizing agent). One of the modes of action is through adsorbing onto nanoparticles, surface stabilizing the nanoparticles and prevent of formation subsequent aggregation (6). Silver nanoparticles contain a wide spectrum of inhibition of bacteria and other microorganisms. Numerous possible mechanism of action for anticancer activity of silver NPs can be proposed.
Cytotoxic activity of the silver NPs might be due to its physiochemical interaction with the intracellular DNA and proteins. Reports have shown that the cytotoxicity might also be due to initiation of apoptosis activated by the caspase-3 enzyme (7). (8) reported that oxidative stress is one of the crucial mechanisms of cytotoxicity induced by silver NPs. The shape and size are important properties that influence the toxicity of silver NPs by elevating reactive oxygen species (9). Because of the physicochemical differences, some silver NPs are broken-down in the lysosomes and then the release of silver ions result in oxidative stress. In addition, oxidative stress can also lead to genotoxic stress and even up regulation of p53 gene (10), which is an important lead for the application of nanomaterial as anticancer Nano-medicine, as up regulation of p53 gene initiates apoptosis (11).

The aim of the present work is to synthesis nanoparticles prepared by biosurfactant produced by L. plantarum isolated from vagina and testing its cytotoxic effect against cell line.

Methods

Synthesis of silver nanoparticles:

Silver nitrate (AgNO3, 99%) (Aldrich/Germany) was used in the preparation of the silver nanoparticles from biosurfactant produced by selected isolate that grown on natural medium (BCDFTM). Silver nanoparticles were synthesized according to a method described by Martinez-Gutierrez et al. (12).

Characterization of AgNPs

Characterization was performed using a variety of analytical techniques including : The X-ray diffraction (XRD) was used to determine phase identification, crystals structure, composition and physical properties of the synthesized materials silver nanoparticles (AgNPs) (13). The AFM was also used to determine surface topography of the silver nanoparticles by SPM-AA300 of angstrom advanced Inc. USA, using AFM contact mode (14). Transmission Electron Microscopy (TEM) analysis was performed to investigate the size, shape, and morphology of bare and lipopeptidebiosurfactant stabilized Ag NPs (15).

The biological activity of biosurfactant

In vitro method was performed to investigate the possible cytotoxic effect of extracted biosurfactant isolated from selected isolate and biosynthesized nanoparticles on two cell lines on of them is human breast cancer cell line (MCF-7) and another is a normal cell line such as human normal liver cell line (WRL 68).

Maintenance of cell cultures: MCF-7 and WRL 68 cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 unit’s/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week, and incubated at 37 °C (16).

Cytotoxicity Assays: To determine the cytotoxic effect of (biosurfactant produced from natural media (BCDFTM), and the effect of the mixture of both biosurfactant and nanoparticles), the MTT cell viability assay was done using 96-well plates. Cell lines (MCF-7 and WRL 68) were seeded at 1 × 10⁴ cells / well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with tested compounds at different concentration (12.5, 25, 50, 100, 200, and 400µg/ml). Cell viability was measured after 24 h of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO followed by 37 °C incubation for 15 min with shaking (16-17).

Statistical Analysis: The obtained data were statically analyzed using an unpaired t-test with Graph Pad Prism 6. The values were presented as the mean ± SEM of triplicate measurements (18).

Results

Noble metal nanoparticles (plasmonic) are distinguished from other nanoparticles such as semiconductor quantum dots, polymeric, and magnetic
nanoparticles by their unique surface Plasmon resonance (SPR). The synthesis of AgNPs was monitored by a color change and UV–Vis spectroscopy. The formation of AgNPs was confirmed by changes in the solution color from colorless to yellow brown. Samples are examined by using the UV–V is spectroscopy and the application of the Nano product as an anticancer agent.

The XRD patterns figure (1) show the distinctive diffraction peaks of AgNPs at $\theta = 27.80^\circ$, $32.02^\circ$, $35.09^\circ$, $46.18^\circ$ and $57.38^\circ$. These peaks were well matched with standard diffraction data of AgNPs (JCPDS file no. 040783) and attributed to the (110), (333), (111), (200) and (220).

![Figure 1](image)

**Figure (1) The results of X-ray Diffraction (XRD) for AgNPs.**

Surface analysis (AFM) requires good attention because of factors that effect on results such as pollutions. The size of silver nanoparticles was estimated by using AFM-SPM shown in table (1). The result shows that the average size of AgNPs was 52 nm figure (3).

<table>
<thead>
<tr>
<th>Sample: AA</th>
<th>Code: Sample Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line No.: linen</td>
<td>Grain No.:141</td>
</tr>
<tr>
<td>Instrument: CSPM</td>
<td>Date:2019-12-08</td>
</tr>
<tr>
<td>Avg. Diameter:52.03 nm</td>
<td>&lt;=10% Diameter:0 nm</td>
</tr>
<tr>
<td>&lt;=50% Diameter:50.00 nm</td>
<td>&lt;=90% Diameter:60.00 nm</td>
</tr>
</tbody>
</table>

TEM is a valuable tool to analyze the size and morphology of nanoparticles. TEM images of AgNPs as figure (2) showed distributed spherical shaped particles with numerous sizes ranging from 20 to 300 nm.
The biological activity of biosurfactant against cell line.

Results in table (2) show that growth inhibition of REF cell line decreased gradually when biosurfactant concentration increased. biosurfactant has significant differences of cytotoxic effect on MCF-7 cell line (P≤0.05), 32.67, 54.47 and 61.49 % growth inhibition was showed at concentrations 100, 200, and 400 µg/ml respectively in biosurfactant produced from natural media (BCDFTM).

Table (2) The effect of different concentration of biosurfactant produced from *L. plantarum* grown on BCDFTM on cell inhibition of MCF – 7 cell line.

<table>
<thead>
<tr>
<th>Concentration of Biosurfactant (BCDFTM) µg/ml</th>
<th>Cell viability (%) by MCF-7 Mean ± SD</th>
<th>No. of dead cells (%)</th>
<th>Cell viability (%) by WRL.68 Mean ± SD</th>
<th>No. of dead cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>96.177 ± 1.280</td>
<td>3.82</td>
<td>95.949 ± 1.028</td>
<td>4.051</td>
</tr>
<tr>
<td>25</td>
<td>95.718 ± 0.810</td>
<td>4.282</td>
<td>95.216 ± 0.821</td>
<td>4.784</td>
</tr>
<tr>
<td>50</td>
<td>88.702 ± 3.183</td>
<td>11.298</td>
<td>95.332 ± 1.183</td>
<td>4.668</td>
</tr>
<tr>
<td>100</td>
<td>67.329 ± 3.151</td>
<td>32.671</td>
<td>92.438 ± 2.713</td>
<td>7.562</td>
</tr>
<tr>
<td>200</td>
<td>45.525 ± 5.157</td>
<td>54.475</td>
<td>82.755 ± 2.842</td>
<td>17.245</td>
</tr>
<tr>
<td>400</td>
<td>38.503 ± 4.125</td>
<td>61.497</td>
<td>76.196 ± 2.224</td>
<td>23.804</td>
</tr>
</tbody>
</table>
Significant cytotoxic effect (P ≤ 0.05) was observed on the growth of MCF-7 cell line at the concentrations of 100, 200 and 400 μg/ml with growth inhibition percentage 25.92, 40.74 and 55.67 % respectively, as shown in the table (3).

### Table (3) The effect of different concentration of Nanoparticles produced from biosurfactant grown on BCDFTM on cell inhibition of MCF – 7 cell line.

<table>
<thead>
<tr>
<th>Concentration of nanoparticles µg/ml</th>
<th>Cell viability (%) by MCF-7</th>
<th>No. of dead cells (%)</th>
<th>Cell viability (%) by WRL68</th>
<th>No. of dead cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>94.599 ± 0.837</td>
<td>5.401</td>
<td>94.907 ± 2.199</td>
<td>5.093</td>
</tr>
<tr>
<td>25</td>
<td>94.599 ± 2.166</td>
<td>5.401</td>
<td>96.952 ± 1.142</td>
<td>3.048</td>
</tr>
<tr>
<td>50</td>
<td>86.921 ± 3.282</td>
<td>13.079</td>
<td>96.180 ± 1.252</td>
<td>3.82</td>
</tr>
<tr>
<td>100</td>
<td>74.074 ± 1.819</td>
<td>25.926</td>
<td>92.130 ± 1.557</td>
<td>7.87</td>
</tr>
<tr>
<td>200</td>
<td>59.259 ± 5.343</td>
<td>40.741</td>
<td>85.262 ± 0.998</td>
<td>14.738</td>
</tr>
<tr>
<td>400</td>
<td>44.329 ± 1.895</td>
<td>55.671</td>
<td>72.068 ± 2.319</td>
<td>27.932</td>
</tr>
</tbody>
</table>

MCF-7 cell line treated with biosurfactant mix with nanoparticles at the same concentrations of 100, 200, and 400 μg/ml and showed growth inhibition percentage of 34.38, 55.75 and 68.44% respectively when used Nano particles with biosurfactant produced from BCDFTM and table (4). Growth inhibition of MCF-7 cell line was increased gradually with the increase of with biosurfactant mix with nanoparticles concentration with significant cytotoxic effect (P ≤ 0.05) between the concentrations when compared with the control (WRL68).

### Table (4) The effect of different concentration of AgNPs mix with same concentration of biosurfactant on cell inhibition of MCF – 7 cell line.

<table>
<thead>
<tr>
<th>Concentration of AgNPs mix with biosurfactant µg/ml</th>
<th>Cell viability (%) by MCF-7</th>
<th>No. of dead cells (%)</th>
<th>Cell viability (%) by WRL68</th>
<th>No. of dead cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>95.370 ± 1.863</td>
<td>4.63</td>
<td>94.907 ± 2.199</td>
<td>5.093</td>
</tr>
<tr>
<td>25</td>
<td>94.599 ± 2.166</td>
<td>5.401</td>
<td>96.952 ± 1.142</td>
<td>3.048</td>
</tr>
<tr>
<td>50</td>
<td>82.745 ± 1.573</td>
<td>17.255</td>
<td>96.180 ± 1.252</td>
<td>3.82</td>
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<td>68.443</td>
<td>77.173 ± 2.278</td>
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</table>
**Discussion**

Biosurfactants usually act as a stabilizing agent of the non-toxic, non-hazardous synthesis of nanoparticles. Biosurfactant use has now emerged as an eco-friendly alternative for enhancing both nanoparticles synthesis and stabilization. Because of the biodegradable nature of biosurfactants, they are more environmentally friendly than chemical surfactants (19).

Biosurfactant produced by microorganisms plays a very important role in aggregation and stabilization process. One of the modes of action is through adsorbing onto metallic nanoparticles, surface stabilizing the nanoparticles and preventing subsequent aggregation. The mechanism of surfactant absorption depends on the type of surfactant and the thickness of the adsorbed layer. So far no comparative study has been published concerning the influence of the biosurfactants nature and composition on the properties and ability to control the production of nanoparticles, Lipopeptidesbiosurfactant has also been reported in nanoparticles synthesis (20).

Clarified that silver with a lattice parameter (21) of a = 4.0862 Å were in good agreement with reference of the face-centered cubic (fcc) crystal lattice of metallic silver, the size of the AgNPs calculated by the Debye-Scherrer equation (D = 0.94λ/d cosθ), and concluded the size of the AgNPs was 38 nm.

Atomic force microscope (AFM) was used to know the surface morphology and to determine topography, the (AFM) gives a two and three-dimensional image of the surface of nanoparticles at an atomic level (21). The average particle diameter was calculated in nanoscale size. The AgNPs prepared by using biosurfactant were studied using (AFM).

In previous studies, TEM images revealed a size of 30–60 nm spherical-shaped polymeric nanoparticles produced from Cs-Hk fungal cultures, *Streptomyces sp.* MBRC-91 and *Bacillus subtilis* MSBN17 (22). AgNPs with a size around 30–50 nm have been reported for bactericidal activity against various pathogens (23). Polymeric nanoparticles ranging from 20 to 300 nm have the maximum potential for *in-vivo* applications (24).

Mentioned that the new therapeutic strategies may be designed by (25), considering that, the use of biosurfactant can alter lipid content to fluidize rigid cancerous tissues and to modulate interfacial properties. While (25) found that the ability of biosurfactant to disrupt cell membranes, leading to a sequence of events that include lysis, increased membrane permeability, and metabolite leakage, have also been suggested as a probable mechanism of antitumor activity. (26) showed that surfactin induces ROS formation, leading to mitochondrial permeability and membrane potential collapse that ultimately results in an increase of calcium ion concentration in the cytoplasm afterwards, cytochrome C released from mitochondria to the cytoplasm activates caspase-9 eventually inducing apoptosis. The apoptotic effect induced was associated with a significant decrease in the unsaturated degree of the cellular fatty acids in Bcap-37 cells due to a reduction in the amount of fatty acids, thereby enhancing membrane fluidization (27).

**Conclusion**

Biosynthesis of AgNPs using produced BS is efficient to convert AgNO₃ to spherical shaped particles with numerous size ranging 20 – 100 nm. The result of sytotoxic effect showed that the potent effect was seen obviously in MCF-7 cell line than WRL68 when used BS and / or AgNPs.

**Ethical Clearance:** Taken from College of Al-Rasheed ethical committee

**Source of Funding:** Self

**Conflict of interest:** Nil

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A Review on Medication Synchronization Programs in Community Pharmacies to Improve Medication Adherence

Rakshith U.R 1 Srikanth M.S 1
1 Lecturer, Dept of Pharmacy Practice JSS College of Pharmacy, SS. Nagar, JSS Academy of Higher Education and Research, Mysuru, Karnataka

Abstract

Adherence to the prescribed medications plays an important role in treatment of chronic diseases. Hypertension, Ischemic heart diseases, Diabetes mellitus, Dyslipidaemia’s are major health challenges of 21st Century and are more expensive. A total 79% of Projected deaths are caused due to chronic diseases in developing countries. Non adherence to prescribed medications is one of major health threat worldwide. Patients who are on chronic diseases are often visits multiple health care practitioners, numerous pharmacies, and who take multiple drugs results in medication non-adherence. The new primary care models suggest that there is no promotion of close physician-patient-pharmacist relationships. Training for the treatment of chronic diseases is seldom satisfactory and there is inadequate consistency and communication between health care providers. Among health care profession community pharmacist are the ideal position to assist the management of patients with chronic disease. The Synchronization services are offered by several community pharmacies to improve medication adherence. These programs work by overcoming the barriers of medication non adherence by refilling their prescription in their single visits. Although there are Numerous medication synchronization programs are available in community pharmacies effectiveness of medication synchronization programs are confined to its type synchronization programs implemented.

Key words: Medication synchronization, Adherence, Medication, Community Pharmacies

Introduction

Health care practitioners describes the definition of medication adherence in their own way. The exact definition of medication adherence is lacking. It is defined as the extent to which patient takes the medication as prescribed by the health care practitioner.1 Non-adherence to prescribed medications occurs in 50% of patients in chronic diseases which leads to increase in emergency visits, hospitalization stay, medical cost. As per world Health organisation chronic diseases kills 38 million people globally every year. Over 20% of Indian populations suffers from chronic diseases which are estimated to cost $6.2 trillion during the period of 2012-2030 42. 70-90% of the population burden of diabetes, hypertension and obesity are having higher social economic status 44. Patient with chronic diseases often prescribed with multiple drugs to treat their conditions, prevent complications, and to improve overall health related quality of life. Elderly populations are at higher risk for chronic disease where they find difficulty in taking the medications and following the medications as prescribed by the doctors.2 chronic diseases patients may feel burden because of increase in visits to the pharmacy to pick up their medications...3 Nonadherence is attributed to multiple factors including like social, economic,
therapy-related, patient-related, condition-related, and health system factors, & Polypharmacy. Community Pharmacist are in better position to improve medication adherence by providing better patient care Medication synchronization program has shown better impact on medication adherence. Medication synchronization programs are implemented and have grown very rapidly. Medication synchronization program works by refilling of prescription as prescribed by medical practitioners in their single visits, which results in convenience for the patients to take their medications. Studies have shown that the rate of medication adherence was increased to 2-6 times when it compared with patients who are managing their disease by own.

Review of Research and Development in the Subject

International status:

In most of the developed part of the world, pharmacist is in better position to improve medication adherence in chronic diseases. Pharmacist can provide drug therapy training, access to all patient’s medication refill data. Medication synchronization programs is one of the popular strategies to improve medication adherence. A study conducted by Jalpa A Doshi et al. mentioned that refilling of prescribed medication using medication synchronization program using health insurance program through mail order community pharmacy results in increase medication adherence to antihypertensive, lipid lowering and antidiabetic agents. A study conducted by Matthew J et al. suggests that pharmacist was positive about medication synchronization programs, although some negative views were present Chayla Stanton Robinson et al. emphasis more about patient specific barriers and pharmacist interventions to improve medication adherence. This study showed that 37.9% were adherent at 90day of follow-up and 69% were adherent at 180days of follow-up Hence More research is needed to carried out to understand the barriers and facilitators to how medication synchronization programs are implemented to achieve better outcomes.

National Status

In India community pharmacy practice is predominantly confined to trade. Majority of registered pharmacists are with diploma in pharmacy qualification and their knowledge regarding professional services is very limited. Pharmacy Council of India and respective state pharmacy councils are providing educational motivation to change their trader attitude. In our health care system, more than 80% of the prescriptions are filled by the community pharmacist. The high number of prescriptions especially to chronic diseases leads to medication non adherence. Hence community pharmacist can sensitize and enroll the patients in medication synchronization programs offers in community pharmacy to prevent medication non-adherence Community Pharmacy based programs are used to enhance medication adherence by incorporating medication synchronization programs. Currently limited available evidences suggestive of medication synchronization might improve patient’s health outcomes in chronic diseases hence more research need to be carried out

Medication synchronization services:

Medication synchronization program defines as synchronized, same day, filling and refilling of all chronic disease medications. Medication synchronization services works by aligning of prescriptions, refilling the quantity of medications which are prescribed for the chronic diseases. These programs are getting benefited for the patients who are on chronic diseases so that they will refill their medications on same day. The chosen date becomes the patient pick-up date. A week before that a patient’s medications are due, a pharmacy team member calls the patient to determine if changes are made in patient’ treatments such as new or changed medications or hospitalizations. Subsequently, based on the discussion, the pharmacy team member fills all of the medications as appropriate. In order to achieve synchronization, some prescriptions will have to be filled for smaller quantities for the first 1-3 months. In day today practice Pharmacist are assisting the patients
in adhering to their prescribed Medications. Several community pharmacies are providing synchronization services to improve medication adherence programs such as Appointment based model, script your future, simply my Meds, Refilling using Auto-refilling, refilling using batching, Refilling using grouping, CVS script sync, patient counselling, pill reminders etc. These services can help patients by remembering to take their medications in a timely manner and to refill chronic medications regularly through patient counselling. Pharmacist are in better position in identifying barriers to nonadherence by providing product information leaflets and educating the patients on their diseases and drugs. Community pharmacists plays an important role on patients in their personalized ways to change a patient’s behaviour and to improved overall health quality of life of patients. These programs run by collecting patients’ past prescribed data from insurance claims to determine their adherence rate which help them achieving higher adherence through patient counselling, providing additional materials which could help by overcoming their specific barriers to nonadherence. The automatic refill programs identify only irregular filing of prescriptions and it will not address the patient specific barrier for medication non adherence Hence there is a need for community-based medication synchronization programs to enhance the medication adherence. Synchronization services allows the pharmacist to check all of medications at one time allowing for more thorough and effective therapeutic check. Refills and/or therapeutic recommendations are sent to the physician and any issues are resolved before the patient picks up the medications. The result is a proactive workflow which reduces the patient’s need to call the pharmacy or wait for extended periods of time while team members resolve last minute issues Medication synchronization can improve both patient satisfaction and pharmacy operations. By helping to improve patient adherence and pharmacy efficiency, the pharmacy team is able to provide a higher level of care. The proactive approach allows patients to feel individually cared for, increasing their confidence in the pharmacy and in optimizing their health.

Advantages:

1) Medication synchronization are the way to promote operation effectiveness 2) It ensures medication appropriateness to improve patient safety and care 3) It improves rapport between pharmacy, patient, physician relationship 4) It allows systematic management, organized and prepared patient appointments to facilitate services like medication reviews, immunizations, medication therapy management, patient counselling etc 5) Patient satisfaction is one of most crucial part of medication synchronization program. 6) It reduces the number of prescription paper works through medication adherence in chronic diseases.

Medication synchronization services offering in community pharmacies.

1) Appointment based model: ABM is one of the patient most important patient care centric model, implemented in the community pharmacies to enhance medication adherence and to build efficiency in pharmacy operations. Appointment based model mainly address on patient and provided based barriers Appointment based model works by pharmacy team members from community pharmacies are actively contacting patients prior to their refill dates. Along with their process of refilling prescriptions pharmacy teams’ members were also actively involved in educating patients on drug-drug, drug-food interactions, adverse drug reactions, direction for use of medication etc. Appointment based model works by scheduling an appointment to the patients so that patient can visit pharmacy at a scheduled time to refill their prescriptions. Before scheduling an appointment, pharmacy team member should contact the patients to identify any changes are made in their prescriptions or in their treatment regimens. These programs develop the rapport between patients and physicians through patient and physicians co-ordinating relationships to improve medication adherence. Appointment based model and their work process are described below.

1) Patient brings new or old refill prescription to the community pharmacy 2) Pharmacy team member explains about Appointment based model and its process,
enrolments & procedures. 3) Patient decided to enrol in appointment-based model where he will enquire more about synchronized appointment with pharmacy team members so that patient can pick up their prescription on a single visit. 4) The Pharmacy team member will review patient profile to prepare their medicaments, where he plans for synchronization services such that patient can pick up all of their chronic medications in a single visit. 5) Pharmacy staff will formulate short refilling of prescriptions (short period of time) or long refilling of prescriptions as advised by their physician (for long period of time) which depends up regimens the doctor was prescribed or based on cost of chronic medication...

6) Every month Pharmacy team member will contact the patients one week prior to the appointment date to check the prescription should be refilled or it required any changes in their prescriptions. 7) Pharmacy staffs reviews for the potential changes in patient medication regimen, prepares for prescription and creates package for the patients so that they can pick up their medications as per scheduled time. 8) Every month community pharmacist reviews the prescription order comprehensively to evaluate any changes in prescription or same prescription can be refilled once again so patient can pick up their medication without any disturbances. 9) On the selected day patients visits the pharmacy to collect all of the prescriptions that are prepared.

3) **Simplify My Meds:** It’s a program which provides tools and training programs to implement the medication synchronization programs for NCPA (Nation community Pharmacist Associations) members. These programs help the pharmacist to consolidate and co-ordinate a patient prescription so that they can re-fill their prescription on a particular time and date in a month, which leads to improving patient medication adherence and change in pharmacy operations. These programs work by improving medication adherence through convenience for synchronizing all of the patient’s chronic medications are refilled in a single day of each month. through this service offered by the several community pharmacies’ patients will get benefited to improve adherence and also it provides an opportunity for the patients to interact with the community pharmacist to clear all their queries.

4) **Refilling using Auto- Refilling:** Pharmacy which have the facilities of Auto- refilling software features and ability to keep electronic record of patient due dates for medications are considered for this type of synchronization programs. Following are the methods that should be considered: 1) Print the auto-refill list at the beginning of each day 2) Review the refill list to ensure all of the patient’s medications are included. If a medication is omitted, complete the syncing process as described above to ensure resynchronization. 3) Call the patient in the afternoon or evening to inform them that their medications have come up in our system and they are ready to be picked up from the pharmacy. 4) If patient informs the team member of any changes, this is taken into account and any resynchronization is completed as required.

5) Review the number of refills left for each medication. If this is the last refill, send a refill request to the physician. If refill request is denied inform patients right away to ensure they book an appointment. 6) Process and refill all the medications.
5) **Refilling – Using Batching:** This type of Synchronization Programs usually offers in Pharmacy whose software does not have the auto refill features, or if the pharmacy prefers not to use this feature, medication can be refilled using batch. Following are the methods that should be considered using batching. 1) Complete the synchronization for each patient.

2) Determine which day of the month patients’ medications will become due and place them into the batch for that respective date. 3) Run the batch each morning and review the batch list to ensure all of the patient’s medications are included. If a medication is omitted, complete the syncing process as described above to ensure resynchronization. 4) Complete steps 3-6 times as per auto refill method.

6) **Refilling using Grouping:** This type of Synchronization Programs usually offers in Pharmacy whose software does not allow or prefer not to use the auto refilling or batching methods. Following are the methods that should be considered using Grouping.

1) Complete the synchronization process for each patient. 2) Determine if patients will be receiving a 28- or 84-day supply of their medications. 3) Based on the patient’s refill date, determine which week of the month medications are to be refilled. a. For those receiving 28 days, on a calendar, mark the first four weeks of your program as Groups A-D. b. For those receiving 84 days of medication, on a separate calendar, label the first 12 weeks of your program as Groups 1-12. 4) Develop a list of patients in each group based on their refill quantity and date. This will be the master list that staff members can refer to each week when determining who is due for medication refills. Additionally, place a note in each file as to which group each patient falls into for easy reference. 5) Create a binder with sections for each group to organize the required patient information. 6) Using the calendar as a guide, call patients the week their medications are due to be filled to determine if any changes have been made. 7) Review the patient’s medication file and complete any synchronization as required. 8) Process and refill all of the medications. 9) Review the number of refills left for each medication. If this is the last refill, send a refill request to the physician. If refill requests are denied, inform patients right away to ensure they book an appointment.
Table 1: Different Medication Synchronization programs offering in community pharmacies to improve medication adherence based on Literature review.

<table>
<thead>
<tr>
<th>SL. NO</th>
<th>Author Name</th>
<th>Study Design</th>
<th>Objective</th>
<th>Synchronization Programs</th>
<th>Outcomes</th>
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<tr>
<td>1</td>
<td>Jalpa A Doshi et al</td>
<td>Quasi Experiment</td>
<td>Impact of refill synchronization program implemented by nation Insurer</td>
<td>Pharmacy-mail order</td>
<td>Synchronized groups have showed larger Proportion of days covered rate (0.86-0.89), adherence rate to (83% - 86%) as compared to control group.</td>
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<td>2</td>
<td>Matthew J Witry et al</td>
<td>Cross section survey</td>
<td>Assess the community pharmacist attitude on medication synchronization programs</td>
<td>A Post card system</td>
<td>45% of all pharmacies reported to have medication synchronization. Pharmacist strongly agreed that program would benefit the pharmacy finically (5.25 SD= 1.41) that would increase the opportunities to interact with patients (4.71SD= 1.45) (P&lt;0.001).</td>
</tr>
<tr>
<td>3</td>
<td>Chayla Stanton-Robinson et al</td>
<td>A Quality improvement design</td>
<td>Assess the patient specific barriers and pharmacist intervention to improve medication adherence</td>
<td>Appointment based model</td>
<td>37.9% were adherent at 90-day follow-up, followed by 69% were adherent at 180days follow-up.A significant increase in the total number of patients achieving adherence occurred at 90 days after baseline (P &lt; 0.001) and at 180 days after baseline (P&lt; 0.001) Study concluded that Pharmacist plays an important role in identifying and addressing patient barrier</td>
</tr>
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<td>4</td>
<td>Kristen L. DiDonato et al</td>
<td>Randomized controlled trail</td>
<td>To examine the effect of medication synchronization program or education program on health outcomes</td>
<td>Mail order system</td>
<td>Synchronization program showed positive change of 40% hypertension knowledge questions. Were as control group showed positive change of 20%. Educations group experienced a 27.1% increase in correct identification of appropriate amount of exercise needed for BP lowering (p&lt;0.001). Significant intervention is needed to impact adherence aside from ensuring that patients have their medication on hand.</td>
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<tr>
<td>5</td>
<td>Alexis A. Krumme et al</td>
<td>Mixed methods</td>
<td>To identify the prevalence, scope, and characteristics existing programs</td>
<td>Telephonic interviews</td>
<td>One observation study, 4 full length studies were evaluating an adherence outcome measured adherence to antihypertensive, hyper lipidemic, and/or oral hypoglycemic therapy.2 peer review article were only one to conduct statistical testing of results, both finding significantly higher adherence in synched patients compared with usual care, with up to 6-fold greater odds of patients being fully adherent. Other studies demonstrated improved adherene or persistence due to synchronization with different lengths of follow-up.</td>
</tr>
<tr>
<td>6</td>
<td>Datar M et al</td>
<td>Retrospectives cohort analysis.</td>
<td>Impact of programs on chronic medication adherence and health care cost</td>
<td>Medicaid claims</td>
<td>Average medical cost was significantly reduced from 5845 to 420S. The new service was not only associated with improved medication adherence, but also with decreased medical expenditures among chronic patients.</td>
</tr>
</tbody>
</table>
Table 1: Different Medication Synchronization programs offering in community pharmacies to improve medication adherence based on Literature review.

<table>
<thead>
<tr>
<th></th>
<th>Author(s)</th>
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<th>Program Description</th>
<th>PDC Range</th>
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<tbody>
<tr>
<td>7</td>
<td>Anthony Pattin et al²⁸</td>
<td>Prospective cohort study</td>
<td>Association between participation in appointment-based model and blood pressure levels among antihypertensive medications</td>
<td>96±9% and 71±21%</td>
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<td>Appointment based model</td>
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<td>8</td>
<td>Emily Ghassemiet al³⁰</td>
<td>Multi centric pilot cohort study</td>
<td>To compare the antiviral therapy with insured HIV infected adult out patient enrolled and not enrolled in medication synchronization program</td>
<td>96±9% and 71±21%</td>
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<td>Script your future</td>
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<tr>
<td>9</td>
<td>Rebecca M. Fitzpatrick et al³¹</td>
<td>Retrospective chat analysis</td>
<td>To identify drug therapy problems (DTPs) associated with patients enrolled in appointment-based model for medication synchronization program,</td>
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<td>Appointment based model</td>
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<tr>
<td>10</td>
<td>David Holdford et al³²</td>
<td>Retrospective cohort study</td>
<td>To compare the impact of community pharmacist chain ABMS program on medication adherence with persistence users who are on chronic diseases which are not enrolled for synchronization program</td>
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<td></td>
<td></td>
<td>Appointment based model</td>
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Discussion

Current Scenario:

This comprehensive review identified 10 research studies relevant to medication synchronization programs offering in community pharmacies to improve medication adherence in chronic diseases among elderly population²²⁻³⁰. Limited available evidences suggestive of medication synchronization programs might improve patient’s health outcomes in chronic diseases. Hence more research needs to carried out to understand better outcomes among elderly populations. More over most
of the studies are conducted in different locations of United States of America, we could not able to identify sufficient evidences other than United States of America. Medication synchronization programs in Community Pharmacies are yet to be implemented and established among Indian population. Majority of registered pharmacists are with diploma in pharmacy qualification and their knowledge regarding professional services are very limited. Practicing pharmacist lacks proper techniques to improve medication adherence hence Pharmacy Council of India and respective state pharmacy councils are providing educational interventions like continuing professional development programs, seminars, webinars, preparing books and manuals to update their knowledge, skills, techniques to increase medication adherence.

Effectiveness of Medication synchronization program:

Medication synchronization program constitutes multiple methods that address patient needs and manage the continuity of care in patients receiving pharmacotherapy for chronic diseases. Literature emphasises that these programs are effective in managing clinical and economic outcomes for patients at various stages. Jalpa A Doshi et al. emphasis on pharmacy mail order programme effectively showed larger proportion of days covered and adherence rate in synchronized group. However, this programme was unable to demonstrate the information on common roots of medication non-adherence (e.g., forgetting of dose, or ambivalence) among the patients through this system. David Holdford et al. addressed that the synchronized patient appointment-based model showed a greater proportion of days covered than the patient not enrolled, but this study has some limitations associated with adherence and persistence measures. Emily Ghassemi et al. demonstrates that script of your future program had a positive impact on patient enrolled in the synchronization program as compared to non-enrolled patients. Synchronized patients have significantly improved ART adherence than non-infected outpatients, but this study does not evaluate patient satisfaction among synchronized groups. Alexis A. Krumme et al. conducted telephonic interviews states that antihypertensive, hyper lipidemic, and oral hypoglycaemic therapy was found to be effective in increasing the medication adherence among synchronized patients as compared with usual care, but this study states that under report of current number of programs and enrolled patients. Anthony Pattin et al. show that participants in appointment-based study model have significant blood pressure reduction, but this study involves lack of randomization at the point of entry into the study. Compared to other programs, the appointment-based model was found to be better medication synchronization program as per the literature finding.

Applicability of Medication Synchronization program in Indian Health Care System:

Although literature contains studies and pilot programs that demonstrated medication synchronization programs can increase in adherence measured by proportion of days covered, decreased overall health care cost, decreased in hospitalization stay, and emergency visits future research is required. However, MSP are not implemented in our system it our belief that strict government regulatory frame works will hinder the progress. Since India is a developing country implementing these programs are quite challenging.

Barriers for Medication synchronization program.

Community Pharmacies have included a medication synchronization program in their workflow to improve the quality of pharmacy care and medication adherence. MSP showed decrease in-patient emergency visits, hospitalization stay & address the barriers of medication non-adherence. These programs are well established in most of the developed countries were as among Indian population pharmacy services are lacking. Common perception about community pharmacists are primary limited to trade, but the majority of registered pharmacists are qualified in pharmacy and have limited knowledge of professional system. Practising pharmacist does not have proper techniques to improve medication
adherence. However, in most of the developed countries pharmacist will play vital role in providing patient educations to minimize medication non-adherence. Majority of patients are having lack of knowledge about their disease, drug usage and their treatment. Developing and implementing medication synchronization program can be quite challenging.

**Conclusion**

Medication synchronization programs are the newer pharmacy services implemented to restore the refilling process. It is one of tools to improve medication adherence and decrease over all health care cost. These programs work by aligning of prescriptions, refilling the quantity of medications prescribed for chronic disease on single visits. Although there are numerous medication synchronization programs are available in community pharmacies to improve medication adherence each MPS has its own pro’s and con’s. Effectives of medication synchronization programs are confined to the type synchronization programs are implemented. Appointment-based model was found to be better MSP as compared to other programs in community pharmacies as per the literature evidence. Since these programs are well established in most of the developed countries among Indian population pharmacy-based services are lacking. Newer pharmacy services are yet to be implemented among Indian health care system Developing and implementing MPS in health care system is quite challenging. This comprehensive review focus on applicability, effectiveness, barrier’s, of medication synchronization programs in India.

**Ethical Approval:** Not Required

**Conflict of Interest:** The Author declares that there are no conflicts of interest.

**Funding:** Nil

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Articaine: Opening up a New Vista For Pediatric Dentists

Ananthu H1, Ashwin P Rao2, Suprabha B S3

1 Post Graduate Student, 2 Associate Professor, 3 Professor and Head, Department of Pediatric And Preventive Dentistry, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education (Mahe), Manipal, Department of Pediatric And Preventive Dentistry, Manipal College of Dental Sciences

Abstract

Lidocaine has remained the gold standard local anaesthetic agent to perform dental procedures both in the adult as well as in the pediatric patients. However, the invent of articaine has offered the clinicians with a newer and more potent local anaesthetic agent, which causes minimal side effects. Articaine is 1.5 times more potent and 0.6 times less toxic than lidocaine. Also, adequate anaesthesia achieved through infiltration route of administration of the drug, almost eliminates the need for the painful and difficult inferior alveolar nerve block in children, thereby minimising the side effects. Thus, achieving adequate anaesthesia through the administration of a small volume of the drug has opened up a new vista for pediatric dentists in managing pain in children, although manufacturers do not recommend the usage of articaine in children less than 4 years of age due to paucity of evidence. So, this review article tries to throw light on the use of articaine in pediatric patients, citing evidence from literature and also tries to portray the recent advances in the research on articaine use in pediatric patients less than 4 years of age.

Key words: articaine, local anesthsia, pain, children

Introduction

Pain management plays a crucial role in the success of any dental treatment in a pediatric patient (1). Minimising the fear and anxiety, a proper management protocol employed, can help develop a positive attitude towards dental treatment in the patient. Though considered a painful and challenging procedure, the administration of local anaesthesia continues to be one of the most commonly used methods for managing pain in the pediatric age group (2). Maximum efficacy through minimum number of injections with negligible adverse events effected, should be the objective of the local anaesthetic agent used (3).

The discovery of the first amide local anaesthetic agent Lidocaine (Proprietary name: Xylocaine), also known as Lignocaine, in the 1940s, marked a revolution in the arena of pain management in dentistry as it offered better potency and triggered less allergic reactions when compared to the then commonly used local anaesthetic Novocain (Procaine) (4). Since its inception, Lidocaine has remained the “gold standard” local anaesthetic agent to perform dental procedures, all around the globe. However, the search for more effective local anaesthetic agents led to the discovery of newer local anaesthetic agents in the subsequent years. Articaine was one among the newer local anaesthetic drugs developed (5). It was originally developed and
synthesised as ‘Carticaine HCl’ by H. Rusching et al in 1969 (6). It was introduced into clinical practice first in 1976 in Germany and Switzerland followed by the other European countries and Canada (7)(8). In the United States, the US Food and Drug Administration approved the use of articaine in April 2000 (9). Gradually, articaine started to gain attention and clinicians began employing articaine in adult dentistry. However, the use of the drug in the pediatric age group has raised concern over times, though literature shows that the efficacy and safety of articaine is comparable to the other commercially available local anaesthetic including lignocaine (10,11) or even superior (12,13). A survey among pediatric dentists in India concluded that the majority of the respondents still preferred lidocaine with epinephrine as local anaesthetic for use in children (14).

**UNIQUENESS OF ARTICACINE**

Articaine or Articaine HCl (“4-methyl-3-[2-(propylamino)-propionamido]-2-thiophene-carboxylic acid, methyl ester hydrochloride”) is an amide type local anaesthetic. The molecular weight of articaine HCl is 320.84 and it contains a thiophene group instead of a benzene ring, a peculiar characteristic that differentiates it from the other local anaesthetic agents. The greater lipid solubility and potency offered by the thiophene ring helps in the entry of a greater amount of the administered anaesthetic solution into the neurons. It is also able to diffuse more easily through soft tissue surfaces and has a high degree of plasma protein binding of 95%, when compared with the other local anaesthetics. Though classified as an amide, owing to its linkage of intermediate chain, articaine also contains an ester side chain, which triggers the plasma esterases and the hepatic microsomal enzymes to bring about the biotransormation of articaine in plasma and liver respectively. The greater half-life of 20 minutes and the primary metabolite obtained is articainic acid which is pharmacologically inactive. This accounts to the decreased systemic toxicity effected by articaine, thereby rendering itself as a safer alternative (15). Articainic acid (64.2 ± 14.4%), articainic acid glucuronide (13.4 ± 5.0%) and the parent drug (1.45 ± 0.77%) are excreted via urine after the metabolism of articaine (16).

4% solution with 1:100,000 or 1: 200,000 adrenals are the commonly manufactured forms of articaine and they are dispensed in 2.2ml and 1.7ml glass dental cartridges. The two articaine formulations have been used in both adults as well as in children.

**SAFETY OF ARTICACINE IN CHILDREN**

Articaine is well tolerated and safe to use in children above 4 years of age. The potency of articaine is 1.5 times that of lidocaine, which enables the administration of a smaller volume of the drug. The reduced volume may help decrease the discomfort felt by the child during anaesthesia administration, especially in cases where an inadequate co-operation of the child is experienced. It is also 0.6 times less toxic as compared to lidocaine (17). There is also a lesser need for supplemental injections in articaine. Articaine can be given via buccal infiltration to children requiring pulp therapy and extractions and can thereby be used to replace inferior alveolar nerve block (IANB). The high risk of inferior alveolar and lingual nerve damage can be avoided by replacing IANB. The extent of soft tissue analgesia experienced by the patient can be reduced and therefore the incidence of complications like lip biting can be minimized (18,19). When buccal and palatal infiltration with other local anaesthetic agents was replaced by a single buccal infiltration using articaine, it yielded favourable results during the extraction of primary molars in the maxillary arch. Also, articaine delivered via intraligamentary injection can be used to replace IANB to extract primary molars (20).

According to Lemay et al, the onset of anaesthesia by articaine was 168±131 sec (nerve block) and 85±60 sec (infiltration) in children; for adults it was 170±131 sec (nerve block) and 119±84 sec (infiltration) (21). For maxillary infiltration, the duration of action on soft tissues ranges from 2.6 to 4.5 hours whereas for nerve block, it ranges from 4.3 to 5.3 hours (6,8). The volume of distribution of articaine is inversely proportional to the patient’s age. For children between ages 4–12 years, the dosage of articaine ranges from 5-7 mg/kg body weight,
however a lower limit of ≤5mg/kg was advocated in children aged 4-12 years by authors who performed studies using articaine in conjunction with sedative agents (22). In any case, it should always be remembered that the concentration of articaine can be considered double as that of lidocaine, and thus will require half the safe number of cartridges.

**COMPARISON WITH OTHER LOCAL ANESTHETIC AGENTS IN PEDIATRIC DENTISTRY**

Multiple investigators have reported their findings and conclusions on the use of articaine as a local anaesthetic agent in pediatric population.

Malamed in 2000 (6) compared the pain control property of “4% Articaine HCl (with 1: 100,000 epinephrine)” with “2% Lidocaine HCl (with 1: 100,000 epinephrine)” in children under the age of 13 years, by employing the Visual Analogue Scale (VAS). He found Articaine to be comparable to Lidocaine and gave a green signal to its use in the branch of pediatric dentistry citing its safety and efficacy.

Ram and Amir in 2006 (11) compared Articaine and Lidocaine during operative procedures in children aged 5-13 years. They stated that “Articaine 4% with 1:200 000 epinephrine is as effective as lidocaine 2% with 1:100 000 epinephrine”. However, articaine produced a longer lasting anaesthetic effect on the soft tissues as compared to lidocaine.

Yilmaz et al in 2011 (23) compared Articaine 4% with (1:100000 epinephrine) and Prilocaine 3% (with felypressin) in 162 children belonging to the 6 to 8 age group, undergoing primary molar pulpotomy. Similar intensity of pain was reported during administration of both the anaesthetic solutions. However, coronal pulp amputation, prilocaine gave a pain score which was 1.5 times higher than that of articaine. The adverse events reported were also comparable.

In a review by Leith et al in 2012 (17), it was also reported that 4% articaine showed better pain control than 2% lidocaine for both simple and complex dental procedures.

In 2012, Odabaş (24) compared 4% articaine with 3% mepivacaine, to study the pain reaction and duration of soft tissue numbness during the administration of local anaesthesia in children. A “randomized, double-blind, split-mouth” design was adopted for the study which included a sample size of 50 children (25 girls and 25 boys) belonging to the 7- to 13-year-old age group, who required comparable operative treatment needs in symmetric primary teeth. The objective evaluation of the children was carried out by the modified behaviour rating scale and the subjective evaluation by using the Wong-Baker FACES pain rating scale which helped assess the post injection and post treatment experience. The duration of numbness felt was recorded by the parents by asking the children. Articaine (140.69±49.76 minutes) presented with a prolonged time period of soft tissue anaesthesia than mepivacaine (117.52±42.99 minutes). Neither did the efficacy of the anesthesia nor did the heart rate, blood pressure, or oxygen saturation show any significant differences during all evaluation spans for both anaesthetic solutions. The two anaesthetic solutions also showed similar post treatment experience. It was concluded that same efficacy and identical child behaviour was observed with 4% articaine and 3% mepivacaine, though articaine reported longer soft tissue numbness.

In 2012, Arrow (25) compared “4% Articaine (with 1: 100,000 epinephrine)” administered via buccal infiltration to “2% Lignocaine (with 1: 80,000 epinephrine)” administered via buccal infiltration (BI) or inferior alveolar nerve block (IANB) during standard restorative procedures in the posterior teeth of the mandibular arch among school children. The study assessed pain response via the Faces Pain Scale – Revised (IASP) and found no statistical difference in pain response between both the solutions. The study declared that both Articaine and Lignocaine, were declared equally effective in pain management in children for routine restorative procedures.

In 2014, Thakare (26) evaluated and compared 4% Articaine and 0.5% Bupivacaine through his randomized
controlled crossover clinical trial, in patients aged 10-18 years who had their premolars indicated for orthodontic extractions. The study showed that 4% Articaine presented with a quicker onset of action and lesser Visual Analog Scale scores as compared to Bupivacaine. However, Bupivacaine presented with a longer time period of analgesia and time to the first uptake of medication for rescue analgesia. Articaine was declared more potent and effective considering its quicker onset of action and low pain scale scores.

In 2015, Arali and Mytri P (27) studied the anaesthetic efficacy of “4% articaine (with 1: 100,000 epinephrine)” delivered via buccal infiltration and “2% lignocaine (with 1: 100,000 epinephrine)” delivered via inferior alveolar nerve block (IANB) by employing a “randomized, double-blind, cross over” study design in children aged 5-8 years, presenting with the clinical condition of irreversible pulpitis. The study also assessed the necessity for the administration of supplemental injections. The trial reported that the anaesthetic onset was quicker with 4% articaine than 2% lignocaine. A shorter span of anaesthesia was observed with articaine infiltration and with a lesser requirement for the administration of supplemental injections. The study concluded that 4% articaine can be given via buccal infiltration to children undergoing management for irreversible pulpitis. The study also highlighted the potential of articaine delivered via buccal infiltration to replace IANB to avoid complications such as lip biting.

A study by Zurfluh et al in 2015 (28) assessed whether an articaine solution could reduce the period of soft tissue anaesthesia and thereby reduce the risk of self-inflicted soft tissue lesions, while still providing an adequate anaesthesia. The study reported that owing to its high efficacy, tolerance, and reduced soft tissue anaesthesia, 4% articaine (with 1: 400,000 epinephrine) was considered safe for treatment in paediatric population.

In 2015, a study was performed by Mittal (29) in 112 children, to compare the anaesthetic efficacy of articaine with lidocaine during the extraction of primary molars in the maxillary arch and assess whether a buccal infiltration injection could achieve sufficient palatal anaesthesia as well. The study reported that buccal infiltration with articaine can be considered a potential substitute for lidocaine in achieving local anesthesia in children, though it did not succeed in achieving sufficient palatal anaesthesia.

In 2016, Chopra et al (30) compared the efficacy of buccal infiltration with articaine against inferior alveolar nerve block with lignocaine for primary molars in children aged 4-8 years, indicated for pulpectomy or pulpotomy. The efficacy of both the anaesthetic agents were assessed using the Pain Scores, the Facial Image score, Sound Eye Motor (SEM) scores and Heft-Parker Visual Analogue Score (HP-VAS). Buccal infiltration with articaine showed significant lower pain scores as compared to IANB (p<0.001). SEM scores at the time of pulp extirpation were also higher for IANB than infiltration (p<0.001).

Kolli et al in 2017 (31) stated that Articaine buccal infiltration can be used as an alternate to conventional local anaesthetic delivered via buccal and palatal infiltration, for extracting primary molars in the maxillary arch.

In 2018, Sharan et al (32) assessed the effectiveness of “2% lidocaine (with 1:80,000 epinephrine)” and “4% articaine (with 1:100,000 epinephrine)” to extract primary mandibular molars in children aged 6-10 years, by employing the intraligamentary injection technique. The study utilised the Sound eye motor scale to assess pain perception. The study showed 80% success rate in Articaine group and 30% success rate in lidocaine group. The study concluded that the intraligamentary injection technique using articaine might serve as an alternative to IANB during extraction of primary molars in the mandibular arch.

Rathi conducted a study in 2019 (33) among 100 children to compare the anaesthetic efficacy of articaine with that of lignocaine. It was concluded that a single buccal infiltration of 4% articaine with 1:100,000 epinephrine is more effective when compared to 2% lidocaine with 1:80,000 epinephrine for extracting
primary molars in children aged 7 to 12 years.

Massignan in 2020 (34) stated that though articaine reported a higher pain incidence during the injection, no difference in efficacy was observed when the anaesthetic administration of articaine was compared to lidocaine in the extraction of primary molars. In the study, the efficacy and the adverse events of “4% articaine with epinephrine 1:100 000” was compared with that of “2% lidocaine with epinephrine 1:100 000” during the extraction of primary molars, by employing anaesthesia via buccal infiltration in forty-three children belonging to the 6-10 age group. 21 children received local buccal infiltration with 4% articaine whereas 22 children received lidocaine 2%. The pain experienced during injection of the anaesthetic solution and during extraction of the tooth was the major outcome assessed. The anaesthetic efficacy of both the solutions was observed to be identical though a higher pain incidence was reported by children belonging to the articaine group. Similar adverse effects were observed in both the groups which included post-operative pain, edema and nausea.

It can be summarised that there is evidence to conclude that 4% articaine is comparable to or more efficacious than the other local anaesthetic agents used in children. However, the parents should be cautioned about self-inflicted soft tissue injuries that may arise due to the prolonged numbness, commonly reported with articaine administration. However, the evidence suggests that articaine is safe in children, as the administration of the drug triggers few adverse events, which are commonly noticed with lidocaine as well.

**EVIDENCE FOR USE IN CHILDREN BELOW 4 YEARS OF AGE**

Some studies have specifically reported the use of articaine in paediatric dental patients less than 4 years of age with positive results, however the manufacturers still do not recommend it for children younger than 4 years.

Wright et al in 1989 (22) conducted a retrospective research which analysed the usage of articaine hydrochloride as an anaesthetic agent in children aged less than 4 years of age. Records of articaine administration on 211 patients, with additional administrations of the agent on 29 patients was retrieved from two pediatric dentistry offices by a record audit. Some cases even reported administration of higher doses of the drug than recommended for older children. The clinicians have not noted the occurrence of any systemic adverse reactions. The report provided initial evidence for the use of articaine in children under 4 years of age.

Jakobs et al in 1995 (35) studied the pharmacokinetics of articaine on 27 children, 3 to 12 years of age, who underwent dental procedures under intubation anesthesia. They determined the serum concentrations after local anesthesia with 2% and 4% articaine solutions in this age group of children. They concluded that articaine shows age-related differences in the pharmacokinetics and hence there is no need to fix a lower mg/kg dose limit while administration in children.

A study was conducted in young children below four years of age by Elheeny in 2020 (36) to assess the efficacy and safety of 4 % articaine as a local anesthetic agent by comparing it with 2 % lidocaine hydrochloride. The study had an equivalent randomized control trial design, with two parallel arms including 184 young children (92 per group) aged from 36 to 47 months, in whom, pulpotomy of mandibular primary molars was indicated after administering anesthesia by buccal infiltration injection. The results of the study indicated that during pulpotomy, children who received articaine showed less pain as compared to their counterparts in the lidocaine group and no statistically significant differences were observed between both the drugs, considering the post-operative complications. It was concluded that the findings supported the efficient and safe use of articaine hydrochloride 4% with epinephrine 1:100 000 as a local anaesthetic agent during dental procedures in children aged between 3 and 4 years.

The evidence supports the use of articaine in children less than 4 years of age. However, limited research demands clinical trials on a larger sample size.
to be performed before employing articaine as a local anaesthetic agent in children less than 4 years of age.

**Conclusion**

Articaine is a safe and effective local anesthetic drug which can be employed in pediatric patients to help achieve dental analgesia during invasive procedures. The evidence that articaine administered via infiltration technique can replace Inferior Alveolar Nerve Block makes it even more interesting. The ability of articaine to penetrate into deeper spaces by diffusing through bone and soft tissue should be understood by the clinicians as it helps to achieve excellent depth of anesthesia, while avoiding block and palatal anesthesia for dental treatment in children. However, advanced research and clinical trials is a necessity, as articaine is not recommended by manufacturers in children below 4 years of age.

**Ethical Clearance** - Not applicable (review article)

**Source of Funding** - Self

**Conflict of Interest** - Nil.

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Maxillary Inter-canine Width at Three Stages of Dentition– A Cross-Sectional Study

Anita Thakur1, Seema Thakur2, Parul Singhal3, Deepak Chauhan3

1Post Graduate Student, 2Professor & Head, 3Assistant Professor, Department of Pediatric and Preventive Dentistry, H.P Government Dental College and Hospital Shimla, Himachal Pradesh

Abstract

Objective: To determine the palatal intercanine width from deciduous dentition to permanent dentition in 5years to 16 years old children.

Materials and Methods: the study sample comprised of 168 children who fulfilled the inclusion and exclusion criteria were examined. Study models were constructed and maxillary intercanine width was measured using Vernier digital caliper.

Results: Statistically significant difference was found in upper intercanine width (UICW) between males and females in primary dentition (p<0.034). Statistically non-significant difference was found in upper intercanine width (UICW) in mixed and permanent dentition. Data were analysed using SPSS 22 (SPSS Inc., Chicago, IL, USA). One-way ANOVA followed by Tukey’s post hoc test and t- test were applied to verify the existence of significant differences between the groups.

Conclusion: The present study found that there is significant increase in intercanine width in upper dental arch from primary dentition to permanent dentition.

Keywords: Intercanine width, arch, maxillary, dimensions

Introduction

The human craniofacial complex and associated dental arches undergo visible alterations as they grow and adapt1 from childhood to early permanent dentition. Morphological variations in the dental arch measurements in primary dentition, mixed dentition and permanent dentition are of great concern to a dentist as they encounter clinical problems associated with developing dentition like tooth anomalies, caries and malocclusion.

Individual occlusal variables are not functionally or anatomically independent. Their interdependence means that sets of occlusal features could have different pattern of inheritance or response to environmental influences than individual attributes2. These morphogenetic characteristics expressed differently in different ethnic people. The size and shape of dental arches presents with different variability within and among different population groups ranging from short and wide to long and narrow.

Thus; Understanding the peculiar features of the developing dentition and changes in dental arches that will take place from primary dentition to permanent dentition for a particular population is important for the Paediatric dentists who are involved in the guidance of eruption, preventive procedures and planning early orthodontic interventions before the peak of growth.

Although various studies have been done on the malocclusion status of the children of Himachal Pradesh, but there are no reported studies showing changes in upper intercanine width (UICW) from primary to permanent dentition. This investigation evaluated the changes in intercanine width cross-sectionally from primary dentition to permanent dentition in 5 years to 16
years old children of Shimla, a district in northern hilly state of India.

**Material and Methods**

This cross-sectional study was conducted in the Department of Pediatric and Preventive Dentistry, Himachal Pradesh Government Dental College and Hospital, Shimla (Himachal Pradesh) between 1st April 2019 to 30th June 2019. The study has been approved by the institutional ethical committee. A total of 823 school going children between the age of 5 years to 16 years were examined and the dentition stage was clinically determined for each subject by a trained examiner. Informed consent was taken from each subject and head of the corresponding school after explaining the purpose, methodology involved, the related risks and benefits. A sample of 150 (each dentition stage consisted of 50 children) was estimated based on the study done by Younes et al\(^3\). Stratified random sampling was done and students were selected from 10 kindergarten, 10 elementary and 10 middle schools which were evenly distributed in the four regions of the district\(^4\) and the patients attending the outpatient department of Pediatric and Preventive Dentistry, as per the defined inclusion and exclusion criteria. Demographic information was taken regarding age and gender. Subjects with their parents and grandparents belonging to Shimla were selected to participate in the study, with an average age of 5.3 year in primary dentition, 9.64 year in mixed dentition and 14.28 years in permanent dentition (Table 1). They were diagnosed as having canines bilaterally in normal occlusion (Normal occlusion was considered as dental and skeletal class I occlusion with a satisfactory clinical occlusion\(^5,6\)) with no history of orthodontic treatment\(^6\).

**Measurement Reliability:** Measurements were obtained for each parameter with a Vernier caliper accurate to 0.01 mm for canine area. Intercanine width was measured as the distance between the crown tips of the canines (transverse width in between deciduous canines in primary and mixed dentition and transverse width in permanent canines in permanent dentition)\(^6\).

**Statistical Analysis:** Data were analysed using SPSS 22 (SPSS Inc., Chicago, IL, USA). One-way ANOVA followed by Tukey’s post hoc test and t-test were applied to verify the existence of significant differences in “Primary Dentition (Group I), Mixed Dentition (Group II), and Permanent Dentition (Group III),” variables among dentition stages respectively. The level of significance was set at \(p < 0.05\).

For the purpose of this study, it was concluded that this level of accuracy was appropriate.

**Results**

A total of 823 subjects were examined in different schools for the study in the age group of 5-16 years.
Among these examined subjects, 168 impressions were poured. The casts which were fractured and had bubbles on the canine tips were excluded. Finally; the study included a total of 150 casts which were divided into three groups equally i.e. Group I, Group II and Group III. The mean age at different stages of dentition were calculated. Measurements of the arch dimension like inter-canine width, was measured on the cast using Vernier caliper.

Table 2 shows the mean (±SD) of Intercanine width using digital caliper in Group I, Group II and Group III which are 28.33±1.01, 33.96±1.29 & 35.07±1.24 respectively. The Group I have statistically significant difference between males and females i.e. p=0.034. In Group II and Group III male and female mean (±SD) do not differ significantly having p=0.088 and p=0.189 respectively. Statistically significant difference is found between Group I & Group II, Group I & Group III and Group II & Group III which are p<0.001, p<0.001 and p<0.001 respectively.

Table 1. Descriptive statics of age among Primary, Mixed and Permanent dentitions.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Mean±SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>5.20±0.50</td>
<td>5.37±0.49</td>
<td>5.30±0.49</td>
</tr>
<tr>
<td>Group II</td>
<td>9.80±0.87</td>
<td>9.48±0.82</td>
<td>9.64±0.85</td>
</tr>
<tr>
<td>Group III</td>
<td>14.18±2.19</td>
<td>13.80±2.76</td>
<td>14.28±0.95</td>
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</tbody>
</table>

Table 2. Comparison of Palatal Intercanine width among Primary, Mixed and Permanent dentition; and between Males and Females.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Male Vs Female</th>
</tr>
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<tbody>
<tr>
<td>Mean±SD</td>
<td></td>
<td></td>
<td></td>
<td>P value**</td>
</tr>
<tr>
<td>Group I</td>
<td>28.64±1.17</td>
<td>28.13±0.84</td>
<td>28.33±1.01</td>
<td>0.034 S</td>
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<tr>
<td>Group II</td>
<td>34.27±1.39</td>
<td>33.65±1.11</td>
<td>33.96±1.29</td>
<td>0.088 NS</td>
</tr>
<tr>
<td>Group III</td>
<td>35.29±1.27</td>
<td>34.82±1.17</td>
<td>35.07±1.24</td>
<td>0.189 NS</td>
</tr>
<tr>
<td>P value*</td>
<td>&lt;0.001 S</td>
<td>&lt;0.001 S</td>
<td>&lt;0.001 S</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33.09±3.08</td>
<td>31.87±3.18</td>
<td></td>
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</table>

P value** 0.019 S

Group I Vs Group II P value <0.001 S
Group I Vs Group III P value <0.001 S
Group II Vs Group III P value <0.001 S

(P value**: Independent sample t test, P value*: ANOVA one way test, P value: Tukey Post Hoc test for multiple comparisons, S: Statistically significant at 0.05 level, NS: Not Significant).
Discussion

India is a developing country and oral health, particularly the treatment of malocclusion is still not the priority of the people. Hence it is necessary to know about the normal dento-skeletal measurements for future diagnosis and treatment planning. Thus, the present study was conducted to evaluate the findings of palatal intercanine width from deciduous dentition to permanent dentition in 5 years to 16 years old children of district Shimla, Himachal Pradesh. In the present study, increase of 5.6 mm from age 5.2 to 9.8 years in overall palatal intercanine width from primary dentition to mixed dentition was found, which was statistically significant (p<0.0001). There was significant difference between males and females in primary dentition. Ahn et al \(^9\) reported that the intercanine width increased from ages 6 to 8 years in maxilla, both in girls and boys.

Most of the studies in literature reported lesser increase in palatal intercanine width from primary to mixed dentition when compared to our study. Rapid increase in palatal intercanine width was reported by the studies done by Barrow & White\(^{11}\) (increase of 4mm), Moorrees et al\(^{14}\) (increase of 3mm) and Sillman\(^{19}\). Very little increase in palatal intercanine width from primary to mixed dentition was found by Hasanali & Odhiambo\(^{13}\) (an increase of 0.21mm from 6-8 to 12 years of age for Kikuyu, Maasai and Kalenjin sample), Ahn et al\(^{9}\) (1.08mm from 6 years to 9 years), and Eslami Amirabadi\(^4\) et al (an increase of 0.47mm from 5 years to 9 years of age). Virgenia B Knott\(^{15}\) and Thialander B\(^6\) reported an increase in palatal intercanine width from primary to mixed dentition due to eruption of permanent incisors in mixed dentition stage which leads to an increase of the anterior segment of maxilla. The studies done by Ruth Elaine Ross -Powell\(^{8}\) & Edward F Harris\(^2\) reported an increase in palatal intercanine width of 4.3mm between ages of 5 and 10 years which he concluded that it could be due to the expansion at maxillary-premaxillary sutures. There was an increase of 1.02 mm in palatal intercanine width from mixed dentition to permanent dentition from the age of 10 years to 14 years in the present study, which was found to be statistically significant (p<0.0001). Similar results were reported in several studies [(0.92mm (Arslan et al\(^{18}\)), 2mm (Sillman\(^{19}\) and Virgenia B Knott\(^{15}\)), Eslami Amirabadi et al\(^4\) (4.60mm for Saudis) and Yang D et al\(^{10}\) (0.95mm from 10 years to 12 years)]. These findings were in contrast with the finding of the studies done by Sinclair & Little\(^{16}\) which showed decrease of 0.31mm, additionally Moorrees et al\(^{14}\) and Tsujino & Machida et al\(^{17}\) also showed a decrease from the mixed to the permanent stage. Samir E Bishara\(^{12}\) stated that the arch width dimensions were established in the mixed dentition by 8 years of age with some, but minimal, increase until the early permanent dentition (13 years) and progressive but minimal decrease in early and mid-adulthood. Burdi and Moyers\(^{20}\) pointed that the direction of the vertical alveolar growth differs significantly in the maxillary and mandibular arches. The maxillary alveolar processes diverge as the teeth erupt, whereas the growth of the mandibular alveolar process is more parallel. Such changes have significant clinical implications because they may allow for a greater differential increase in the maxillary arch width during treatment. The Study done by Thialander B\(^6\) stated that with the eruption of permanent canines, a further minor increase was observed in the maxillary arch. Ahn SJ\(^9\) stated that the permanent canines establish their final position and get stabilized by surrounding functional structures. Because of this stability, teeth are generally considered to move within bony confines and surrounding bone would change according to these functionally stabilized teeth which should be analyzed in future studies\(^9\). Furthermore; Ciusa V et al\(^{21}\) stated that “in children with a complete deciduous dentition, the lack of age-related modifications in maxillary intercanine width implies careful consideration of treatment timing of patients with crossbites and when a functional cause has been ruled out, the diagnosis of a crossbite at the deciduous canines should be a priority, because a relatively reduced maxillary intercanine distance will probably not correct spontaneously with growth”.

The present study indicated that there is increase of 6.62mm in intercanine width from primary to permanent
dentition. However, it is important to construct more precise findings for the width in anterior maxillary arch in canine region for children of Shimla origin of Himachal Pradesh. These progressive changes from deciduous to permanent dentition could not be indicated due to our cross-sectional research. Hence; further longitudinal studies are required to be done for the follow up of the dental arch development in children throughout the whole growth period to ascertain changes that may occur during the transitional periods from primary dentition to permanent dentition22.

**Conclusion**

Intercanine width varies among different populations. To summarize, the present study found that there is significant increase in intercanine width in upper dental arch from primary dentition to permanent dentition. Statistically significant difference was found in upper intercanine width (UICW) between males and females in primary dentition. Statistically non-significant difference was found in upper intercanine width (UICW) in mixed and permanent dentition.

**Conflicts of Interest:** The authors declare that there are no conflicts of interest regarding the publication of this paper and the study has not been funded by any source.

**References**


Effectiveness of Structured Teaching Programme on Knowledge Regarding Post Exposure Prophylaxis Following Needle Stick Injury among B. Sc Nursing Students

Anjana A.P1, Reshma Thomas2, Renjitha2
1Assistant Professor, Department of Medical Surgical Nursing, 2 IV Year B. Sc Nursing Students, Amrita College of Nursing, Amrita Vishwa Vidyapeetham, Kochi, Kerala, India

Abstract

Background and Objective: A needle stick injury is a percutaneous piercing wound typically set by a needle point, but possibly also by other sharp instruments or objects. Needle stick injuries are more common among health workers during night shifts, and for less experienced people, fatigue, high work load, high pressure, or high perception of risk can all increase the chances of needle stick injury. The present study was conducted with an Objective, to evaluate the effectiveness of structured teaching programme on knowledge regarding post exposure prophylaxis following needle stick injury among B. Sc Nursing students. Methods and Materials: Approach was quantitative approach. The study design was Quasi experimental one group pre test post test design. 157 samples were included in the study by using convenience sampling technique. The knowledge of nursing students regarding post exposure prophylaxis following needle stick injury were assessed before and after the implementation of education programme. The data was collected by using semistructured knowledge questionnaire. Post test was done seven days after the intervention. Results: Application of paired t test revealed a statistically significant increase in knowledge score regarding post exposure prophylaxis following needle stick injury after the implementation of the education programme (t= 9.40, p<0.001). There was a significant increase in the knowledge of each components of post exposure prophylaxis also. Conclusion: Based on the study findings it was concluded that the education programme was effective in improving the knowledge level of nursing students regarding post exposure prophylaxis following needle stick injury.

Key words: Knowledge, Needle stick injury, post exposure prophylaxis

Introduction

A needle stick injury is a percutaneous piercing wound typically set by a needle point, but possibly also by other sharp instruments or objects. Needle stick injuries are more common among health workers during night shifts, and for less experienced people, fatigue, high work load, high pressure, or high perception of risk can all increase the chances of needle stick injury1. Generally needle stick injuries cause only minor visible trauma or bleeding, however, even in the absence of bleeding the risk of viral infection remains. The world health organization estimated annual global needle stick injuries at 2 million per year, and another investigation estimated 3.5 million injuries yearly. The European biosafety network estimated 1 million needle stick injuries occurs annually in Europe 2. In India, authentic data on NSI are scarce. More than 90% infections occur in developing countries.3 CDC estimates that each year 3,85,000 needle stick injuries are sustained by hospital based health care personnel. According to WHO study, the annual estimated proportions of HCWs exposed to
blood borne pathogens globally were 2.6% of HCV, 5.9% for HBV & 0.5 % for HIV. 4

Anjana A. P., Gisha Joseph, Revathy A.Valsan conducted a descriptive study to assess the knowledge regarding Post Exposure Prophylaxis following needle stick injury among 134 B. Sc. Nursing Students at Kochi using convenience sampling technique. The data were collected using semi structured knowledge questionnaire. The results revealed that out of 134 respondents 125 (93%) had an average knowledge regarding post exposure prophylaxis following needle stick injury. Regarding students’ knowledge on different variables on PEP following needle stick injury, they had good knowledge on prevention, average knowledge on meaning and risk factor of needle stick injuries and the lowest mean score in the area of management and complications of PEP 5.

Mittal Garima, Taneja Anmol. R K, Garwal R K, Gupta Pratima and Gupta Priyanka conducted a cross-sectional observational study to assess the knowledge, awareness and prevalence of needle stick injury among undergraduates, postgraduates and nursing students (100 in each category) of the SRHU, Uttarakhand, India. Data was collected on a pretested structured questionnaire distributed among the students which consisted of questions to assess the knowledge and awareness towards needle stick injuries. Out of 300 students, needle prick injury was reported in 6 undergraduates, 7 postgraduates and 20 nursing students in past twelve months. Out of 300 students, 22% (66) knew the definition of needle stick injury and 58.6% (176) knew the immediate measure to be taken i.e. to wash the wound with soap and water. Out of the 33 students who contracted NSI, 38.5% cannot remember the cause of needle stick injury, while 34.9% mentions the cause of NSI due to the carelessness/accident and 21.7% reports the NSI due to poor disposal of needle. Only 56.6% reported the incident, whereas only 21.7 filled an incident report at integrated counselling and testing centre.6

Based on the findings of the above studies it is concluded that the nursing students have an average knowledge regarding post exposure prophylaxis following needle stick injury. Therefore, there is a need for regular training and education to update the awareness and knowledge about post exposure prophylaxis which serves as an effective strategy to prevent the spread of blood borne disease. Hence the topic was selected for study.

Methods and Materials

This study was conducted in Amrita College of Nursing, Kochi, Kerala during the month of January 2019. Quantitative approach was used for the study. The design adopted for the study was quasi experimental one group pretest posttest design. By using convenience sampling technique samples were selected. 157 Second- and Third-Year B Sc Nursing students, who were present on the day of data collection was selected for the study.

Data collection began with pretest by using semi structured questionnaire which includes sociodemographic data and knowledge questionnaire. After that teaching was given with lecture cum discussion method. After seven days posttest was obtained.

Data were analyzed by using descriptive and inferential statistics. Continuous data were expressed in terms of mean and standard deviation (SD). Comparison of mean pre test and post test knowledge scores using paired t test.

Results

Regarding the sociodemographic data most of the sample 128 (81.5%) were belongs to the age group of 18-20 years. 153 (97.5%) were females. 152 (97%) were heard about needle stick injury and majority of them 141 (90%) were vaccinated against blood borne infection. 27(17%) had experienced needlestick injury and the frequency of needle stick injury were 1-2 times. Only 24 (89%) were reported on needle stick injury and 3 (11%) were not reported. Reason for not reporting the incident was lack of knowledge 23 (86%). Among the sample 10 (37%) had needlestick injury while disposing the
waste and 10 (37%) had needle stick injury while giving injections. 124 (79%) had previous knowledge on post exposure prophylaxis and they got training regarding the same. 145 (92%) sample agreed postexposure prophylaxis is effective in preventing blood borne infections.

Regarding the level of knowledge, in pretest 133 students had average knowledge and 3 had poor knowledge. Only 21 students had good knowledge regarding postexposure prophylaxis. After the intervention 73 students gained good knowledge and 84 had average knowledge.

### Table 1: Comparison of pretest and posttest knowledge score

<table>
<thead>
<tr>
<th>Tests</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretest</td>
<td>12.45</td>
<td>1.906</td>
<td>9.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Posttest</td>
<td>14.21</td>
<td>2.133</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Mean and standard deviation of knowledge score in the pretest and posttest with respect to the components

<table>
<thead>
<tr>
<th>Component</th>
<th>Maximum Score</th>
<th>Pretest</th>
<th>Posttest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard Deviation</td>
<td>Mean</td>
</tr>
<tr>
<td>Mean</td>
<td>1.82</td>
<td>0.44</td>
<td>1.89</td>
</tr>
<tr>
<td>Risk Factors</td>
<td>2.81</td>
<td>0.74</td>
<td>2.96</td>
</tr>
<tr>
<td>Management</td>
<td>3.77</td>
<td>1.14</td>
<td>4.32</td>
</tr>
<tr>
<td>Complication</td>
<td>1.31</td>
<td>0.765</td>
<td>2.18</td>
</tr>
<tr>
<td>Prevention</td>
<td>2.75</td>
<td>0.64</td>
<td>2.87</td>
</tr>
</tbody>
</table>

### Discussion

The study shows an overall average knowledge among nursing students before participation in education programme. The present study was supported by another study conducted by Sudhir Sing. A pre-experimental study was conducted to assess the effectiveness of structured teaching programme regarding knowledge of needle stick injury and its prevention among nursing students in selected Nursing institutes Faridabad, Haryana. 60 sample were selected for the study. The study finding revealed that, in pre-test, out of 60 sample, most of them 42 (70%) had inadequate, 18(30%) had
moderate level of knowledge. In post-test, out of 60 sample most of them 40 (66.66%) had adequate and 20 (33.33%) had moderate level of knowledge and none of them had Inadequate level of knowledge. In pre-test overall mean score was 5.41 with the SD of 3.29 and in post-test, the overall mean score was increased to 14.10 with SD of 3.71. The mean difference of pre and post-test was 8.69. The calculated ‘t’ value was 35.58, which is significant at p <0.05. This indicates that structured teaching programme was effective. 

Aswin Kumar, G. K. Ratnaprabha conducted a cross-sectional study to assess the Awareness about HIV and post exposure prophylaxis among students of a nursing college from central Karnataka. Out of 108 students 72 (66.67%) students heard about post exposure prophylaxis. Majority were unaware about time of initiation (94.6%) and duration of PEP (86.1%). The study concluded that knowledge about HIV/AIDS and PEP are inadequate among nursing students.

Conclusion

Continuous education about postexposure prophylaxis among nursing students helps to improve their knowledge, which serves as an effective strategy to prevent blood borne diseases.

Conflict of Interests: Nil

Source of Funding: Self

Ethical Clearance: Prior to the study the investigators obtained approval from research committee of Amrita College of Nursing and Ethical clearance from Institutional Review Board. Permission from the Head of the Department was obtained before conducting the study. An informed consent was obtained from each participant.

Reference


Fatal Poisoning Cases in a Teaching Hospital of West Bengal: a Descriptive Cross-Sectional Clinico-epidemiological Study

Ankur Bhattacharjee¹, Saptarshi Chatterjee², Partha Sarathi Hembram², Sobhan Kumar Das³
¹Assistant Professor, Department of Forensic Medicine and Toxicology, N.R.S. Medical College, Kolkata, West Bengal, ²Associate Professor, Department of Forensic Medicine and Toxicology, Bankura Sammilani Medical College, Bankura, West Bengal, ³Professor, Department of Forensic Medicine and Toxicology, R.G. Kar Medical College, Kolkata, West Bengal

Abstract

**Background:** Poisoning is a global health issue and one of the leading causes of morbidity and mortality in India. Pattern of poisoning depends on factors, such as availability of poisons, socioeconomic status of the population, religious and cultural influences, thus creating varied epidemiological trends across time and regions. The present study is planned to address the epidemiological profiles of fatal poisoning cases, with reference to the gastric findings during autopsy.

**Methods:** This is a descriptive, observational, cross-sectional study analysing 184 fatal poisoning cases, who attended to R.G. Kar Medical College Police Mortuary during the period of 2014-2015.

**Conclusion:** People of age group 20-29 years were mostly affected, with a male preponderance and an incidence of 8.03%. People from the rural regions constituted more than 80% of the study sample, with suicide as the commonest manner of death. Organophosphorus compounds and acids were the mainly detected, with mucosal congestion and submucosal hemorrhage of stomach as the most reported findings. The authors feel that a prospective study is an option, which could accurately identify the cases and deaths, which could formulate stringent measures so as to decrease the morbidity and mortality due to poisoning.

**Keywords:** Demography, Gastric findings, Intentional poisoning

Introduction

Poisoning is a global health issue and one of the leading causes of mortality and morbidity in India.¹ According to World Health Organization (WHO), approximately 0.03 million people die annually due to various types of poisoning.² In India, it has been estimated that about 5 to 6 persons per lakh of population die due to poisoning every year.³

Corresponding author:
Dr. Mainak Tarafder
Assistant Professor, Department of Forensic Medicine and Toxicology, BankuraSammilani Medical College, Bankura, West Bengal

Wide availability, easy access and extensive use in medical, industrial, household and agricultural applications increase the risk, exposure and incidence of poisoning.⁴ Pattern of poisoning in a region depends on a variety of factors, such as availability of poisons, socioeconomic status of the population, religious and cultural influences,⁵ thus creating varied epidemiological trends across time and regions. Similarly, the mortality and morbidity in any case of acute poisoning depends upon a number of factors, such as the nature of poison, dose consumed, availability of medical facilities, treatment by qualified persons and the time interval between intake of poison and provision of medical help.

Though literature on poisoning in India is widely available, there is a significant lack of data from the
eastern parts of the country. Moreover, most of the studies, so conducted, aimed at the clinical presentation and management protocols of the poisoning cases, based on in-hospital records, or are retrospective studies. The present study is hence planned to address the epidemiological profiles of fatal poisoning cases in West Bengal, with reference to the gastric findings during autopsy. In due course, it may estimate the nature and severity of poisoning, with demographic profiles of the victims, thus providing the stakeholders some recommendations in order to take appropriate preventive measures.

Aims and Objectives

1. To study the prevalence of deaths due to poisoning from 2014 to 2015
2. To study the socio-demographic profiles of the victims of fatal poisoning
3. To analyse the type of poisons
4. To study the gastric findings in fatal poisoning cases

Materials and methods

a. Place of study: R.G. Kar Medical College Police Mortuary, Kolkata, West Bengal, India.

b. Period of study: 1st April, 2014 to 31st March, 2015

c. Study population: All the victims of fatal poisoning, attended to R.G. Kar Medical College Police Mortuary for autopsy examination between 2014 to 2015.

- Inclusion criteria: All the victims of fatal poisoning between 2014 to 2015, who attended for autopsy examination at R.G. Kar Medical College Police Mortuary.

- Exclusion criteria:
  i. Victims of fatal animal, snake and insect bites
  ii. Cases with incomplete or inadequate history
  iii. Grossly decomposed bodies
  d. Study design: Descriptive observational cross-sectional study
  e. Statistical analysis: Details regarding the cases were obtained from the inquest report, interviewing the eyewitnesses and the family members of the deceased. All the data were manually checked and edited for completeness in a pre-determined format and were then coded for computer entry. Collected data was recorded in Microsoft Excel worksheet and SPSS IBM 19.

Results

1. Prevalence of Fatal Poisoning cases

The number of victims due to poisoning was 184 (8.03%) among 2290 cases sent for autopsy.

2. Distribution according to age and gender

Majority of the victims of fatal poisoning belong to the age group of 20-29 years, with a male predominance in almost all the age groups (except victims below 10 years and between 40-49 years).[Table 1].

3. Distribution according to religion and residence

In our study, 141 out of total 184 victims (76.63%), were Hindus and the rest 43 victims (23.37%) belonged to the Muslim community. 148 of the victims (80.4%) were from the rural regions and the rest 36 of the victims (19.6%) belonged to urban areas.

4. Distribution according to the type of poison

Majority of the fatal poisoning cases were due to Organophosphorus poisoning (22.8%), followed by 21.1% victims of acid ingestion [Figure 1].

5. Distribution according to manners of poisoning (as per Police inquests)

Deaths due to suicide (91.3%) were maximum, followed by accidental (7%) and homicidal poisonings (1.7%).
6. Distribution according to smell of gastric contents

Gastric contents mostly exhibited pungent odours (47.4%), followed by 23.9% of the victims with no untoward smell [Table 2].

7. Distribution according to appearance of gastric mucosa

Congested mucosa (46.2%) was mostly found, followed by submucosal hemorrhage (30.5%) and 15.8% of victims with charred mucosa and perforation [Figure 2].

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>&lt;10</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>10-19</td>
<td>13</td>
<td>44.83</td>
</tr>
<tr>
<td>20-29</td>
<td>20</td>
<td>42.55</td>
</tr>
<tr>
<td>30-39</td>
<td>9</td>
<td>29.03</td>
</tr>
<tr>
<td>40-49</td>
<td>15</td>
<td>51.72</td>
</tr>
<tr>
<td>50-60</td>
<td>5</td>
<td>19.23</td>
</tr>
<tr>
<td>&gt;60</td>
<td>9</td>
<td>42.86</td>
</tr>
</tbody>
</table>

Table 1 Distribution of fatal poisoning cases according to age and gender

Fig 1 Distribution according to type of poison
### Table 2 Distribution according to smell of Gastric contents

<table>
<thead>
<tr>
<th>Smell of Gastric content</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholic</td>
<td>6</td>
<td>3.2</td>
</tr>
<tr>
<td>Faint uncharacteristic odour</td>
<td>23</td>
<td>12.5</td>
</tr>
<tr>
<td>Kerosene like</td>
<td>21</td>
<td>11.4</td>
</tr>
<tr>
<td>Noodour</td>
<td>44</td>
<td>23.9</td>
</tr>
<tr>
<td>Phenyl like</td>
<td>3</td>
<td>1.6</td>
</tr>
<tr>
<td>Pungent</td>
<td>87</td>
<td>47.4</td>
</tr>
</tbody>
</table>

### Fig 2 Distribution according to appearance of gastric mucosa

#### Discussion

The present study examines the prevalence, socio-demographic profiles and nature of fatal poisoning among cases who was brought to the Institutional Police Mortuary for autopsy examination. The higher prevalence among males by 1.5 times that of females is also noted in other parts around India. In a study from Karnataka, the males dominated the study with male:female ratio of 3:1. In continuation to the data from Southern parts of the country, the majority of the fatal poisoning cases in our study belonged to the age group of 2nd to 3rd decade. The possible reasons for increased poisoning in this age group may be because of problems in family, studies, marriage, life settlement and employment which may themselves act as stressors to suicidal ideation and attempts. Owing to the geographical location, though religion was not considered for analysis, but the people...
from the rural areas contributes to a significantly higher percentage (80.4%) of the study sample. Similarly, a study from North India shares that 60% of their victims were from rural background, with lesser resources available to them than their urban counterparts. This compounded by the usually large family size, high illiteracy, ignorance and superstitions, complete dependence on the fate of their crop- both in the field and in the market may be responsible for the trend observed in our study.

Various national and international studies have projected the rise of prevalence of suicidal poisoning cases. During the study period, 184 fatal poisoning cases were collected, with more than 90% of the deaths attributable to suicides. This endorses the view that the demands put forth by the materialistic modern society is the main factor for deaths due to poisoning. Studies across the country have revealed a rapidly increasing trend of Organophosphorus poisoning. Similarly, Organophosphorus compound was the single most culprit agent in about 23% of cases in our study, followed by acids in 21%. India is a country, where agriculture is the prime profession for majority of people in rural areas and pest control is one of the most common problems faced by the farmers in agriculture. In order to eradicate the weeds and pests, farmers procure and keep pesticides at their houses. Because of easy availability of the pesticides, people tend to use them for intentional poisoning.

Though odour of the gastric contents being a subjective data was not considered for analysis, stomach was affected in all the poisoning cases. This may be due to the fact that stomach being a reservoir, the contact period of poison with stomach is more. In the present study, congestion was noted in more than 45% of cases which is in accordance to other studies, conducted elsewhere around the country. However, there are numerous factors that contribute to the stomach mucosal finding in poisoning cases. These are the dosage, biological factors, route of administration, treatment interventions and the post-mortem interval. With all these factors affecting, it is difficult to predict the gross mucosal findings attributable to specific poisons. Generally, the findings are seen in combination, and are not consistent with a type of poison. However, if the poison suspected is mentioned in the requisition to the Forensic Science Laboratory, it shall help in early analysis and also avoids blind testing and wastage of chemicals.

**Conclusion**

The present study is an observational, descriptive, cross-sectional study. Other than reporting an annual prevalence of 8.03% of fatal poisoning cases, it also reflects that the individuals between 2nd to 3rd decade as the most vulnerable, with a male preponderance in almost all the age groups. People from the rural regions were mostly the victims of fatal poisoning, with suicidal poisoning being shared as the most common manner of death. Organophosphorus compounds and acids were the mainly involved in almost 50% of the cases, with mucosal congestion and submucosal hemorrhage of stomach as the most reported findings. However, the authors feel that a prospective study is the need of the hour, which could accurately identify the cases and deaths, which could formulate stringent measures so as to decrease the morbidity and mortality due to poisoning.

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**Conflict of Interest:** No conflict of interest is associated with this work.

**Ethical Clearance:** Taken

**Source of Funding:** None declared

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A Study to Assess the Knowledge and Practice Regarding Prevention of Deep Vein Thrombosis among Bedridden Patients in a Selected Hospital, Mangalore

Ann Sangeetha James¹, Fiona George¹, GraceViolet¹, Jomol Sebastian¹, Prashma Bharath²

¹IV B.Sc. Nursing Students, ²Lecturer, Department of Medical Surgical Nursing, Yenepoya Nursing College, Yenepoya (Deemed to be University) Mangaluru, Karnataka, India

Abstract

Introduction: The vascular system is a vast network of vessels through which blood circulates in the body. Blood leaving the ventricles is distributed through arteries and arterioles in progressively smaller branches to the capillaries. Deep vein thrombosis is a medical condition that occurs when a blood clot forms in a deep vein. A world wide survey conducted by CDC shows that 900,000 people could be affected by DVT (1 to 2 per 1,000) each year in the United States. Among people who have had a DVT, one-half will have long-term complications. DVT is a serious condition that can be life-threatening. However, it’s largely preventable and treatable. Aim: To assess the knowledge and practice regarding prevention of deep vein thrombosis among bedridden patients. Materials and Methods: A cross-sectional study was carried out among the bedridden patients of Yenepoya Medical College Hospital, Mangalore. The content validity of the tool was established in consultation with 7 experts. The reliability of the tools were found to be $r(6) = 0.8$ and 0.7 which was statistically significant. The tools were found to be reliable. Non probability convenience sampling was used to select the subjects for the study. Pilot study was conducted to find out the feasibility of the study. Data collected from the 95 subjects were analyzed by descriptive and inferential statistics using SPSS (Version 23). Results: The findings of the study demonstrated that among 95 bedridden patients, that maximum percentage (61%) were belonged to the age group of 47 years and above. Majority (72%) were males. (41%) of were completed primary education. Majority (62%) of the respondents were physically active workers. 58% of subjects had inadequate knowledge and 42% of subjects had moderate knowledge and none of the subjects had adequate knowledge on prevention of deep vein thrombosis and 22% of subjects had low practice, whereas majority 66% of respondents had moderate practice level and 12% of subjects had high practice level. Mean knowledge score was 11.14 and mean practice score was 9.85. There existed a positive relationship between knowledge and practice (correlation coefficient =0.662*) at 0.05 level of significance. The study also indicated that chi-square value of demographic variables have no significant association with knowledge scores and practice scores of bedridden patients.

Keywords: Knowledge, Practice, Deep Vein thrombosis.

Introduction

Venous thrombosis, including deep vein thrombosis and pulmonary embolism, occurs at an annual incidence of about 1 per 1000 adults. Rates increase sharply after around age 45 years, and are slightly higher in men than women in older age ¹. Death from DVT-associated massive pulmonary embolism (PE) causes as many as 300,000 deaths annually in the United States ². VTE is a major healthcare problem worldwide. In 2007, over 500,000 deaths in the EU were associated with an estimated 1.1 million venous thromboembolic events approximately one-third of these events manifested as
PE. In the US, DVT and PE together affect an estimated 350,000–600,000 people each year, leading to an estimated 100,000 –300,000 deaths ³.

According to a study done on 60,000 patients in more than 32 countries, almost one out of every two hospitalized patients in medical and surgical wards worldwide and in India was at risk of developing DVT. The study revealed that although the risk of DVT was very high, only 17 per cent of these patients in India received any prophylaxis ⁴. DVT is a serious condition that can be life-threatening. However, it’s largely preventable and treatable ⁵. DVT is preventable and treatable if discovered early ⁶.

In the 21st century, venous thromboembolism still accounts for 10% of deaths in hospital patients. Venous ulcers develop in at least 300 per 100,000 population and the proportion due to DVT is approximately 25%. ⁴, ⁵ It has been estimated that the management of venous ulcers in the UK costs £100-300 million every year, nursing time accounting for most of this cost ⁷.

Global public awareness is substantially lower for pulmonary embolism (54%) and deep-vein thrombosis (44%) than heart attack (88%) and stroke (85%). Over time, the incidence and MRs of these conditions have improved in developed countries, but are increasing in developing countries. Public health efforts to measure disease burden and increase awareness of symptoms and risk factors need to improve in developing countries ⁸.

**Material and Methods**

A descriptive cross-sectional study was conducted from 19 September 2019 to 29 September 2019 at Yenepoya Medical College Hospital, Mangalore after getting ethical permission (Ref.no: YEC-1/061/2019). By using the Non-probability convenience sampling technique, 95 bedridden patients of Yenepoya Medical College Hospital were selected. The patients were informed and explained the objective of the study. The written informed consent duly signed individually by them was obtained. The inclusion criteria were: Patients Confined to bed for duration of 14 days and more and patients in medical ward, surgical ward, orthopedic ward and intensive care units. Patients who were unconscious and who were seriously ill and sedated were excluded from the study. Demographic variables were collected in terms of Age, gender, education, marital status, monthly income, place of residence, occupation, and duration of hospitalization.

A structured knowledge questionnaire has 32 multiple choice questions and these were classified in different areas, such as i) Information regarding deep vein thrombosis, ii) General information about deep vein thrombosis iii) Causes and symptoms for deep vein thrombosis iv) Complications of deep vein thrombosis v) Prevention of deep vein thrombosis. A self reported checklist consisting of 20 items were prepared to assess the practice. The both tools were prepared based on the extensive review of the literature. Each correct answer carries one mark and the total score was 32 and 20 respectively. The prepared tool was validated by the seven experts, out of this 6 were from the nursing department, one was from the General medicine department and another from the General surgery department. The Reliability analysis was computed by Guttman split-half coefficient and Kuder-Richardson Formula 20(KR-20). The reliability obtained was 0.8 and 0.7 respectively, hence the tools were found reliable, valid and feasible.

**Statistical Analysis**

The collected data were coded, tabulated and analyzed by using descriptive and inferential statistics. Association of knowledge and practice with demographic variables was done by Chi-square test and Karl Pearson’s correlation co-efficient was calculated to find the correlation between knowledge and practice scores.

**Results**

**Distribution of subjects according to their demographic characteristics.**

The study shows that majority (61%) of the subjects belonged to the age group of 47 and years above.
Majority (72%) were males. (41%) of subjects were completed primary education. Majority (62%) of the respondents were physically active workers, (77%) were married, majority (39%) had income between 1001-3000, 65% of subjects belonged to rural area, (72%) were admitted between 14 days -30 days, Majority (76%) of the respondents got information from verbal report.

Table 1: Classification of respondents knowledge score on knowledge level on Prevention of deep vein thrombosis

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Knowledge scores</th>
<th>Knowledge level</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-11</td>
<td>Poor knowledge</td>
<td>55</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>12-22</td>
<td>Average knowledge</td>
<td>40</td>
<td>42</td>
</tr>
<tr>
<td>3</td>
<td>23-32</td>
<td>Good knowledge</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Depicts that 58% of subjects had poor knowledge and 42% of subjects had average knowledge and none of the subjects had good knowledge on prevention of deep vein thrombosis.

Table 2: Classification of respondents practice score on practice level on prevention of deep vein thrombosis

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Practice scores</th>
<th>Level of practice</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-6</td>
<td>Low practice</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>7-13</td>
<td>Moderate practice</td>
<td>63</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>14-20</td>
<td>High practice</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 2. Depicts that (22%) of subjects had low practice, whereas majority (66%) of respondents had moderate practice level and (12%) of subjects had high practice level.

Table 3: Correlation between the knowledge and practice scores on prevention of deep vein thrombosis among bedridden patients

<table>
<thead>
<tr>
<th></th>
<th>Max. scores</th>
<th>Response</th>
<th>Correlation significant (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Mean %</td>
</tr>
<tr>
<td>Knowledge</td>
<td>32</td>
<td>11.14</td>
<td>12</td>
</tr>
<tr>
<td>Practice</td>
<td>20</td>
<td>9.85</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 3. Depicts that mean knowledge score is 11.14 and mean practice score is 9.85. There exists a positive relationship between knowledge and practice (correlation coefficient $r = 0.662^*$) at 0.05 level of significance.

Association between level of knowledge and selected demographic variables

The study reveals that the calculated chi-square value with regard to the age ($\chi^2= 29.931$ $p>0.05$), gender ($\chi^2=13.698$), educational status ($\chi^2=57.691$), occupation ($\chi^2=30.631$), marital status($\chi^2=18.992$), monthly income ($\chi^2=42.108$), place of residence($\chi^2=13.524$) duration of hospitalization ($\chi^2 =32.965$) and source of information ($\chi^2=26.448$) $p> 0.05$ were less than table values at 0.05 level of significance.

Association between practice scores and selected demographic variables

The study reveals that the calculated chi-square value with regard the age ($\chi^2=49.131$) ,gender ($\chi^2=18.762$), educational status ($\chi^2=78.707$) ,occupation($\chi^2=35.080$), marital status($\chi^2=23.821$), monthly income ($\chi^2=60.272$), place of residence($\chi^2=25.313$), duration of hospitalization ($\chi^2=43.726$) and source of information($\chi^2=36.613$) $p>0.05$ were less than table values at 0.05 level of significance.

Discussion

Alberto Okuhara et al., (2014)- A study was conducted in Brazil to determine the incidence of deep vein thrombosis and prophylaxis quality in hospitalized patients undergoing vascular and orthopedic surgical procedures. The total sample comprised 335 patients. Regarding the distribution of sex, 98 (33.3%) were women and 198 (66.6%) men. The mean age was 57.7 years.9

A study conducted to assess knowledge of venous thromboembolism among hospitalized patients. Among the study samples (83%) were aware that they were receiving injections to prevent blood clots and (81.2%) reported hearing of DVT, of the participants who had heard of DVT knew immobility was a risk factor but had limited knowledge of symptoms and prevention modalities.10

A study was conducted to assess the knowledge and practice of immobilized orthopedic patients and their caregivers regarding prevention of complication of immobility. The sample consisted of 40 patients and their caregivers and study results showed that the highest mean percentage practice score of patients was in the area of constipation (61.75%) and least mean percentage of practice score of patients was in the area of pressure sore (35.5%).11

The current study findings were supported by a study conducted to assess correlation between the knowledge scores and practice scores on prevention of deep vein thrombosis among bedridden patients. The study showed that correlation between the post test level of knowledge with skill regarding prevention of DVT in the experimental group was calculated by using Karl Pearson’s correlation coefficient and it was found that the ‘r’ value of $r =0.15$,was not statistically significant .10

Almodaimegh H et al., (2017) - A cross sectional study was conducted in Saudi Arabia to assess the awareness of venous thromboembolism and thromboprophylaxis among hospitalized patients. The percentage of respondents reporting awareness of DVT or PE was significantly higher among those with a personal or family history of VTE: 68% versus 32%, $p = 0.001$, and 57% versus 35%, $p = 0.046$, respectively. Awareness of DVT was not associated with any of the other factors such as age, gender and level of education.12

Jemini K - A study was conducted to assess the knowledge and practice of immobilized orthopedic patients and their caregivers regarding prevention of complication of immobility. The sample consisted of 40 patients and their caregivers and study results showed that association between practice score in relation to age ($r= 0.13$), gender ( $p=0.45$), level of education ($r=.00$), marital status ($p=0.72$) was not significant.11
Conclusion

The findings of the study showed that majority of the bedridden patients have poor knowledge and low practice regarding prevention of deep vein thrombosis. This study will help the bedridden patients to understand about the seriousness of this condition thereby they have to learn and practice about how to tackle the particular condition in safer way. The present Study was conducted on a Small group within a specific areas and only confined to bedridden patients of selected hospital. A similar study can be replicated on a large sample and in different settings.

Financial support and sponsorship: NIL

Conflicts of Interest: None

Acknowledgment: The authors are thankful to all the patients who took part in the study. Authors are grateful to editorial board members and a team of reviewers of medico legal updates who have helped to bring quality to this manuscript

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A Descriptive Study to Assess the Knowledge and Practice Regarding Prevention of Osteoporosis among Group D Female Workers of Selected Tertiary Care Hospitals in Mangaluru

Anumol Shaju¹, Beeda K Jose¹, Geeta Fernandes¹, Jeevan¹, Josna Johny¹, Indumathi²
¹Yenepoya Nursing College, 4th BS.c(N) Student, Deralakatte, Mangaluru, Karnataka, India, ²Lecturer, Yenepoya Nursing College, Yenepoya (Deemed to be University), Department of Community Health Nursing, Deralakatte, Mangaluru, Karnataka, India

Abstract

Background: Osteoporosis knowledge is one of several factors that are associated with osteoporosis preventive behaviour. Moreover, the educational level of individual has the potential to change the perception of health and illness to a much better level. The objective of the study was to assess the level of knowledge regarding prevention of osteoporosis among Group D female workers, to determine the level of practice regarding prevention of osteoporosis among Group D female workers, to find correlation between knowledge and practice on prevention of osteoporosis among group D female workers, to find association of knowledge and practice with selected demographic variables. Methodology: A descriptive study design was adopted for this study. The samples were drawn through Purposive sampling technique and the sample comprised of 87 female group D workers. The tool used for this study was demographic proforma, OKAT questionnaire, and self reported practice checklist. Conclusion: The mean percentage of knowledge score was 37.05% and the mean percentage of practice score was 37.18%.

Key words: osteoporosis, OKAT, Group D female workers

Introduction

Osteoporosis knowledge is one of several factors that are associated with osteoporosis preventive behaviour. Moreover, the educational level of individual has the potential to change the perception of health and illness to a much better level. Thus, highly educated people usually seek knowledge and have an opportunity to learn about health preventive behaviour more than those with lower education¹. Bone density peaks when a person is in their late 20s, and it starts to weaken at around 35 years of age, as a person grows older, bone breaks down faster than it rebuilds. It occurs more in women after menopause because of the sudden decrease in estrogens².

Worldwide, osteoporosis cause more than 8.9 million fractures annually, resulting in an osteoporotic fracture every 3 seconds. It is estimated to affect some 200 million women globally – approximately one – tenth of the aged 60, one fifth aged 70, two fifth aged 80, two third aged 90, also one in every three women over the age 50 years will experience osteoporotic fracture, as in one in every 5 men of the same age group.³ It is projected that more than about 50 % of all the osteoporotic hip fractures will occur in Asia by the year 2050.⁴

Osteoporosis develops in older adult when the normal process of bone formation and resorption become impaired. Treatment program should therefore

Corresponding Author:
Mrs. Indumathi Vasanth
Lecturer, Department of Medical Surgical Nursing, Yenepoya Nursing College, Yenepoya (Deemed to be) University, Deralakatte, Mangaluru
Phone No: 9964140748
Email ID: reachinduvasanth@gmail.com
A focus on strategies that reduce falls, optimum treatment and prevention of osteoporosis require modification of risk factor particularly smoking, physical activity, and diet in addition to pharmacology interventions.

**Material and Methods**

A descriptive correlational research design was adopted for this study. The respondents in the study were Group D female workers in selected Tertiary care hospitals in Mangaluru. The 87 respondents were selected using purposive sampling technique.

**Sampling Criteria**

**Inclusion criteria:**
- Women’s between 30-60 years of age
- Able to communicate in Kannada or English.
- Person available at the time of data collection

**Exclusion criteria:**
- Women’s above 60 and below 30 years of age
- Women’s taking calcium supplementation
- Women’s those who are suffering from mental illness

Sample is selected through purposive sampling method. The tool used for the study consists of demographic variables like Age, educational status, marital status, type of family, habits, regular exercise, previous family history, history of earlier menopause, previous source of information, Modified OKAT and self reported practice checklist was developed by the investigator and used to collect the data. The reliability of knowledge questionnaire was calculated by split half method, and practice scale by cronbach’s alpha. The reliability co-efficient was \( r=0.91 \) for OKAT which indicated that the tool is reliable. The data collection period extended from 14th September to 22nd September 2020. The investigator explained the purpose of the study and requested the participants’ full cooperation and assured the confidentiality of the data. Written consent was taken from subjects. Participants cooperated well during the time of data collection process. Formal written permission was obtained from the authorities to conduct the study. Data collected from the sample were analyzed using descriptive and inferential statistics using SPSS version 23.

**Results**

**Section I: Sample Characteristics**

The findings of the study demonstrated that among 87 group D female workers who are working in Yenepoya Medical college Hospital surveyed, the highest percentage of the subject (49.43%) belonged to an age group of 40-50 years. Maximum number of subject had Higher secondary education (85.06%), majority of the subjects (90.80%) were married, maximum number of the subjects (87.36%) were belongs to nuclear family. Most of the subjects (82.76%) had caffeine intake. Almost (78.16%) of the subjects had no exercise. Majority of the subjects (88.51%) had no previous family history. The maximum number of subjects (87.36%) had no history of earlier menopause. Majority of the subjects (88.51%) were not received any information.

**Section II: Distribution of subjects according to their knowledge score**

**Table 1: Frequency and percentage distribution of subjects according to the level of knowledge**

<table>
<thead>
<tr>
<th>Level of knowledge</th>
<th>Frequency (f)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent knowledge</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Good knowledge</td>
<td>61</td>
<td>70.1</td>
</tr>
<tr>
<td>Poor knowledge</td>
<td>25</td>
<td>28.7</td>
</tr>
</tbody>
</table>

The data presented in Table 1 shows that, majority (70.1%) of the subjects had good knowledge, 28.7% of the subjects had poor knowledge. This is shown in figure I.
Section III: Distribution of subject according to their practice score

Table 2: Frequency and percentage distribution of subjects according to level of practice

<table>
<thead>
<tr>
<th>Levels of practice</th>
<th>Frequency (f)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor practice</td>
<td>18</td>
<td>20.7%</td>
</tr>
<tr>
<td>Average practice</td>
<td>63</td>
<td>72.4%</td>
</tr>
<tr>
<td>Good practice</td>
<td>6</td>
<td>6.9%</td>
</tr>
</tbody>
</table>

Maximum score 11  n=87

The data presented in Table 2 shows that, majority of the subjects (72.4%) had average practice, 20.7% of the subjects had poor practice, and 6.9% of the subjects had good practice.

Correlation between knowledge and practice

Finding of the study revealed that there is a positive correlation between knowledge and practice. As the knowledge increase practice also increase. The result shows that r =0.277.

Association between knowledge score and selected demographic variables

The findings of the study revealed that there was a significant association between knowledge score and the following demographic variables such as Type of family, Previous Information but rest of all there is no significant association between knowledge score and demographical variables.

Association between practice score and selected demographic variables

The findings of the study revealed that there is a significant association between practice score and demographic variables such as type of family, earlier menopause, previous information whereas rest of all there is no significant association between the practice score and demographic variables.

Discussion

Distribution of subjects according to their knowledge score

Majority of the subjects (70.1%) having good knowledge on prevention of osteoporosis, 1.2% of the subjects having excellent knowledge and 28.7% of the subjects having poor knowledge. The mean percentage of overall level of knowledge was 37.05%.

The following study supports the findings of current study

A cross sectional study was conducted to assess Knowledge on Osteoporosis prevention among Bahraini women. Sample consisted 400 Bahraini women using convenience sampling technique in a community setting. The data were collected using Osteoporosis Knowledge Assessment Tool (OKT). The study revealed that Most of the women (73%) had moderately adequate knowledge on osteoporosis.5

Association between practice score and selected demographic variables

The findings of the study revealed that the majority (72.4%) of the subjects had average practice, 20.7% of the subjects had poor practice and 6.9% of the subjects had good practice. The mean percentage of overall level of practice was 37.18%.

The following study supports the findings of current study:

A descriptive study was conducted to assess knowledge, beliefs and practices regarding osteoporosis among female medical school entrants in Sri Lanka. A self administered questionnaire was used to assess knowledge, beliefs and practices on osteoporosis, including a food frequency chart to assess the calcium intake. Majority of the participants (51.6%, n=96) had an average score (40–60) on the knowledge test, while 40.8% (n=76) had a poor score (<40). However, in depth knowledge on risk factors, and protective factors was
lacking. Perceived susceptibility for osteoporosis was low with only 13.9%. Only 7(3.8%) participants were currently engaged in specific behaviours to improve bone health whilst 20(10.8%) had thought of routinely engaging in such behaviour.

**Conclusion**

The following conclusions were drawn on the basis of the findings of the study:

- v majority of the subjects had good knowledge (70.1%), 28.7% of the subjects had poor knowledge.

- v Most of the subjects (72.4%), had average practice 20.7% of the subjects had poor practice, and 6.9% of the subjects had good practice.

**Limitations:**

- v The study is limited to group D female workers in Yenepoya Medical College Hospital, Mangaluru.

- v Study is limited to Knowledge Questionnaire and Practice Checklist

**Disclosure of potential conflicts of interest:** The authors declare that they have no conflict of interest

**Ethics approval:** Approval was granted by the Yenepoya Ethics Committee-2, Yenepoya (deemed to be university) of Mangaluru.

**Consent to participate:** Written informed consent was obtained from the study participants.

**Source of Funding:** Self-funding

**References**


Assessment and Comparison of Total Salivary Protein and Salivary Flow Rate among Type I, Type II Diabetics and Healthy Controls

B.Niveditha¹, M. Kavitha², Mutum Sangeeta Devi¹, J. Manju³, C.K. Vishnu Priya⁴, D.K.S.Lakshminrusimhan⁵

¹Senior Lecturer, Madha Dental College & Hospital Chennai, ²Reader, Madha Dental College & Hospital, Chennai, ³Senior Lecturer, Thai Moogambigai Dental College & Hospital, Dr MGR Educational and Research Institute, Chennai, ⁴Senior Lecturer, Ragas Dental College & Hospital, Chennai, ⁵Senior Lecturer, R.V.S. Dental College & Hospital, Coimbatore

Abstract

Introduction: Diabetes mellitus characterized by either absolute insulin deficiency (Type I) or target tissue resistance (Type II) is associated with oral complications like xerostomia, gingivitis, periodontitis, odontogenic abcesses and soft tissue lesions of the oral mucosa. Saliva is a unique biological fluid and is also a characteristic biomarkers for different diseases. Several classes of drugs are found to be associated with dry mouth or salivary gland dysfunction or hypofunction, which in turn influences concentration of salivary proteins. This leads to changes of oral health status among individuals using these drugs.

Aim of the Study: To evaluate and compare the total salivary protein and salivary flow rate among Type I Diabetics, Type II Diabetics and healthy controls.

Materials and Methods: A total of 60 individuals have participated in the study which include 20 with Type I diabetes, 20 with Type II diabetes and 20 healthy controls. The study was prospective in nature. Patients were asked not to eat or drink 2 hours before the time of saliva collection. The samples were collected in the same time of the day to avoid circadian variations. Unstimulated saliva was collected using spit technique. Patient was instructed to spit the saliva in graduated containers for a period of two minutes. The flow rates were determined visually from graduated salivary containers as ml/min. After measuring the saliva volume the saliva sample was stored in deep freeze until protein estimation. The total salivary protein in each salivary sample was determined using BioRad Protein Assay Dye Concentrate method using BSA standard.

Results: On comparing the total salivary protein among Type I, Type II Diabetics and healthy controls, a significant difference in total salivary protein was found among Type I Diabetics and healthy controls and also among Type I and Type II Diabetics and there was an insignificant difference in Type II Diabetics and controls. There was an insignificant difference in total salivary flow rate among Type I and Type II Diabetics and healthy controls. Conclusion: A significant difference in total salivary protein level among the diabetic and non diabetics emphasized that protein utilization by other biochemical metabolic pathways has an overall systemic response to glucose intolerance. With regards to salivary flow rate, the inconsistent results obtained may be due to the duration of diabetes, age range of patients and metabolic control of patients, class of drugs taken by the patient.

Keywords: Diabetes mellitus, Salivary flow rate, Protein, Glucose

Introduction

Diabetes mellitus is a syndrome characterized by abnormalities in carbohydrate, protein and fat metabolism that result either from a profound or absolute deficiency...
of insulin (Type I), or from target tissue resistance to its cellular metabolic effects (Type II). It is found to have a multitude of oral manifestations in the oral cavity.

Saliva is a unique biological fluid, which when compared to other diagnostic media, such as tissue samples, serum, CSF, saliva sample is easily collectable, cost effective, non invasive diagnostic tool for research and is often preferred as an alternative diagnostic approach.

Alterations in salivary parameters cause disorders of the hard and soft tissues of the mouth leading to gingival lesions, increased prevalence of caries and finally bad oral health. Dry mouth is a complaint among diabetic patients and may be associated with several classes of drugs, which in turn influence concentration of salivary proteins. This leads to changes of oral health status among individuals using these drugs.

Since saliva is a non diagnostic tool the present study aims to detect and compare the difference in salivary flow rate and total salivary protein among Type I and Type II diabetics and healthy controls, which may be useful in describing and further understanding of oral findings in this condition.

**Methodology**

The present study is a prospective study which includes a total sample of 60 patients which includes 20 in the Type I diabetic group, 20 in the Type II diabetic group and 20 in the healthy control groups. Patients with symptoms of diabetes including polyphagia, polydipsia and polyuria, with a non fasting glucose level of greater than 200 mg/dl and HbA1c level greater than 6.5% were included in the study. The control group consisted of gender matched non diabetic subjects without symptoms of diabetes. Patients with other systemic illness, smoking/alcohol habits, those treated on radiotherapy and pregnancy were excluded from the study.

Patients were asked not to eat or drink 2 hours before the time of saliva collection. The samples were collected in the same time of the day to avoid circadian variations. Unstimulated saliva was collected using spit technique. Patients were instructed to spit the saliva in graduated containers for a period of two minutes. The flow rates were determined visually from graduated salivary containers as ml/min. After measuring the saliva volume the saliva sample was stored in deep freeze until protein estimation. The total salivary protein in each sample was determined using BioRad Protein Assay Dye Concentrate method using BSA standard. Bio-Rad Protein Assay Dye Reagent Concentrate is a colorimetric assay for protein concentration similar to the Lowry assay, but with the following improvements: Within 15 minutes, the reaction reaches its maximum colour change and the change in colour is not more than 5% in one hour. A standard curve is prepared each time the assay is performed.

BioRad assay is prepared by diluting the assay dye in the ratio of 1:5, where 2 ml of Bio Rad dye is mixed with 10 ml of distilled water and the mix is stirred well. The volume of the bovine serum albumin sample used as standard is 5µl and the sample is also 5 µl. 100 µl of diluted dye is then treated with 5µl of the standard and 5µl of the sample and then ultraviolet spectrometer with a wavelength of 595nm is then used to determine the total protein in each sample of saliva.

**Statistical Analysis**

Turkey HSD test was used to correlate the difference in salivary proteins and salivary flow rate among diabetic and healthy controls. The mean difference is taken as significant at the .05 level. The SPSS 13 software analysis is used.

**Results**

A comparison of total salivary protein among Type I, Type II Diabetics and healthy controls were performed in male and female subjects. The total salivary flow rate was also compared among Type I, Type II Diabetics and healthy controls with the following results:

On comparing the total salivary protein among the Type I, Type II diabetics and healthy controls in male subjects, it was found that the mean difference between the total salivary protein in controls and type
I diabetics was -1.96046 with a P value of 0.015 which was statistically significant. The mean difference between the total salivary protein in controls and type II diabetics was -1.3536 with a P value of 0.977 which was statistically insignificant. The mean difference between the total salivary protein in type I and type II diabetics was 1.82510 with a P value of 0.025 which was statistically significant.

The results of our study are in accordance to the study conducted by Laisi T.J et al (2012) and Syed Shabaz et al (2017).

In the study conducted by Laisi T.J et al (2012) in a sample of total of 40 subjects of 20 type II diabetics...
and 20 healthy controls, it was found that the P value was greater than 0.005 which was statistically insignificant. In the study conducted by Syed shahbaz et al (2017)\(^8\), in a sample of total of 100 patients of 50 type 1 diabetics and 50 healthy controls, it was found that the P value was <0.01 which was statistically significant.

The results of our study are in contradiction to the results of the study done by Prathibha et al (2013)\(^9\), Indira M et al (2015)\(^10\), Juan Aitken, Saveendra et al (2015)\(^11\).

In the study conducted by Prathibha et al (2013)\(^9\), in a total sample of 60 subjects, 30 in the diabetic group and 30 as healthy controls, it was found that the P value was 0.000 which was statistically highly significant. In the study conducted by Indira et al (2015)\(^10\), in a total sample of 40 subjects, 20 in the type 2 diabetic group and 20 in the healthy controls, it was found that the P value was <0.001 which was statistically significant. In the study carried out by Juan Aitken Saveendra et al (2015)\(^11\), in a total sample of 74 patients, it was found that the P value was 0.000 which was statistically highly significant.

The contradiction could be explained by the fact that there is a variation in the salivary fluid secretion among the diabetic patients and varying oral health conditions like periodontitis influence the leakage of proteins from the gingival crevicular fluid.

On comparing the salivary flow rate of controls with type I and type II diabetics, it was found that the mean difference in salivary flow rate between controls and type I diabetics was 0.2245 with a P value of 0.089 which was statistically insignificant. The mean difference in salivary flow rate between controls and type II diabetics was 0.0790 with a P value of 0.731 which was statistically insignificant.

The results of our study are in accordance with the results of the study done by Arati S Panchbai (2010)\(^6\) and Juan Aiket Saveendra (2015)\(^11\).

In the study done by Arati S Panchbai (2010)\(^6\), in a total of 120 subjects, 40 in the uncontrolled diabetic, 40 in the controlled diabetic group and 40 in the healthy controls group, it was found that the P value was 0.56 which was not statistically significant. In a study done by Juan Aiket Saveendra et al (2015)\(^11\), in a sample of 72 patients with type 2 diabetes, it was found that the P value was 0.518 which was not statistically significant.

The results of our study are in contradiction to the results of the study done by Laisi T J et al (2012)\(^7\) and Prathibha et al (2013)\(^9\).

In the study performed by Laisi et al (2012)\(^7\) to compare the salivary flow rate among diabetic and non-diabetic subjects in a total of 40 subjects, the P value was 0.004 which was statistically significant. In a study performed by Prathibha et al (2013)\(^9\) to compare the salivary flow rate among the diabetic and non-diabetic subjects in a total of 60 subjects, the P value was 0.002 which was statistically significant.

The reason for such contradiction may be due to the fact that oral dryness occurring in diabetic patients could be multifactorial, either due to fatty infiltration of cells into the salivary glands, or physical alteration of mucosal cells subsequent to dehydration due to polyuria or microvascular disease, local inflammation and irritation in the oral cavity, infections, metabolic disturbances, and neuropathy affecting the salivary glands, and may be due to drug therapy for diabetes or concomitant drugs.
Graphs: Representation of the result in graphs:

Graph 1: COMPARISON OF TOTAL SALIVARY PROTEIN IN TYPE I, TYPE II DIABETICS AND HEALTHY CONTROLS IN MALE SUBJECTS AND FEMALE SUBJECTS:

Graph 2: COMPARISON OF TOTAL SALIVARY FLOW RATE IN TYPE I, TYPE II DIABETICS AND HEALTHY CONTROLS:
COMPARISON OF TOTAL SALIVARY PROTEIN AMONG TYPE I, TYPE II DIABETICS AND HEALTHY CONTROLS:

Multiple Comparison of total salivary proteins:

Dependent Variable: Protein (µg/ml)

Tukey HSD

<table>
<thead>
<tr>
<th>Gender</th>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>P value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
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* The mean difference is significant at the .05 level.

COMPARISON OF TOTAL SALIVARY FLOW RATE AMONG TYPE I, TYPE II DIABETICS AND HEALTHY CONROLS:

Multiple comparison of total salivary flow rate:

Dependent Variable: Flow rate (ml/min)

Tukey HSD

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Conclusion

A significant difference in total salivary protein level among the diabetic and non diabetics emphasized that protein utilization by other biochemical metabolic pathways has an overall systemic response to glucose intolerance. Insulin is known to have the potential to alter protein metabolism. Further many studies have confirmed that increase in whole salivary proteins was not associated with dental caries, except for the 17kDa protein which might be a risk marker for dental caries. Nevertheless, the overall results obtained from various studies regarding salivary proteins in diabetics remains controversial.

Various studies conducted to assess the salivary flow rate among diabetic patient have found to give inconsistent results, which may be attributed to the duration of diabetes, age range of patients and metabolic control of patients, class of drugs taken by the patient.

The present study has certain limitations like a small sample size, and hence the results are entirely conclusive, the study has provided a platform for further research.

Ethical Approval:

All authors at this moment declare that all the experiments have been approved by the appropriate ethics committee ( Institutional Review Board, Ragas Dental College and Hospital, Chennai) and have therefore been performed by ethical standards.

Financial Support: Nil

Conflict of Interest: Nil

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Antifogging Measures for Protective Eyewear

Bharti Sachdeva¹, Parul Saini¹

¹Assistant Professor, Faculty of Nursing, SGT University, Gurugram

Abstract

Fogging of protective eyewear is commonly encountered by healthcare workers and there exists a number of ways to combat this. This article presents a comparison various anti-fogging measures of protective eyewear in terms of their mechanism of action, advantages and disadvantages.

Keywords: Fogging, Misting, Condensation, Anti-fogging, Protective eyewear

Introduction

Coronavirus disease (COVID-19) is an infectious disease caused by SARS-CoV-2 Virus. It can cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). COVID-19 outbreak has severely abolished the world’s health, economics and social progress. In India, over 10.8 million confirmed COVID-19 cases and more than 1, 55,000 deaths have been reported as of 11th February, 2021 [1]. COVID-19 predominantly spreads by contact, droplet or airborne transmission necessitating the use personal protective equipment (PPE) to prevent cross infection. Before we understand the use of Protective eyewear and the challenges associated with it, let’s first explore the mode of SARS-CoV-2 viral transmission.

Mode of viral transmission

Droplet transmission generally occurs via larger respiratory particles which tend to settle down and travel not more than one meter. Once the virus settles on a surface, it remains there for a no. of hours or possibly days and becomes the source of potential infection to the ones who come in contact with these. Although the SARS-CoV-2 is not considered to be airborne but the risk is associated with production of smaller respiratory particles during certain procedures such as endo-tracheal intubation and cardiac compressions. These smaller particles remain suspended in air for longer and can travel distances more than two meters. Thus the use of PPEs needs to be at par with the level of contact, droplet and airborne transmission [1].

Therefore, the challenge arises where both mask and eyewear are required and that is fogged up eyewear [2]. It happens on escaping of warm exhaled air through ridge of the mask and accumulating on the eyewear. This commonly occurring but rather risky phenomenon interested the authors to compiling the data on various antifogging measures. We started with interviewing our nursing officers at SGT Hospital, Gurugram and observed that the fogging of goggles was a great occupational health hazard. As the healthcare workers tend to remove the protective eyewear once it is mistified, putting at risk the safety of their own as well as their patients. In order to support healthcare workers overcome this problem, we decided to retrieve the existing data on antifogging measures. We performed an extensive search on PubMed using keywords ‘anti-fogging of eyewear’, ‘fogging of protective eyewear’, ‘fogging and eyewear’, ‘misting of eyewear’, ‘condensation of eyewear’, ‘anti-fogging measures’. The search displayed 12 results that were relevant to the purpose of study.
We concised the findings of our study as under:

1. Tightly sealing the mask against nasal ridge by crossing over the ear loops or mask ties, by pressing nasal rim hard, by putting on adhesive tape over the junction of mask and nasal ridge [2]. A tight seal blocks the exhaled air escaping from superior margin of the mask and thus prevents it from accumulating on the eyewear. It is the most convenient and practical option but tight seal may cause face marks and may be uneasy to wear for long. Adhesive tape may cause skin irritation, if hypoallergenic one is not used.

2. Applying soap-water solution [3,4,5,7-9]. Soap is a good surfactant, known to reduce surface tension. The reduced surface evenly spreads out water vapors and prevents fogging. It is cost-effective and suitable for low resource areas but may distort the vision, if not wiped adequately.

3. Alcohol based sanitizer [3, 4, 7]; Alcohol is believed to decrease scattering of light. It is readily available in good resource settings. The down side is that alcohol may be irritating to eyes causing burning sensation, may cause complications in some cases such as conjunctivitis, keratitis and rarely corneal scarring.

4. Anti-fogging sprays or gels [3, 4, 7]: It works on the same principle as that of soap by decreasing surface tension and spreading of water molecules to prevent scattering of light. These are commercially available surfactants exclusively meant for anti-fogging of eyeglasses, goggles or wind shields. Their use is limited in healthcare settings due to cost constraint.

5. Filtered eye mask [4]; Since it is airtight, it prevents fogging and also gives a good protection against COVID-19 infection.

6. Iodophor [3,7]; These are iodine based substances which are readily available but they take longer (approx. 10 minutes) to dry and leave yellow stain on eyewear.

7. Application of hydrogel patches on the upper surface of mask [9]; These are better than other measures for achieving a tight fit without compromising the comfort.

**Conclusion**

As the COVID-19 cases continue to rise across the world, wearing mask and protective eyewear is going to stay in practice for uncertain times. This article provides a range of antifogging measures to choose from; in terms of cost, availability, feasibility and comfort.

**Ethical Clearance:** NA

**Source of Funding:** Self

**Conflict of Interest:** Nil

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Women and Sexual and Reproductive Health Rights: Issues and Challenges due to Environmental Pollution and Covid-19 Pandemic

Bijaylaxmi Mohapatra¹, Itishri Sarangi²
¹Research Scholar, ²Associate Professor, Department of Humanities, Kalinga Institute of Industrial Technology, Deemed to be University, Bhubaneswar, Odisha

Abstract

The situation emerging out of COVID-19 pandemic is not only just a health issue that has affected people but it has entrenched structural and gender inequalities and inequities in addition to the economic shock. Women as care giver, at home and communities are at the more challenging end in terms of getting attention and care across the societies, be at the rural, tribal or urban areas. degree of vulnerability has increased due to COVID-19 in every sphere be it in domestic space, labour market or access to health care. Worse situation is the provision of sexual- and reproductive-health services where women face innumerable challenges to address them. (Linde & Gonzalez,2020). Further, rising environmental pollution has further worsened the health situation, particularly in case of sexual and reproductive health for women and girls. This paper explores how women’s health is affected by Air pollution during COVID-19 situations. The paper discusses how women’s health in general and particularly, sexual reproductive health is affected by COVID-19 and environmental pollution, particularly, Air pollution.

Keywords: COVID-19, Environment, Pollution, Women, Sexual and Reproductive Health

Introduction

The situation emerging out of COVID-19 pandemic is not only just a health issue that has affected people but “it has also profoundly impacted economy worldwide, socio-cultural compatibility, political situations, academic atmosphere, and moreover people’s living standards particularly in developing and underdeveloped countries” [1]. The COVID-19 was declared as global pandemic by the World Health Organization on March 11, 2020. The first confirmed case of COVID-19 in India was on January 30, 2020. Since then, there has been a consistent increase in the number of cases within the country. “As on 1st April, 2021, the highest number of active cases of COVID-19 were found in Maharashtra, followed by Kerala, Karnataka, Andhra Pradesh, Tamilnadu and Delhi” [2].

Before the COVID-19 situation, the world has experienced severe health crisis such as the Spanish Flu of 1918, outbreak of HIV/AIDS, MERS (Middle East Respiratory Syndrome), SARS (Severe Acute Respiratory Syndrome) and Ebola. “India has also witnessed and dealt with diseases such as the small pox, polio and plague. All of these individually have also affected the economy and loss to humanity in some way” [3,4]. However, the Covid-19 which was found for the first time in China in December 2019 and rapidly spread to almost all countries of the world and turned out to the worst biggest health crisis in the history. It has also caused a great damage to world economy. “It has had devastative effects on various section of society such as the poor, socially marginalised groups, people those who are working in the informal sectors and those employed in contact-intensive sectors.” [5] Further, rising environmental pollution has further worsened the health situation.

For girls and women, degree of vulnerability has increased as a result of COVID-19 pandemic in every
sphere be it in domestic sphere, labour market or access to health care. Worse situation is the provision of sexual- and reproductive-health services where women face innumerable challenges to address them on, particularly in case of sexual and reproductive health for women and girls. This paper explores how women’s health is affected by Air pollution during COVID-19 situations. The paper discusses impact of air pollution on reproductive health and health of women in general in different locations both in rural and urban areas.

Objectives

The study aims:

- To understand the Social, Economic and health Impact of COVID-19 and Environmental Pollution at national and international level
- To explore the issues of issues of Sexual and Reproductive health Rights of women being affected by COVID-19 Pandemic and Environmental pollution
- To suggest mechanism, need to be provided to women to address issues of sexual and reproductive health.

Research Questions:

1. What was the Social, Economic and health Impact of COVID-19 and Environmental Pollution at national and international level.

2. How are sexual and reproductive health Rights of women affected by COVID-19 Pandemic and Environmental pollution?

3. What kind of solution be provided to address the sexual and reproductive health issues of women.

Methodology

This study is based on non-empirical method. For the purpose of the research, secondary data such as books, articles, newspapers and various reports of national and international agencies such as ILO, UNFPA, UN Women and UN were referred and analyzed Social, Economic and health Impact of COVID-19 and Environmental Pollution

“COVID-19’ is also likely to set back human capital development”.[6]. The spells of unemployment which is currently in a longer mode will depress workers from the left-out labour force, that could noticeably erode skills and lead to more job losses. “In earlier crises of economy, susceptible groups confronted with complex rates of school dropout and compacted skills development, which amplified income differences.”[7] As per the estimation of International Labour organization (ILO), full or partial lockdown measures has affected almost 2.7 billion workers, constituting around 81% of the world’s total workforce.

In India, where the economy was already deteriorated before the pandemic by stress in non-bank financial corporations, has pushed as many as 75 million people into the ranks of the poor (those who earn $2 or less a day) as per the estimation by the Pew Research Centre. Similarly, the numbers of India’s middle class (Those who earns income of $10.01–$20 per day) are projected to have shrunk by 32 million to about 66 million. The level of poverty may increase as there are other risks involved such as financial distress due to an abrupt shrinking of financing conditions or corporate bankruptcies, extreme weather and climate change, and further a possibility of worsening of policy- and security-related uncertainty.

The impacts are not just economic. The loss of earnings and livelihoods has higher chances of hampering women’s access to sexual and reproductive health as they are not able to spend the money on their health aspects. There were also rising cases of Violence against women as widespread stay-at home orders compel women to stay for a long time at one place with their abusers and often with disastrous consequences. Further, the burden of unpaid care and domestic work also exacerbated the situation for women and girls during Covid outbreak. Women and girls those who are in the vicious circle of institutionalized poverty, domestic violence and other forms of discrimination.
are particularly at high risk. “Their probability of being affected by COVID-19 transmission and fatalities are higher and are most exposed to more adverse situation such as loss of job and livelihood.” [8]

Women as care giver, at home and communities are at the more challenging end in terms of getting attention and care across the societies, be at the rural, tribal or urban areas. Further, School closures during COVID situation have put additional stress and demand on women and girls. As formal and informal provision of childcare were on decline, the demand for unpaid childcare and education was falling more heavily on women. This has further constrained their ability to work, particularly during the situation when it is difficult to carry out jobs remotely.

The lack of childcare support during covid-19 has not only costs economic loss but it also caused poor physical and mental health for women. A recent study done on young people in three Indian states of Uttar Pradesh, Rajasthan and Bihar by Population Foundation of India points out that “51% female adolescents experienced an increase in workload as a result of the nationwide lockdown due to COVID-19, against 23% male adolescents. In Uttar Pradesh, 96% females experienced an increase in workload was experienced by 96 percent female and out of them 67% are below 18 years of age”.[9]

Environmental pollution particularly air pollution has been a major contributor to ill-health. As per The State of Global Air (SoGA) 2020 report, long-term exposure to outdoor and household air pollution has caused to over 16.7 lakh annual deaths across age groups in India. With Covid-19 situation, the health challenges become very acute. Although there is not enough study to substantiate the link between long-term exposure to air pollution and COVID-19 outcomes, a recent study done to find out “the link between air pollution and COVID-19 outcomes done in the united states reveals that someone who lives for decades in a county with high levels of fine particulate pollution(PM$_{2.5}$) is 8%* more likely to die from COVID-19 than someone who lives in a region that has just one unit (one microgram per cubic meter) less of such pollution.”[10] Another study done also reveals that “around 15% deaths worldwide happened due to exposure to air pollution for a long period of time. While in Europe, the proportion of COVID death happening due to exposure to air pollution for along period of time was about 19%, proportion of death in North America was 17%, and it was about 27% in East Asia.” [11]

Most fine particulate matter that cause air pollution comes from sources such as fuel combustion, like automobiles, refineries and power plants and also from some indoor sources like tobacco smoke. Breathing in such microscopic pollutants inflames and damages the lining of the lungs over time, weakening the body’s ability to fend off respiratory infections. While exposure to such fine particulate matter have caused heightened risk for lung cancer, heart attacks, strokes and even premature death for people, it has really put life at more risk during COVID-19 periods.

### Environmental Pollution and issues of Women’s Sexual and Reproductive health Rights during COVID-19

The COVID-19 pandemic has potentially disastrous direct and indirect impacts on the health of women and girls around the world. As COVID-19 demands health care and caution at every level, it is less likely that women will have adequate access to sexual and reproductive health services and rights. This will lead to sharp rises in maternal and neonatal mortality. “During such times, some sexual and reproductive health services, such as contraception and safe abortion care, are also often considered as non-essential or even illegitimate. These services are deprioritized considering that as it is not urgent.”[12] In this regard, “The WHO has strictly noted such restrictions on access to services are a violation of human rights.” [13] This has provided rights-based interim operational guidance on how States should maintain essential services in the context of the pandemic, including sexual and reproductive health services.
One important lesson from the “West Africa Ebola outbreak of 2014-2016 is that the major threat to women’s lives was not the Ebola virus itself, rather the closure of routine health services and people’s fear that going to health centers/hospitals where their chance of being infected could be higher. Death of thousands more women happened as safe delivery, neonatal, and family planning services became inaccessible due to the Ebola outbreak. Now, the same dynamics are being faced by women on a much larger scale and more affected are aspiring mothers."[14,15,16]

“A study by United Nations Population Fund (UNFPA) held in April 2020 suggests that as result of COVID-19 Pandemic, 47 million women in 114 low- and middle-income countries would not be able to access the facilities of modern contraceptives and 7 million unintended pregnancies would have occur if the lockdown or related disruptions continues for the period of six months. The study further points out that for every three months the lockdown continues, up to an additional two million women would be unable to access modern contraceptives.”[17]

Another study conducted by UNFPA and Avenir Health done in 115 low- and middle-income countries in January 2021 estimated that as many as “1.4 million unintended pregnancies may have occurred lasting an average of 3.6 months as a result of COVID-19. At the higher end of projections, it could be as high as 2.7 million and on the other hand, it could be as low as 500,000 at the lower end of projections. “[18]A detailed study done by The Foundation for Reproductive Health Services India, in May, 2020 suggests that in likely cases, the disruption caused from lockdowns could leave up to 25.63 million couples in Indian will be unable to access contraceptives. In addition, “COVID-19 is also going to result in 2.38 million unintended pregnancies and 1.45 million abortions.”[19]

With environmental pollution, particularly air pollution, the risk is higher for women, particularly pregnant women. “Air pollution has largely impacted the lives of women too particularly when it comes to pregnancy and infant mortality. Bad air quality impacts the intrauterine growth retardation (IUGR) in the first month of gestation which leads to many challenges, including premature birth or major health complication or deaths in new born babies. Mothers’ exposure to airborne pollutants during pregnancy has much adverse effects as babies may either be born prematurely or with a lower weight. If a woman during pregnancy is exposed to air pollution, there is a chance of both women and new born to be affected to respiratory disorders like Chronic Obstructive Pulmonary Disease (COPD), asthma, chronic bronchitis, cancer”.[20]

The pregnant women being in the environment affected by toxic air are at the disadvantage as there are higher chances of some amount of damage occurring to the baby being nurtured in their uterus. When the new born baby comes out after delivery and breathes for the first time, if the air quality level PM 2.5 which is close to 500-600 [AQI] – equal to about 30 cigarettes of smoke in terms of damage – the new born baby becomes a smoker from the first breath. It is going to have sever health impact on the new born baby. When tiny particles enter the foetal side of the placenta, it impacts the development of the unborn baby. This increases the high risk of infant mortality. With COVID-19 pandemic, the situation has become much worrisome for the women.

As per The State of Global Air 2020 published by Health Effects Institute (HEI) in the year 2020, 21 percent of all neonatal deaths in India has happened due to air pollution. 116,000 infants die in India in the first month alone, due to the impact of air pollution on newborns. The report further states that half of these infant deaths were caused by outdoor air pollution. “…Various south Asian nations like India, Bangladesh, Pakistan and Nepal were among the top 10 countries contributing highest PM 2.5 levels in 2019 and thus, affected the life of infants. Among these countries, the level of air pollutants in India was dangerously higher in 2019. The annual average concentration of outdoor air pollutant was measured at 83.2 micrograms per cubic meter in India which was eight times higher than the World Health Organization’s air quality guideline of 10 µg/m.
This is being recorded highest in the world, which puts higher risk for women and children.”[21]

During November, 2019, World Health Organization reported that out of the world’s 15 most polluted cities, 14 cities are in India. “During winter season every year, smoke generated through farmers’ fires along with industrial and vehicle emissions turn towns and cities located in northern India into smog-blanketed hellholes, which heavily pollutes the air. As air get devastatingly polluted, this becomes challenging both for pregnant mothers and new born kids. New born infants breathe the noxious air twice as fast as adults as they have the smaller lungs and this causes respiratory problems and even impairing brain development.”[22] With COVID-19, the pregnant women are in doubly disadvantaged position struggling with both health challenges from environmental pollution and COVID-19 pandemic.

The COVID-19 has also put disastrous impact on the couples experiencing infertility and undergoing fertility treatment to have their babies. However, due to COVID-19, fertility treatment of these couples either postponed or cancelled. “While Women experiencing infertility are already affected with a disproportionate share of the psychological burden and consistently reporting anxiety, lower self-esteem, more depression and lower life satisfaction.”[23,24] The situations arising out of COVID-19 has disastrously affected the women’s health. These fertility treatment postponement or suspension has considerable effect on mental health and quality of life of women.

In India, COVID-19 has posed challenging situation for 3 million infertile couples in India seeking fertility treatment. “As per the data analysis done in 1100 IVF centers across India from April to June, almost 90% drop was observed in number of clientele undergoing IVF cycles.”[25] It is not only due to the decision of government or health centers arising out of COVID-19 but also due to the lack of public transport and communication, travel restriction and also due to the concern that COVID-19 may affect pregnancy. Although there are rare studies in India depicting the impact of COVID-19 done on fertility treatment and overall quality of life of women, a study conducted on “92 women from United States and Canada, the aged between 20–45 years whose fertility treatments had been cancelled revealed that majority of them (52%) endorsed clinical levels of depressive symptoms.”[26] This also causes various social and emotional problems terms of adjustment between couples and in the family.

### Conclusion and Future Recommendations

It is important that Sexual and Reproductive Health services and rights be considered as a significant public health issue during pandemic and it should be a priority call for the nations to address this issue with care and attention. Women particularly aspiring mothers and their new born babies may be more likely to require specialist care and attention. This is particularly true for women who are located in urban areas where the cases of air pollution are widespread and therefore, more precautions are to be taken for them. It is also important to note that there are high chances of the increased stress and anxiety may be experienced by pregnant women and their partners, and families. Therefore, it is important that healthcare providers have to play a crucial role in to deal with pregnant women with proper attention and care and compassionate manner.

Provision of uninterrupted abortion and contraceptive counselling, devices and care services are vital to protect and safeguard the reproductive rights of women. Efforts must be made to continue the services through various measures such as telemedicine including those on telemedicine medication abortion, and extending digital access, ensuring continued coverage of birth control methods and detailed counseling and resolving drug supply chain disruptions etc.

More importantly, there is need to strengthen operational strategies and actions to protect Sexual and Reproductive Health and rights of women, young people, and marginalised populations during the epidemic. There should be concerted effort not only from the scientists and physicians, but also policy-
makers, community-based organizations, and national and international agencies, need to work tandem with each other to deal with such a human rights issues as this are integral to well-being of women and also the larger benefit of society and nation.

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**References**


Relapse Prevention in Mental Illnesses: Concepts, Issues and Strategies with Perspectives of Psychiatric Nursing- Review Article

Mudakavi I1, Rentala S.2, Irasangappa M3
1Ph.D Nursing Scholar, INC, New Delhi. Nursing Tutor, College of Nursing, All India Institute of Medical Sciences, Basni Phase II, Jodhpur, Rajasthan. India, 2 Professor & Head of the Department, Department of Psychiatric Nursing, Dharawad Institute of Mental Health & Neurosciences (DIMHANS), Dharawad, Karnataka. India, 3Nursing Tutor, College of Nursing, All India Institute of Medical Sciences, Basni Phase II, Jodhpur, Rajasthan.

Abstract
The aim of this review article was to explore and understand the concepts, issues and preventive strategies of mental health relapse with psychiatric nursing perspectives and also to present a comprehensive concept of relapse and its prevention in a narrative form based on the evidences from the various sources of literature. The review materials were included from the books, journals, news papers, online data base and grey literature of last three decades to 2018. The significant articles were searched by using key words: “Relapse”, “Mental illness”, “Prevention of Relapse”, “Relapse AND Mental illness”, “Relapse Prevention AND Mental illness” in PubMed, CINHAL, PsychINFO SCOPUS and Google Scholar data bases. Based on the relevant and significant facts found with respect to the aim of the review, concepts were evolved and evidence based narration was made under each concepts to understand prevention of mental health relapse with regards to identification of issues and formulating strategies to overcome such issues with psychiatric nursing perspectives. The study concluded that, relapse is a major obstacle for recovery of mental illness, but can be preventable by addressing underlying issues with preventive approaches across various health care settings.

Keywords: Relapse, Mental illness, Prevention of Relapse, Relapse and Mental illness, Relapse Prevention and Mental illness.

Introduction
“What remains in diseases after the crisis is apt to produce relapses.” -Hippocrates

World Health Organization (WHO) reported mental illness constitutes the highest burden of disease in the world with relapse being one of the most pertinent barriers to recovery and rehabilitation.1 In spite of the availability of various treatment modalities and advancement of the evidences, relapse rate among the mentally-ill is relatively high, reports among those with schizophrenia has put relapse rate between 50% and 92%.2

Relapse is a recurrence of symptoms of mental illness similar to those that have previously been experienced. Relapse is generally agreed to have occurred when the person experiencing the symptoms is not able to cope using their usual supports and requires a greater intensity of intervention.3 Relapse prevention is an important element of the recovery process. It focuses
on maximizing wellness for people with mental illness by reducing the likelihood and impact of relapse.4

Relapse prevention is based on communication and understanding between the person experiencing mental illness, their family and caregivers, primary health care, the specialist mental health system, and community support services about access to support or treatment if there are early signs of relapse.3

Relapse prevention immensely improves the quality of life of individuals with mental illness and enables them to more fully participate in work, leisure and relationships. Effective relapse prevention enables people to gain mastery over their symptoms, which increases their sense of control over their lives (Mueser et al 2002).4

Methods

The aim of this review article was to explore and understand the concepts, issues and preventive strategies of mental health relapse with psychiatric nursing perspectives and also to present a comprehensive concept of relapse and its prevention in a narrative form based on the evidences from the various sources of literature. The review materials were included from the books, journals, news papers, online data base and grey literature of last three decades to 2018. The significant articles were searched with following key words: “Relapse”, “Mental illness”, “Prevention of Relapse”, “Relapse AND Mental illness”, “Relapse Prevention AND Mental illness”. Based on the relevant and significant facts found with respect to the aim of the review, concepts were evolved and evidence based narration was made under each component to understand prevention of mental health relapse with regards to identification of issues and formulating strategies to overcome such issues with psychiatric nursing perspectives.

Findings

Findings of the review on evolved concepts are discussed under following headings:

I. Relevance / Need for Understanding Prevention of Relapse of Mental Illnesses

Global and National scenarios related to prevalence of relapse of mental illness presents apprehensive data, which made every stake holders to think about it.

a) Prevalence of Relapse among Mentally Ill Patients: Global and Indian

A study conducted in Ethiopia to assess the prevalence of relapse of schizophrenia in 2011. They stated that relapse rates vary from 50% to 90% globally. In different studies, the prevalence of relapse of schizophrenia ranged from 44% to 50% and 48% in developed and developing countries.

Severe mental disorders (SMDs), including schizophrenia, bipolar disorder, schizoaffective disorder and depressive psychosis, have a relatively low prevalence (1%-2%), and they are associated with increased risk of relapse and hospitalizations in USA.7

Relapse is most common and carries a serious burden in patients with schizophrenia. Patient with schizophrenia has been estimated to be 3.5% risk of relapse per month, and about ~40% of patients experience a relapse within a year following hospital admission.8, 9

A study conducted in institute of psychiatry, London, indicated that up to 90% of patients with bipolar disorder have at least one relapse in their lifetime, with an average of 0.6 relapses per year. After recovery from a mood episode, nearly 50% subsequently have a relapse within two years of remission.7

A study conducted by Chabungbam G in Chandigarh on 40 schizophrenic patients to examine socio-demographic and clinical factors associated with relapse in schizophrenia. The findings suggested that a severe illness, psychological stress and inappropriate treatment (side-effects of medicines) may be causally related to relapse in schizophrenia10

A similar study was conducted to assess the relapse rates among first episode psychosis patients identified in Nottingham. The reassessment of 168 cases of first
episode psychosis after 3 years later, they found high rates of relapses (21/32, 65.6%), when patients with diagnostic changes were excluded, only 4 of 11 patients (36.3%) with acute transient psychotic disorder had more than one episode.\(^{11}\) A follow-up study of acute transient psychotic disorder (ATPD) over 3–7 years found that 58% of patients relapsed.\(^{12}\)

**b) Gap in Health Care Burden of Mental Disorders and Resources**

WHO report (2003) on Investing in Mental Health has detailed explanation of global scenario regarding gap between the healthcare burden of mental disorders and the resources. In developed countries with well-organized health care systems, between 44% and 70% of patients with depression, schizophrenia, alcohol-use disorders and child and adolescent mental illnesses do not receive treatment in any given year. In developing countries, where the treatment gap is likely to be closer to 90% for these disorders, most individuals with severe mental disorders are left to cope as best they can. Worldwide more than 40% of all countries have no mental health policy and over 30% have no mental health programme.\(^{13}\)

Further reported, over 90% of countries have no mental health policy that includes children and adolescents. Out-of-pocket expenditure was the primary method of financing mental health care in many (16.4%) countries. Even in countries where insurance cover is provided, health plans frequently do not cover mental and behavioral disorders at the same level as other illnesses; this creates significant economic difficulties for patients and their families.\(^{13}\)

**c) Common Conditions of Relapse in Mental Illnesses**

There is a high rate of relapse within 5 years of recovery from a first episode of schizophrenia and schizoaffective disorder (Robinson D et al.).\(^{14}\) People with severe depression were more likely to relapse or commit suicide than people with milder depression (Goldney R D).\(^{15}\) High prevalence of relapse among patients with psychotic disorder. Nearly one-fourth of participants who were diagnosed as having schizophrenia (n=70), schizophreniform and schizoaffective disorders (n=13) had psychotic relapse (Mahlet F, Matiwos S, Garumma T F).\(^{16}\)

**d) Triggering factors of relapse in major mental illnesses**

Patient and caregivers of mentally ill perceived non adherence to antipsychotic medication as a leading risk factor of relapse; other risks included poor family support, stressful life events and substance use (Adellah ES, Anne HO, Khadija IY).\(^{17}\) Similarly, it is reported non adherence to antipsychotic medication, substance related factors, low social support and co-morbid psychiatric illness were major factors for relapse.

**e) Economic burden of mental illness due to relapse**

The economic burden during the first post discharge year is relatively higher due to loss of efficacy; whereas the burden from noncompliance is higher in the second year. (Weiden PJ, Olfson M.\(^{18}\) The costs of treatment were found to be high but with wide variations in the range. Costs for bipolar disorder were somewhat higher than those for schizophrenia at least for the period of study (Sharma P, Das S.K, Deshpande S.N).\(^{19}\)

**f) Evidences on relapse prevention in mental illnesses**

The Relapse Prevention Programme was efficient in detecting prodromal symptoms of relapse early in an episode. Crisis intervention including increased antipsychotic medication use during the prodromal phase reduced relapse and rehospitalization rates (Marvin I. Herz et al.).\(^{20}\)

Specialist Psychosis programs are effective in preventing relapse. Cognitive-based individual and family interventions may need to specifically target relapse to obtain relapse prevention benefits that extend beyond those provided by specialist FEP programs. First and second generation antipsychotics have potential
to reduce the relapse rates (Maria A J, Alexandra GP, Sarah EH, Patrick DM, John FG.).21

II. Existing Practices / Situation/ Knowledge on Relapse Prevention in Mental Illnesses

a) Factors Associated With Relapse in Mental Illnesses

Early identification and prevention of relapse in patients with mental illness has significant therapeutic and socioeconomic implications. Relapse is common in the cases of patients who had documented evidence of either re-emergence or aggravation of psychotic symptoms. Globally, the factors commonly associated with relapse include deprived treatment adherence, substance abuse, co-morbid psychiatric illness, a co-morbid medical and/or surgical condition, stressful events in life, and the treatment setting.

A meta-analysis conducted on factors associated with relapse of schizophrenia in University of Johannesburg22. As mentioned in the results, the figure 01 depict following factors are most commonly associated with the mental illness:

![Figure 01: Factors Associated with Relapse of Mental illnesses](image)

b) Awareness of Early Warning Signs of Relapse

Fundamental aspects of relapse prevention are recognition and awareness of early warning signs and of the risk and protective factors for mental health. This awareness should focus on individual, family and service levels. Early warning signs vary among individuals, and a personal set of early warning signs is referred to as a ‘relapse signature’.

As mentioned by Van Miejel et al., in 2002 there are two types of monitoring that are relevant to recognition of early warning signs: direct monitoring, where the existence or nonexistence of symptoms is ascertained; and indirect monitoring, where situations are assessed to determine the presence or absence of risk and protective factors. Therefore, it is essential to include both types of monitoring for a thorough and holistic relapse prevention approach.23 Box 02 illustrate early warning signs of relapse (Birchwood, Spencer & McGovern 2000)
Early Warning Signs of Psychotic relapse (Birchwood, Spencer & McGovern 2000)

<table>
<thead>
<tr>
<th>Thinking/Perception</th>
<th>Feelings</th>
<th>Behaviors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoughts are racing</td>
<td>Hopeless or useless</td>
<td>Difficulty sleeping</td>
</tr>
<tr>
<td>Senses seem sharper</td>
<td>Afraid of going crazy</td>
<td>Speech comes out jumbles filled with odd words</td>
</tr>
<tr>
<td>Thinking you have special powers</td>
<td>Sad or low</td>
<td>Talking or smiling to yourself</td>
</tr>
<tr>
<td>Thinking that you can read others mind</td>
<td>Anxious and restless</td>
<td>Acting suspiciously as like being watched</td>
</tr>
<tr>
<td>Thinking that others can read your mind</td>
<td>Increasingly religious</td>
<td>Spending time alone</td>
</tr>
<tr>
<td>Receiving personal messages from the TV or radio</td>
<td>Feeling like you are being watched</td>
<td>Neglecting your appearance</td>
</tr>
<tr>
<td>Having difficulty making decisions</td>
<td>Feeling isolated</td>
<td>Not seeing people</td>
</tr>
<tr>
<td>Experiencing strange sensations</td>
<td>Tired or lacking energy</td>
<td>Not eating</td>
</tr>
<tr>
<td>Preoccupied about 1 or 2 things</td>
<td>Feeling in another world</td>
<td>Not leaving the house</td>
</tr>
<tr>
<td>Thinking you might be somebody else</td>
<td>Feeling strong and powerful</td>
<td>Behaving like a child</td>
</tr>
<tr>
<td>Seeing visions or things others cannot see</td>
<td>Unable to cope with everyday tasks</td>
<td>Refusing to do simple request</td>
</tr>
<tr>
<td>Thinking people are talking about you</td>
<td>Feeling like you are being punished</td>
<td>Drinking more</td>
</tr>
<tr>
<td>Thinking people are against you</td>
<td>Feeling like you cannot trust others</td>
<td>Smoking more</td>
</tr>
<tr>
<td>Nightmare</td>
<td>Irritable</td>
<td>Movements are slow</td>
</tr>
<tr>
<td>Difficulty concentrating</td>
<td>Feeling like you do not need sleep</td>
<td>Unable to sit down for long</td>
</tr>
<tr>
<td>Bizarre things</td>
<td>Feeling guilty</td>
<td>Aggressive</td>
</tr>
<tr>
<td>Thinking your thoughts are controlled</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hearing voices</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Box 02: Early Warning Signs of Relapse (Birchwood, Spencer & McGovern 2000)

**c) Importance of Relapse Prevention in Mental Illnesses**

Relapse prevention is desirable for several realistic reasons, namely

- It reduces the negative impact of mental illness on individuals and their families and caregivers, as well as their communities.

- Prolonged and recurring periods experiencing the symptoms of mental disorder severely affect a person’s life and also erode their confidence in work and wellbeing.

- Evidence shows that each relapse increases both residual symptoms and social disabilities.

- Relapse greatly increases the burden on families and caregivers, contributing to their distress and reducing their quality of life.

- Preventing relapse reduces the cost of mental illness to the community.

**III. Existing Problems/Shortcomings Regarding Relapse Prevention**

With reference to many evidences from the literature, relapse prevention of mental illnesses is literally challenging task for the health care providers. The most important problems experienced by the health care providers, patients and caregivers are as follows:
a) **Revolving Door Syndrome**

The tendency of clients to get better for a while, and then end up relapsing is known as revolving door syndrome refers to. It most often applies to those with severe mental illness, such as schizophrenia, but a person with a mental health disorder could potentially be at risk.25

A cross-sectional study was conducted on revolving-door patients in public psychiatric hospital in Israel which aim to study social, demographic, clinical, and forensic profiles of frequently re-hospitalized (revolving-door) psychiatric patients. They suggest that, revolving-door patients had significantly shorter mean interval between hospitalizations, showed less violence, and were usually discharged against to medical advice from health care providers. 26

b) **Major Issues of Relapse Prevention in Mental Illnesses**

A critical review of selected literature regarding relapse among schizophrenics identifies following major problems of relapse:

1. Relapse rates are extremely high among mentally ill after treatment discontinuation, even after a single episode of psychosis.

2. Risk of relapse does not decrease even after longer treatment period prior to discontinuation.

3. Many patients relapse very soon after treatment discontinuation.

4. The transition from remission to relapse may be abrupt, with few early warning signs.

5. Once relapse occurs, symptom severity rapidly returns to previous psychotic episode.

6. The majority of patients after relapse respond rapidly to re-introduction of antipsychotic treatment, the response time is unpredictable and whereas frank treatment failure may emerge in a subset of patients.27

c) **Growing Health Care Costs: Consequence of Relapse**

A significant toll on patients, caregivers, and society in terms of direct and indirect costs of schizophrenia.28

Total per year excess cost of schizophrenia in the US was estimated at $62.7 billion in 2002. In this economic burden, 52% was due to indirect costs ($32.4 billion, including unemployment, reduced workplace productivity, premature mortality from suicide, and family care giving), 36% was due to direct healthcare costs ($22.7 billion, including outpatient, inpatient, long-term care, and medications), and the remaining 12% was due to direct non-healthcare costs ($7.6 billion).29

Healthcare Cost and Utilization Project show that in comparison with patients of other mental health and substance abuse disorders, patients with schizophrenia admitted for treatment have the second-longest average length of stay (11.1 days), highest average total price per stay ($7500), and highest aggregate cost of hospitalization ($2.7 billion).30

A primary driver of medical costs in schizophrenia patient is relapse and associated care (including hospitalizations). A study shown that patients who experienced a relapse of psychotic symptoms within the previous six months incurred four times higher costs than schizophrenia patients without a recent relapse (p<0.01).31 Similarly, in another published, prospective, observational study (1997–2003); the 12-month direct mental healthcare costs for patients with a prior relapse (within the previous six months) were three times higher than costs for patients without a relapse ($33,187 vs $11,771; p<0.01).32 These results underscore the economic importance of relapse prevention.

d) **Knowledge and Skill Gap Regarding Relapse Prevention**

Tricia Nagel and associates conducted a retrospective study to review the quality of care provided in the Top End in-patient setting, and the involvement of Aboriginal Mental Health Workers (AMHWs) in the care of Indigenous people who are hospitalized for mental
illness. This review was a part of relapse prevention project in Australia. The findings suggest that, low rates of AMHW engagement in care. They were involved in the care of only 55% of Indigenous patients in 2004. There is a need to explore strategies for reorientation of services to deliver consistent quality care and promote relapse prevention, self-management and empowerment of all clients and families.33

Mental health professionals are not skilled in working with families. Some recent programs now train mental health workers to provide families with the skills needed to be active, positive caregivers rather than passive victims to the difficult situations created by a relative’s illness. The nature of this care giving role is often not understood by professional mental health workers. Families’ still experiencing blame from others for causing these illnesses, although it is diminishing as evidence suggest for a neurobiological explanation of mental illness.34

IV Strategies to Improve the Situation / Minimize or Solve Problems of Relapse Prevention in Mental Illnesses

a) Major Approaches to Relapse Prevention and Evidence of their Effectiveness

In the medical and academic literatures, relapse prevention interventions have been categorized in following types of approaches5:

1. **Awareness of early warning signs**: The primary intervention approach of relapse prevention are programs that focus on teaching people how to recognize their early warning signs and the environmental triggers of their symptoms. Such programs generally involve training in identification of early warning signs and stress management.

2. **Compliance with medication**: Medication non-compliance is a major risk factor for relapse. This is the case for psychotic illnesses as well as depression. There is clear evidence that maintenance medication and taking medication as prescribed significantly reduces the risk of relapse.

3. **Coping skills training and cognitive behavioral approaches**: Coping skills programs aim to help people manage stress or deal with persistent symptoms. Review of randomized controlled trials for four coping skills programs were shown to be effective in terms of reducing symptom severity.
4. Broad-based psycho-education programs (BBPP): It is a commonly held belief that greater knowledge empowers people to make better decisions regarding many behavioral choices, including those affecting their health. BBPPs provide information people about their mental illness, generally focusing on symptoms, stress-vulnerability, and treatment options.

5. Self-help programs: Consumer self-help groups developed partly out of dissatisfaction with the mental health care system and the need for consumers to combat the stigma of mental illness (Emerick 1990). These groups have extended to include a wide range of programs and to also encompass family and caregiver support groups.

6. Risk and protective factors: A more holistic approach to prevention focuses on identifying the multiple risk and protective factors for mental health. Risk factors are those that always responsible to a individual’s susceptibility to relapse, whereas protective factors alleviate relapse by enhancing wellbeing; “risk factors increase the possibility that a disorder will develop and can aggravate the burden of existing condition, while protective factors provide people resilience in the face of difficulty and moderate the impact of stress and transient symptoms on social and emotional wellbeing, thereby decreasing the probability of disorders.

b) Basic Elements of Relapse Prevention

Essentially, prevention of relapse requires awareness, planning, and the provision of timely and appropriate intervention responses. Laurie identifies prevention of relapse as the 4As of crisis prevention: 1) awareness, 2) anticipation, 3) alternatives and 4) access. To implement such an approach, actions need to be undertaken by all those concerned in the continuing care and recovery of people with mental illness: by people who have experienced mental illness, their families and caregivers, clinical service providers and planners, non-clinical service providers and planners, policy makers, and communities.35

Assertive Community Treatment Model

 Assertive Community Treatment (ACT) is planned and implemented by an interdisciplinary team that ensures service availability for whole day, all the days of week and is prepared to carry out all possible treatment functions as and when needed. A person may referred to the ACT team service when it has been determined that his/her needs are so pervasive and/or not expected that it is unlikely that they can be met efficiently by other combinations of accessible community services, or in situation where other levels of outpatient care have not been successful to maintain stability in the community.36

Assertive community treatment offers significant advantages over standard case management models in reducing homelessness and symptom severity in homeless persons with severe mental illness.37

V Nursing Implications in Relapse Prevention in Mental Illnesses

a) Role of Nurse in Relapse Prevention in all Possible Settings

Traditionally, the public health concept of disease prevention has conceptualized prevention as primary, secondary or tertiary depending on whether the strategy prevents the disease itself, the severity of the illness or the associated disability.38

Preventive strategies are always reducing risk factors; hence to get maximum effect, these strategies need to be implemented at specific periods before the onset of the disorder. On the other hand, it is still possible to decrease severity, course, duration, and associated disability of disease once it has developed, by taking preventive measures throughout the course of the disorder.

The following three categories of primary prevention have been identified:

- Universal prevention: In universal prevention, will focusing on the general public or a whole population group.
**Selective prevention:** Selective prevention targeting individuals or subgroups of the population whose risk of developing a mental illness is considerably higher than that of the rest of the population.

**Indicated prevention:** Persons at high-risk for mental disorders will be targeting for indicated prevention.

**Secondary prevention** refers to preventive measures undertaken to decrease the prevalence of all specific treatment-related strategies.

**Tertiary prevention** would embrace interventions that reduce disability and all forms/types of rehabilitation as well as prevention of relapses of the mental illness.

**b) Psychiatric Nurse-Led Preventive Strategies/Recommendations for Mental Health Relapse**

Psychiatric nurses become involved in prevention of relapse programs in many different ways. They can act as a referral source for the program, trainer or leader of the programs, or an after care sources for patients when the program is completed. In addition mental health nurses can help the patient and family by promoting optimism, sticking to goals and aspirations, and focusing on individual strength.

- Psychiatric nurses serve in various pivotal functions across the continuum of care. These functions can involve both direct care and coordination of the care delivered by others.

- The intensification of research in nursing practice is of great importance because a considerable portion of the therapeutic efforts is and can be executed with the context of nursing care. Reasons for these results indicated that effectiveness research should be continued on psychotic relapse.

- Nurse-led psycho-education program showed an increased level of knowledge about the disease and health resources as well as a positive displacement in terms of some Outcome Indicators such as Participation, Attention and greater Satisfaction with health services. These programs may result in greater health gains.

- Psychiatric nurses facilitate the incorporation of shared decision making into clinical practice to improve medication follow-through. Globally, the most trusted health care professionals are nurses. They continuously assess treatment effectiveness and medication side effects.

- Statistically significant improvement in attitude toward medication and quality of life of patients with schizophrenia after implementation of a psychiatric nursing intervention. In improvement of medication adherence and quality of life of schizophrenic patients, the psychiatric nursing intervention was effective.

**Available Resources in Indian Context for Relapse Prevention**

**Human resources**

- “Tapasya” Mental Health Rehabilitation And De-Addiction Centre, Indore
- Shraddha Rehabilitation Foundation, Mumbai
- Chaitanya Centre for Psycho-social Rehabilitation, Cochin, Kerala
- Tulasi Best Psychiatrist Rehabilitaiton and Rehab centre, Delhi
- PapayaCare Assisted Livivng and Long Term Care, Ahmedabad
- SuVitas-Rehabilitation Center, Hyderabad

**Physical infrastructure**

National Institute of Mental Health and Neurosciences, Bengaluru

Central Institute of Psychiatry, Delhi
Institute of Human Behavior and Allied Sciences
- Schizophrenia Research foundation, Chennai
- Institute of psychiatry and Human Behavior, Goa

**National Programs Related to Relapse Prevention**
- National Mental Health Program, 1982
- District Mental Health Program, 1996
- National Program for Health Care of the Elderly, 2010
- National Mental Health Policy, 2014
- National Health Mission, 2017
- Mental Health Care Act, 2017

**Conclusion**
Relapse prevention must become a routine component of continuing care for all people who have been seriously affected by mental illness. It needs to be incorporated alongside rehabilitation as one of the tools within a recovery oriented mental health care system. Relapse prevention should commence at the earliest possible opportunity, even during treatment of the first episode, and then be adapted according to each person’s changing needs across the lifespan, across the course of their ongoing experience of mental health and mental illness, and across changing life circumstances.

**Ethical Clearance**
Since it is a review article and has been written as narrative concept article. Further, not needed informed consent as human subjects were not involved in this scientific work. Review is based upon available primary and secondary literatures, therefore does not require ethical permission. Journal guidelines have been followed in writing references. However, based on the conclusion of the present review a study has been undertaken which aims to prevent relapse and readmissions among patients with severe mental illness and ethical permission was taken to conduct the present study from Institutional Ethical Committee of AIIMS, Jodhpur.

**Conflict of Interest:** No conflict of interest

**References**


Histopathological Changes in Brain Tissues associated With Oral Administration of Tramadol in Male Rats

Abbas Ch. Mraisel1, Sawsan, A. Ibrahim2, Muntadher H. Dawood3

1Prof. Assistant, Clinical Libratory Science Department /Pharmacy College / Misan University, Iraq. 2Lecturer Assistant, Basic Science Department /Nursing College / Misan University, Iraq. 3Lecturer, Pharmacy College, Misan University

Abstract

Background: Repeated and long treatment with tramadol might lead to accumulation of toxic metabolites in the body and increase the risk for pharmacokinetic interaction and decrease the clearance of tramadol, therefore this study was performed to investigate the toxic impact of the tramadol on the tissues of the brain in the male rats.

Method: The experiment was carried out at Environmental Toxicology Laboratory, Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt. Thirty-two Albano Waster male rats weighing (200-250 g) were obtained from the animal house of the Faculty of Medicine, Alexandria University, and grouping into four groups (8 rats for each group in each cage). The Control group was fed a basal diet and given tap water daily for ten days. In group two the rats were fed basal diet and given Tramadol HCL orally in dose 45mg/ kg .B.W dissolved in (5ml) normal saline (0.9%) by gastric tube, daily for Ten days. In group three the rats were fed with basal diet and given Tramadol HCL orally in dose 45mg/ kg .B.W dissolved in (5ml) normal saline (0.9%) by gastric tube, daily for Twenty days. The group for the rats was fed with basal diet and given Tramadol HCL orally in dose 45mg/ kg .B.W dissolved in (5ml) normal saline (0.9%) by gastric tube, daily for Thirty days. At the end of the experimental period Kidney tissues of each rat were immediately removed and after weighted put into 10% neutral buffer formalin as a fixative solution and stained with Hematoxylin –Eosin stain.

The Results: The results observed a significant decrease in the weight of the brain in the groups of the rats that were given the Tramadol HCL in dose 45mg/ kg .B.W with increasing the time of administration as compared with the control group. Histopathological changes were observed in rats brain tissues section the rats that given Tramadol HCL orally in dose 45mg/ kg .B.W dissolved in (5ml) normal saline for ten days revealed a mild degree of tissue injury in the cerebral cortex, with few vacuolar degeneration and dilatation of blood vessels, and the tissue sections of group two after ten days revealed a mild degree of tissue injury in the cerebral cortex, with few vacuolar degeneration and dilatation of blood vessels, while the three groups observed increase in the vacuolar degeneration, with neural atrophy and degeneration of neurons with reduction the neural process and pyknosis of the nuclei dilatation of blood vessels after twenty days of tramadol administration. The tissue Sections obtained from group four after thirty days revealed an increase in the vacuolar degeneration, with more atrophy of the neural cells and complete reduction of the neural process and pyknosis of the nucleus in the injured neural cells and gliosis.

The conclusion of this study there are harmful toxic effects when administrated tramadol for long period on the brain tissues, therefore an abuse of tramadol should be avoided except with medical prescription owing to its toxic effects.

Key words: Tramadol HCL, Histopathological changes, Brain tissues
Introduction

Clinically the tramadol has been used for relieving mild and moderate pain in human and veterinary medicine and used as anesthesia especially in veterinary medicine. Tramadol is marketed as the hydrochloride salt and is available in a variety of pharmaceutical formulations for oral (tablets, capsules), sublingual (drops), intranasal, rectal (suppositories), intravenous, subcutaneous, and intramuscular administration. It is also available in combination with acetaminophen (paracetamol), as immediate- and extended-release formulations, and for once-a-day dosing described variously as ‘controlled’, ‘sustained’, or ‘delayed’ release.

The tramadol is rapidly absorbed orally and 30% of tramadol excreted through the kidney with half-life elimination (5-6) hours, while the remaining dose is metabolized in the liver. Tramadol in the liver is converted to O-desmethyl-tramadol by cytochrome P 450 which is an active substance and is two to four times more potent than tramadol. Persistent tramadol administration might lead to the accumulation of toxic metabolites in the body, increase the risk for its toxic kinetics effects, and/or lower the clearance of tramadol, thus increasing its potential for toxicity. The most common mechanisms of death after tramadol overdose are cardiorespiratory depression, resistant shock, asystole, and liver failure. Fatal toxicity of tramadol has been reported after coadministration of other medications including propranolol, ethanol, barbiturates, and benzodiazepines.

Repeated administration of tramadol might lead to toxic metabolites in the body and cause many adverse effects such as headache, constipation, nausea, dizziness, and central nerve disturbances. Neurotoxicity of tramadol has been reported in patients administrated tramadol both at the recommended dosage and the high dosage ranges in animal and human studies. The neurotoxicity of tramadol commonly manifests as generalized tonic-clonic seizures. Chronic use of tramadol in increasing doses causes neuronal degeneration in the rat brain, which probably contributes to cerebral dysfunction.

Many types of research were performed to detect the biochemical and histopathological changes due to long-term abuse the tramadol on the liver, kidney, and brain, also some studies dealt with abnormal histological changes in the testis.

The study was performed by Atici et al. (2005) founds biochemical and histological changes in the rat’s liver with significantly higher serum Alanine aminotransferase (ALT), Aspartateaminotransferase (AST), Lactate dehydrogenase (LDH), and creatinine, also severe congestion and focal necrosis in the hepatocytes.

Youssif et al. (2016) found hemorrhage and cytolysis in the hepatocytes of the liver with complete cell membrane degeneration with changes in testicular tissues and atrophy in the somniferous tubules accompanied with interstitial calcification after administration of different doses of tramadol in experimental rats for 60 days. Hafez et al. (2015) observed that the toxic effect of tramadol on the parenchymatous organs such as liver, kidney, and thyroid glands in rats after intramuscular injection in different doses (12.5mg, 25mg, 50mg, and 300mg /Kg B.W) respectively for two weeks.

Many researchers have evaluated the effects of chronic use of tramadol on many organs, for example, liver, kidney, testes, heart, and thyroid gland. and scanty data dealt with the effects use of tramadol on the brain, therefore the objective of this study was designed to evaluate the toxic impact of the tramadol on the tissues of the brain in the male rats.

Material and Methods

Tramadol (tramadol HCL) 200mg/Kg B.W Tablet (Indian origin), were purchased from the outer pharmaceutical, Missan, Iraq. Experimental animals: Thirty-two Albano Waster male rats weighing (200-250 g) were obtained from the animal house of the Faculty of Medicine, Alexandria University. Animals were handled following the principles of laboratory animal care as contained in the NIH Guide for laboratory animal welfare and the experimental protocol was approved by the Local Ethics Committee and Animals Research. The
rats were housed in stainless steel bottomed wire cages after grouping into four groups (8 rats for each group) and maintained at a temperature of 22 ± 2°C, relative humidity of 40-60%, with a 12 h/12 h light/dark cycle and allowed free access to food and water, the test substances were administrated to the animals according to the following experimental protocol:

- Group I: Control rats were fed basal diet and given tap water as drinking water daily for Ten days.

- Group II: Rats were fed basal diet and given Tramadol HCL orally in dose 45mg/kg .B.W dissolved in (5ml) normal saline (0.9%) by gastric tube, daily for Ten days.

- Group III: Rats were fed with basal diet and given Tramadol HCL orally in dose 45mg/kg .B.W dissolved in (5ml) normal saline (0.9%) by gastric tube, daily for Twenty days.

- Group IV: Rats were fed with basal diet and given Tramadol HCL orally in dose 45mg/kg .B.W dissolved in (5ml) normal saline (0.9%) by gastric tube, daily for Thirty days.

At the end of the experimental period, the rats were overnight fasted (control and experimental animals) and sacrificed after 24 hours of the last dose of different administration under light ether anesthesia. Brain tissues of each rat were immediately removed taking care to handle specimens gently to minimize trauma, weighted, and put into 10% neutral buffer formalin as a fixative solution. Fixation time was limited to 24 hours and the fixed tissues were stored in 70% ethyl alcohol until they were processed. The fixed tissues were dehydrated through a graded series of ethanol and embedded in paraffin, sectioned according to the Luna (1968) method for histopathological examination, and stained with Hematoxylin –Eosin stain.16

**Statistical Analysis**

Statistical analyses were made with one-way analysis of variance (ANOVA) to compared the experimental groups(SPSS for windows version 17). P < 0.05 was considered statistical significance.

### The Results

**Table (1) Relative Brain weights(G) of the control group and Tramadol groups in different periods of the experimental protocol**

<table>
<thead>
<tr>
<th>Exp rats</th>
<th>Control group</th>
<th>Group II(Tramadol within 10 days)</th>
<th>Group III(Tramadol within 20 days)</th>
<th>Group IV(Tramadol within 30 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.53±0.63</td>
<td>1.44±0.07</td>
<td>1.33±0.47</td>
<td>1.23±0.55</td>
</tr>
<tr>
<td>2</td>
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<td>1.29±0.50</td>
<td>1.36±0.04</td>
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</table>
*Dead rat.

Table (1) showed the weight of the Brain obtained from the rats in the experimental groups, the results observed a significant decrease in the weight of the Brain in the groups of the rats that given the Tramadol HCL in dose 45mg/kg B.W with increasing the time of administration incompared with the control group.

Histopathological changes in the brain:

**Group I** (Control group): Microscopic examination of brain tissue sections observed normal cerebral cortex with a normal distribution of neurons and fibers (N) in the neuropit, also the glial cells (GC) and oligodendroial cells (ODC) were found in normal structure (Fig 1).

**Section of group (II)** which represent the rats that given Tramadol HCL orally in dose 45mg/kg B.W dissolved in (5ml) normal saline for ten days revealed a mild degree of tissue injury in the cerebral cortex, with few vacuolar degeneration and dilatation of blood vessels (Fig 2).

Figure (1): High power micrograph of rat brain section in control group stained with Haematoxylin & Eosin (H&E, X400), N: neural cells, GC: glial cells, OD: oligodendroial cells.
Section of group (III): Observed the rats that given Tramadol HCL orally in dose 45mg/kg .B.W dissolved in (5ml) normal saline for Twenty days characterized by an increase in the vacuolar degeneration, with neural atrophy and degeneration of neurons with reduction the neural process and pyknosis of the nucleidilatation of blood vessels(Fig 3).

Figure (2): High power micrograph of rat brain section of group (II ) stained with Haematoxylin & Eosin (H&E, X400), V: Vacuolar degeneration, BV: Blood Vessels.

Figure (3): High power micrograph of rat brain section of group (III ) stained with Haematoxylin & Eosin (H&E, X400), V: Vacuolar degeneration, BV: Blood Vessels, NCA: Neural Cell Atrophy, NP: Neural Process.
Section of group (IV): Which represent the rats that given Tramadol HCL orally in dose 45mg/kg B.W dissolved in (5ml) normal saline for Thirty days revealed an increase in the vacuolar degeneration, with more atrophy of the neural cells and complete reduction the neural process and pyknosis of the nucleus in the injured neural cells and gliosis (Fig 4).

Figure (4): High power micrograph of rat brain section of group (IV) stained with Haematoxylin & Eosin (H&E, X400), V: Vacuolar degeneration, BV: Blood Vessels, NA: Neural Atrophy, NP: Neural Process.

Discussion

Oxidative stress, an imbalance between oxidant and antioxidant mechanisms in animal bodies, this imbalance may result in either form excessive exposure to pro-oxidants or from compromised anti-oxidant mechanisms. Which may result from deficiency of essential elements or incapacitation of disease, while the former might emanate from exposure to exogenous toxins or the pathologic stress of disease\textsuperscript{17,18}.

Tramadol hydrochloride used as analgesic drugs, therefore in the 70s used for treating moderate and severe pain but in recent years the tramadol abuse among youngers and teens in different countries especially between males, therefore the present study performed to investigate histopathological changes in the brain tissues accompanied with the toxic effect of tramadol in male rats.

The result revealed a significant decrease in the weight of the brain in a group of rats that administrated tramadol at different times as compared with the control group, this result agrees with Balhara \textit{et al} (2018) that founds the administration of tramadol caused a reduction in the cells volume and nuclear condensation in the brain of rats which probably contributes to cerebral dysfunction\textsuperscript{19}.
In present study observed varied adverse effects in morphological and histological structures of the brain tissues with increasing the time of given dose of tramadol. Essam et al (2015) found histological changes in the brain tissues of rats after continuous administration of tramadol for a long period.

Mohamed et al (2013) found changes in the pyramidal cells which lost the shape and increase the hemorrhage in the brain and disrupted ependyma and the choroid plexus become hypertrophoid.


Chronic administration of tramadol with increasing the doses of the drug may cause degeneration in the red neurons and apoptosis in the brain which contributes to cerebral dysfunction (Atici et al, 2005).

Some researchers have reported the adverse effect of tramadol on other organs in the body, where Azari et al (2014) referred the long term administration of tramadol can cause to the testicular tissues and deposition of acidophilic PSA-positive materials in male rats. Yousef and Sheweita et al (2018) reported that administration of morphine and tramadol can cause degeneration in the hepatocytes and dilatation in the central vein with dilation in the sinusoid.

The study was performed by AbouEluaga et al (2020) to investigate the effect of tramadol on the histological structures of the testes in Albano rats which observed abnormal changes in the seminiferous tubules with long-term administration of tramadol.

Salma et al (2003) referred that tramadol may increase the accumulation of free radicals and ROS which can cause an increase in nitric oxide level in the brain and lead to hypofunction of Leydig cells with consequent reduction of the testosterone secretion.

Caju et al (2012) reported that exposed the mature rats to high doses of tramadol and morphine for a long time can cause testicular changes due to disorders in the endocrine and paracrine functions with reduction of Sertoli and Leydig cells leading to disorders in LH, estradiol, somatotropin, somatostatin, and gonadotrophin-releasing hormone.

Hussein et al (2017) recorded an increase in the area and creatinin levels in rats after received tramadol (22.5 mg/Kg B.W/day for nine weeks) due to evidence of renal damage and impaired renal function.

In conclusion, the results of this study observed a harmful toxic effect on the histological structures and function of the brain in male rats when administrated the tramadol for long period, therefore abuse of tramadol should be avoided except with medical prescription owing to its toxic effects.

Ethical Clearance- Taken from Farmacy college/ Misan University /Ethical committee

Source of Funding- AUTHORS OWN MONEY ONLY

Conflict of Interest – NIL

References


Legal Civil Liability of the Forensic Medicine as a Judicial Expert from a Jurisprudential Perspective

Abdelawal Bassiouny1, Saad Ramadan2, Majd Manasra3

1Faculty of Law/ The American University in the Emirates in the United Arab Emirates, 2Faculty of Law/ Umm Al Quwain University/ United Arab Emirates, 3Faculty of Law/ Applied Science Private University/ Jordan

Abstract

This study dealt with the long-term liability of the forensic medicine expert as a judicial expert. This study clarifies the concept of civil liability for the judicial expert, the forensic medicine, for the implementation of it is expertise, through introducing the judicial expert to the forensic medicine, and what is the nature and types of this liability, then the study also addressed elements of the civil liability of the forensic medicine.

The study concluded that the civil liability of the expert is a tort liability, provided that the elements of this civil liability arising from the breach and the occurrence of damage, and the existence of causation where the strength of this liability is the damage resulting from the expert’s evacuation of the obligations incumbent upon expert.

Keywords: Forensic Medicine, Legal Expert, Civil Liability, Contractual Liability, Tort Liability, Causation, Damages, Breach.

Introduction

This research deals with the issue of civil liability of the judicial expert, forensic medicine. It is known that many courts of different types and degrees seek help in many cases, whether criminal, civil, commercial or religious, the opinion of people of experience and know-how about certain issues that are difficult or prevented for the judge to decide on them spontaneously.

Forensic medicine knows the doctor who devotes all his time to the job and is not allowed to practice his profession abroad in order to go to study the technical cases and issues that are presented to him and to have ample time to see and follow up on new scientific research in the various branches of forensic medicine.

The judicial expert forensic medicine is considered an assistant to the judiciary to clarify and remove the ambiguity of some of the difficulties encountered by the judge during the exercise of his profession in adjudicating the disputes presented to him.

From this standpoint, the courts are seeking the help of forensic medicine to determine the cause of death for the victim, estimate the period of suspension for it, or indicate whether the injury that the victim received poses a threat to his life or not, or determine the mental and psychological state of the offender to ensure his eligibility for trial and punishment And many other things.

The civil liability of the expert means the positive and negative actions that commit during the practice that accommodates the issue stipulated in the legislation and laws, as civil liability is a person’s breach of a spot on him and imposed on him to implement it either as a law or an obligation. It resulted in damage and this damage
is matched by compensation and liability here means a personal obligation to compensate for the damage that he caused to the expert, either as a result of a breach of a legal rule or a breach of the obligation he owes.

**Discussion**

Forensic medicine is the specialist in treating and studying cases that are considered by lawmen from a medical point of view and in which an opinion has been expressed. He also knows that every person undertakes a technical examination or gives a professional opinion specialized in a judicial incident and thus includes a doctor, a weapons expert, a specialist in examining fingerprints, and a forensic photographer. And the investigation expert is every doctor who undertakes an examination of a judicial incident and issues an oral or written opinion, and he is not required to be a specialist, for example, a general practitioner who examines simple daily judicial medical facts and gives preliminary judicial medical reports. The internal diseases specialist who undertakes the examination and treatment of a poisoned, the dermatologist examines and treats an injured or infected venereal disease and links it to a time related to a sexual accident, and the surgeon who treats a person with a gunshot wound, acute instrument, or a traumatic wound caused by a traffic accident, and the radiologist gives him a radiological report on a fracture Updated with a specific mechanism.

Forensic medicine in the capacity as a judicial expert when the judge resorts to the election in the event that a technical or medical scientific issue outside the jurisdiction of the judge is presented. A civil liability asks the forensic medicine in the capacity as an expert for every mistake he commits. Contractual liability and tort liability resulting from what the expert does to others in terms of the harm that commits against them.

Regarding the civil liability of forensic medicine in the capacity of a judicial expert, many disputes have arisen between the jurists regarding the legal nature of the civil liability of forensic medicine. Is it a contractual liability or a tort liability?

And considering that forensic medicine, in the capacity of a judicial expert, does not enjoy any of his immunity, he is subject to civil liability provisions based on the legal rule. Any damages to others obliges the perpetrator to pay compensation.

The civil liability of the forensic medicine is proven as soon as its elements are available, but there is a variation in cases in which the responsibility of the forensic medicine is contractual and in which it is negligent in the work of the expert, so the work of the expert is subject to an oversight during the performance of the tasks assigned to him, and the lack of commitment to caution and prudence in his work exposes forensic medicine civil liability as a judicial expert.

Most of the legislation, judicial jurisprudence and jurisprudential opinions are unanimously agreed that forensic medicine is responsible for the capacity as a judicial expert. It is responsible for the damages that may occur during the exercise of the profession.

A debate arose in jurisprudence about the nature of the judicial expert’s civil liability. There is an aspect of jurisprudence that considers the civil liability of the judicial expert to be a moral responsibility. That this point of view from a personal or subjective can only trigger his moral responsibility. In order to enable the expert to carry out his responsibility to the fullest and free from the influence of potential legal liability. Another aspect of jurisprudence in France is considered by the forensic medicine expert being subject to the responsibility of the judge if he issues his report with a final judgment.

A dispute also arose over the nature of the forensic medicine expert civil liability, as he is a judicial expert about whether it was a contractual or tort liability.

The Tort Liability of Forensic Medicine as a Judicial Expert:

The liability of forensic medicine is considered one of the most important manifestations of civil liability at all. Social coexistence requires individuals to respect the controls on which this coexistence is based, such
as respecting the rights of others and not violating their property or rights and any breach of this system unless the owner bears the consequences of this breach.

The legal civil liability of forensic medicine is proven as soon as the expert commits a professional mistake that harms the litigants, this responsibility is subject to the general rules of limitation.

For the establishment of the tort liability of forensic medicine, the three founding elements of the tort, breach, Damages and Causation, must present.

Breach: An error on the part of a forensic medicine expert considers every deficiency or breach thereof. The jurisprudential definitions that were given to the breaches have varied so that the idea of breach is not controlled in terms of limits, and one of the most famous definitions of breach is the definition of the jurist Baniol and the jurist Mancini, but the jurist SulaimanMorcos defined it based on the moral element, and the scholar Al-Sanhouri sees it as a deviation in the due behavior and he had the ability to distinguish so that he realizes that has deviated.

The forms of the contemporary breach of the task and the responsibility of the forensic medicine expert in the delay in carrying out the mission and omission in its implementation or refusal to do it without justification, and the acceptance of the task despite his knowledge of his inability to accomplish it and his deviation from the ethical principles that must be met such as objectivity and integrity. As for the forms of the breach during the execution of the task, they may be legal or technical.

As for the legal breach that arises as a result of the neglect of the legal principles and the obligations that the expert under the law regulating the experience, such as the failure of the forensic medicine expert to undertake the task himself, exceeding the limits of the task, or the delay in filing the report and receiving any fees from the litigants directly under any form. And the technical errors of the judicial expert, which makes during the exercise of the work and deviates from the established technical and scientific principles in relation to the specialization. As for the forms of breach after the implementation of the task, such as failure to respect the principle of confrontation, attend sessions and discussion, lack of respect for confidentiality.

The majority of jurisprudence is unanimous that the civil liability of the forensic medicine expert is fulfilled for all errors that make regardless of their seriousness. As long as the mistake committed by the forensic medicine expert is not committed by another expert with the same circumstances.

Damage: damage is the second element in tort liability, so there is no negligence if no damage occurs to others, and the damage is of two types, material and moral. Material if the forensic medicine expert deviates from the behavior of the common man taking into account the circumstances of time and place and leads to injury to the injured in money or a diminution of rights, and it will be a moral act of awareness and will, and it will strike the affected person, not with money, but in emotion and feeling.

In the field of compensation for the damage caused by the forensic medicine expert, the effort and expenses spent by the litigant, and the opportunities that were lost from him, represented in the loss of his right in whole or in part, according to events and circumstances, and the delay in deciding the case by changing its course in a way that harms him. It is stipulated that the damage be done personally, and that the damage be actuality and affect an acquired right.

Causation: It is not sufficient for the tort liability of the forensic medicine expert to be a breach on the one hand and the damage on the other hand. The tort liability for the breaching act must not be realized until it is proven that that act is the direct cause of the damage. In the area of expertise, causation is established between the breach of forensic medicine expert and the damage caused to the plaintiff litigant in the liability lawsuit.

Although causation is an essential element in the realization of tort liability. However, in the report of the forensic medicine expert, the judge does not base judgment on it, to some extent the judge has the right to
recognise it or not recognise it\textsuperscript{10}.

The Contractual Liability of Forensic Medicine as a Judicial Expert

The forensic medicine expert, usually appointed by the court regarding the understanding of facts of a technical or scientific nature concerning a dispute before it awaiting an urgent solution.

An opinion in French jurisprudence went to regard the responsibility of forensic medicine expert as a liability of a contractual nature, supporting their position that the appointed expert is a joint agent of the litigants. Accordingly, the responsibility of forensic medicine expert is a liability of a contractual nature. The DEMOGUE\textsuperscript{5} jurist went to support this trend, considering that the forensic medicine expert had contracted with the litigants as soon as accepted the mission. However, these trends have been criticized because the forensic medicine expert works under the supervision, direction and control of the judge and court, and he is not an attorney for the parties to the case\textsuperscript{11}.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Ethical Clearance was not necessary this study

Conclusion

Forensic medicine expert is every person who undertakes a technical examination or expresses a professional opinion on judicial cases, after reviewing the civil liability of the forensic medicine expert, whether it is a contractual liability or tort liability, it was found that the forensic medicine expert has the legal civil liability responsible based on tort liability. Forensic medicine expert has legal liability in the capacity as an expert for every breach commits by forensic medicine expert, as he finds himself a litigant defendant if the elements of tort liability, including a breach, damage, and causation for every damage caused by forensic medicine expert for the judicial experience.

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References

Association between Gestational Diabetes and Proinflammatory Cytokine (IL-1β)

Abdullah F. Yousif 1, Raid M. H. Al-Salih 2, Alaa H. Al-Naser 3
1 Assistant Lecturer, Department of Chemistry, Directorate of Education Thi Qar, Ministry of Education, Iraq,
2 Professor, Department of Chemistry, College of Science, University of Thi-Qar, Iraq, 3 Professor, Doctor Obstetricians and Gynecologists, College of Medicine, University of Thi-Qar, Iraq

Abstract

Gestational diabetes mellitus (GDM) defined according to the (World Health Organization) as glucose intolerance of varying degrees of severity with start or first recognition during pregnancy. This definition applies whether or not insulin is needed and whether or not it disappears after pregnancy. It is not apply to pregnant women with a pre-pregnancy diagnosis of T1DM or T2DM. This study included (150) pregnant women in first trimester (9-13 weeks) of pregnancy, during follow up in [late second trimester and early third trimester (24-28 weeks) of pregnancy], 75 of them diagnosed of GDM, and 75 of them without GDM (Control group). The FBG, Insulin, HOMA-IR, OGTT, HbA 1c and IL-1β levels were increase in women with GDM than control group (P≤0.05). Conclusions: In late second trimester and early third trimester of pregnancy, IL-1β levels were increase in women with GDM compared with healthy pregnant, therefore, IL-1β as an early biomarker may help to predict the development of GDM later in pregnancy.

Keywords: Interleukin-1 Beta (IL-1β), Hemoglobin A 1c Ratio, Insulin, HOMA-IR.

Introduction

The most recent definition of GDM is by the (American Diabetes Association), as GDM has been defined as diabetes diagnosed in the second or third trimester of pregnancy (24-28 weeks) that was unclear of diabetes before pregnancy.1 GDM is a pregnancy complication describe by the impaired glucose tolerance, and it develops when the pancreatic b-cell reserve is not enough to recompense for the arise IR during pregnancy. As a result, there is an increase in hepatic gluconeogenesis, severe insulin resistance and thus hyperglycemia.2

The dominant protein in red blood cells is hemoglobin it’s also oxygen carrying tincture and that gives blood its red color. Approximately 97% of hemoglobin is (hemoglobin A). 92 percent of (hemoglobin A) is made up of one chemical component, while approximately 8 percent is made up of minor components that are chemically slightly various. The minor components have (hemoglobin A 1c, A 1b, A 1a 1 and A 1a 2). Glucose is bound with hemoglobin A 1c (HbA 1c). Sometimes HbA 1c is also referred to as “glycosylated hemoglobin or glycohemoglobin”. In addendum to random FBG levels, HbA 1c levels are routinely measured in monitoring diabetes patients.3

HbA 1c concentration are depend on the levels of glucose in the blood. That is, any arise in the of glucose level in the blood leads to an increase in the HbA 1c concentration. HbA 1c concentration are not influenced by daily fluctuations in the blood glucose levels. HbA 1c is a beneficial index of how well blood glucose level has been controlled in the recent past (more than two to three months) Also it may be used to monitor the effects of diet, exercise, and drug therapy on blood glucose in people with diabetes.3

Corresponding author:
Abdullah F. Yousif
E-mail: Abd.fak_ch@utq.edu.iq
Interleukin-1 Beta (IL-1β) is a strong pro-inflammatory cytokine that is decisive for host-define responses to injury and infection. It is also the best characterized and most studied of the 11 IL-1 family members. Also known as leukocyticpyrogen, lymphocyte activating factor, leukocytic endogenous moderator, mononuclear cell factor and other names, is a cytokine protein that in humans is encoded by the IL-1β gene.

This cytokine is secreted and producting by a diverse of cell types although the vast majority of studies have focused on its production within cells of the innate immune system, such as macrophages and monocytes. It’s a precursor is cleaved by cytosolic caspase1(Interleukin 1 Beta convertase) to form mature IL-1β.

Cytokine IL-1β is a major mediator of the inflammatory response, and is includes in a assortment of cellular activities, involved apoptosis, cell proliferation, and differentiation. Necessary for host - response and resistance to pathogens, as well as exacerbation of damage through acute tissue injury and chronic disease. So it is not surprising that there has been a great deal of interest in how this protein is produced and exported from cells. However, the IL-1β release mechanism has proven elusive. It does not secrete through the traditional ER-Golgi pathway. The literature full of diverging observations arising from the many experimental systems contributed to a complex mixture of diverse propositions.

Materials and Methods

The present study included (150) pregnant women in the first trimester (9-13 weeks) of pregnancy, during follow up in [late second trimester and early third trimester (24-28 weeks)], 75 of them diagnosed of GDM, and 75 of them without GDM (Control group). It was determined sample size according to the equation Stephen Thompson. Serum blood glucose estimated by assay kit (Randox, England). Serum insulin and IL-1β were measured using ELISA Kits (Demeditec Diagnostics GmbH, Germany). HbA1c in whole blood measured by NycoCard™ HbA1c analyzer. Biochemical parameters measured in the [first trimester (9-13 weeks)] and [second and third trimester (24-28 weeks)].

Calculate Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) :

Serum of (glucose and insulin) estimated from fasting morning blood samples were utilised to calculate IR by the homeostatic model assessment-insulin resistance (HOMA-IR), which is approximated using the equation below.

\[
\text{HOMA-IR} = \frac{\text{Glucose (mmol/L)} \times \text{Insulin (µIU/mL)}}{22.5}
\]

Protocol of OGTT

1- The subjects should have a normal mixed diet for the prior three days previous to the test.
2- The subjects is fasted overnight and blood is drawn for glucose measurement before 75 grams is administered orally in 200 mL of water.
3- Blood glucose is measured every sixty minutes for two hours.

Collection of Blood Sample

After an overnight fasting for about 8 hr, (5 mL) of venous blood was collected from pregnant women with and without GDM, then divided into the following:

A) 2 mL of blood sample was transferred to tubes containing EDTA (Ethylene diamine tetra acetic acid) and used for HbA1c estimation.

B) 3 mL were transferred to plain tube and allowed to clot at room temperature to get serum by putting it in empty disposable tubes and centrifuged to separate it at 3000 rotor per minute (rpm) for 10 min, the serum samples were separated and stored at (-20°C) for later measurement biochemical parameters, unless used immediately.

Statistical Analysis

Statistical analysis was done using statistical package.
for the social sciences version 23, results were expressed the (mean ± standard deviation). T test was applied to compare parameters in studied groups. P-values (P≤0.05 ) were considered.

**Result and Discussion**

The characteristics of the studied groups are presented in Table (1). The results show no marked difference in the age of gestational, age of maternal and BMI in GDM group in comparison with the Controls group (p≤0.05).

<table>
<thead>
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<th>GDM Mean±SD</th>
<th>Controls Mean±SD</th>
<th>P-value</th>
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</thead>
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<td>75</td>
<td>-</td>
</tr>
<tr>
<td>Age of gestational 1st (weeks) at sampling</td>
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<tr>
<td>Age of gestational 2nd &amp; 3rd (weeks) at sampling</td>
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<tr>
<td>Age of maternal (Yrs)</td>
<td>31.42±3.67</td>
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<td>0.951</td>
</tr>
<tr>
<td>BMI 1st trimester</td>
<td>28.78±2.21</td>
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<tr>
<td>BMI 2nd &amp; 3rd trimester</td>
<td>30.08±2.81</td>
<td>29.37±2.75</td>
<td>0.515</td>
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</table>

Table (1): General characteristics data of the patients and controls groups

**Fasting Blood Glucose Concentration**

Table (2) shows a marked a rise in the levels of serum FBG in GDM-2 group in compare with the Cont-2 group (p≤0.05). The results obtained in this study show a marked a rise in the levels of serum FBG in the GDM-2 group in compare with the GDM-1 group (p≤0.05).

FBG is a strong predictor for diagnosing GDM, but it has been stated that using FBG as a diagnostic basis for GDM in early pregnancy is inappropriate because FBG is lower with rising gestational age. Riskin-Mashiah _et al._ it has been found that FBG useful to be a risk assessment screening tool but not as a diagnostic test in early pregnancy: Although FBG rises in the first trimester and remains within normal limits, it increases the risk of GDM later in pregnancy.

**Serum Insulin Concentration**

The results in Table (2) illustrating a marked a rise in the levels of serum insulin in GDM-2 group in compare with the Cont-2 group (p≤0.05). The results obtained in this study show a marked a rise in the levels of serum insulin in the GDM-2 group in compare with the GDM-1 group (p≤0.05).

Insulin needs are raised during pregnancy due to the presence of insulin antagonists, such as the lactogen secreted by the human placenta, and these hormones stimulate lipolysis and reduce glucose utilization. Various inherited disorders of the β-cells, insulin function, exocrine pancreas diseases, and endocrinopathies may result in varying degrees of glucose intolerance. As a result, glucose intolerance increases in GDM when β-cells are unable to compensate for the increased insulin resistance that occurs during pregnancy. In T2DM the cells of body be resistant to the action of insulin as the receptors that bind to the hormone become less receptive.
to insulin levels. This results in a rise in insulin levels (hyperinsulinemia) and insulin secretion disruptions, as well as a decreased insulin response. Hyperinsulinemia occurs when beta cells release elevated levels of insulin in response to persistently high levels of glucose in the blood. Insulin resistance is a hallmark of gestational diabetes mellitus. Insulin’s effect is thought to be hampered by gestational hormones and other factors because it binds to the insulin receptor. This interfering could happen at the level of the cell signaling pathway following the insulin receptor.17

Accurate measures of insulin sensitivity applied in the last trimester show slightly larger insulin resistance in females with gestational diabetes mellitus than in healthy pregnant females. The further resistance occurs for insulin’s effects to stimulate glucose elimination and to suppress glucose creation and decrease fatty acid concentrations.47 As a results the level of insulin in pregnant women with gestational diabetes mellitus are higher than insulin level in pregnant women without gestational diabetes mellitus due to the a raise levels of insulin resistance in women with gestational diabetes mellitus.17

Hemostatic Model Assessment-Insulin Resistance (HOMA-IR)

Table (2) illustrating a significant increase in the HOMA-IR in GDM-2 group in compare with the Cont-2 group (p≤0.05). The results obtained in this study show a significant increase in the HOMA-IR in the GDM-2 group in compare with the GDM-1 group (p≤0.05).

HOMA-IR method requires measuring single fasting serum glucose and the conformable fasting serum insulin level, that have been used to estimate insulin resistance.18,19

Insulin excretion in female with GDM can arise extremely over weeks or months in linked with the acquired insulin resistance of pregnancy. However, the arise occurs along an insulin sensitivity–excretion curve that is approximately 50% lower than that of normal pregnancy.20

Most GDM patients are linked with insulin resistance as noticed previously.21 GDM occurs in female who are unable to compensate for the natural metabolic changes of pregnancy.22 In order to enclose sufficient energy supply to the fetus, there is a rise in the levels of several hormones that oppose the effects of insulin, thus creating a state of insulin resistance. These hormones include human placental lactogen, placental growth hormone, estrogen, progesterone, cortisol, human chorionic gonadotrophin and prolactin.23 Norma glycaemia in pregnancy can only be preserve with a near doubling of basal insulin levels, as well as enhanced nutrient catalyze insulin excretion In humans, it appears that enhanced function of existing beta cells accounts for the majority of the a rise in insulin secretion, rather than hyperplasia. The arise insulin resistance reverts with delivery of the placenta.24

<table>
<thead>
<tr>
<th>FBG concentrations (mg/dL)</th>
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<tr>
<td>Control Groups</td>
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<tr>
<td>Mean±SD</td>
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<tr>
<td>Cont-1 75.27±6.09</td>
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<tr>
<td>Cont-2 80.52±6.43</td>
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<td>p-value 0.288</td>
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</table>

Table (2): FBG, Insulin and HOMA-IR concentrations for studied groups
Control Groups Mean±SD No. GDM Groups Mean±SD No. p-value
Cont-1 7.53±1.02 75 GDM-1 7.82±1.15 75 0.118
Cont-2 7.95±0.85 GDM-2 12.07±2.53 0.000

p-value 0.772 0.000

Cont-1: Healthy Pregnants at (9-13 weeks) / Cont-2: Healthy Pregnants at (24-28 weeks) GDM-1: Pregnants with GDM at (9-13 weeks) / GDM-2: Pregnants with GDM at (24-28 weeks)

Oral Glucose Tolerance Test (OGTT)

The results in Table (3) show a marked increase in the OGTT in GDM-2 group in compare with the Cont-2 group (p≤0.05).

Oral glucose tolerance test used to diagnosis of GDM during pregnancy. The thresholds and method for diagnostic screening tests in GDM have changed according to the results of some recent studies. The Hyperglycemia and inverse Pregnancy Outcome study showed continuous correlation between carrying out a 75-g OGTT at (24-32 weeks’ gestation) and perinatal outcomes. The International Association of Diabetes and Pregnancy Study Groups (IADPSG) does not recommend a 75-g OGTT at 24 weeks of pregnancy due to a lack of evidence on the connection between 75-g OGTTs and perinatal outcomes. At <24 weeks of pregnancy, fasting blood glucose (FBG) is used to diagnose GDM. GDM is diagnosed using a 75-g OGTT at 24-28 weeks of pregnancy.

Table (3): OGTT for studied groups

<table>
<thead>
<tr>
<th>Control Groups</th>
<th>Mean±SD</th>
<th>No.</th>
<th>GDM Groups</th>
<th>Mean±SD</th>
<th>No.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont-2 1-h</td>
<td>129.72±6.15</td>
<td>75</td>
<td>GDM-2 1-h</td>
<td>182.06±7.79</td>
<td>75</td>
<td>0.013</td>
</tr>
<tr>
<td>Cont-2 2-h</td>
<td>103.25±8.52</td>
<td>75</td>
<td>GDM-2 2-h</td>
<td>158.13±6.31</td>
<td>75</td>
<td>0.005</td>
</tr>
</tbody>
</table>
**Hemoglobin A₁c Ratio:**

Table (4) shows a marked increase in the ratio of HbA₁c in GDM-2 group in comparison with the Cont-2 group \( (p \leq 0.05) \). The present study shows no marked difference in the ratio of HbA₁c between GDM-1 group and Cont-1 group \( (p \leq 0.05) \). The results obtained in this study show a marked increase in the ratio of HbA₁c in the GDM-2 group in comparison with the GDM-1 group \( (p \leq 0.05) \). Also the results show no marked difference in the ratio of HbA₁c between Cont-2 group and Cont-1 group \( (p \leq 0.05) \).

Some authors do not find statistically marked differences throughout the whole period of pregnancy\textsuperscript{25}, other authors find decreases \textsuperscript{26,27}, and still others find increases in the third trimester\textsuperscript{28,29}.

In this study both groups of female pregnant showed a little a rise in HbA₁c in the 2\textsuperscript{nd} trimester comparison to the first one, though only the GDM group reached statistical marked, most probably due to an a rise in the medium postprandial glycemia.

Female with GDM are at a rise risk of perinatal morbidity, impaired glucose tolerance, and type 2 diabetes in the years after pregnancy. Many researcher have tried to apply HbA₁c to the diagnosis and monitoring of GDM patients. Rajput \textit{et al.} studied HbA₁c in Asian Indian female pregnant and reported higher mean HbA₁c levels in GDM patients than in normal female pregnant\textsuperscript{30}.

High levels of glucose can arise the glycation of common proteins such as hemoglobin, forming HbA₁c. However, it is important to note that HbA₁c is neither considered dysfunctional nor harmful. The levels of the HbA₁c predicts diabetes complications because it reflects more harmful glycation sequelae of diabetes, such as retinopathy and nephropathy, which are understood to be due to harmful advanced glycation end products\textsuperscript{31}.

### Table (4): Hemoglobin A₁c ratio for studied groups

<table>
<thead>
<tr>
<th></th>
<th>HbA1c (%)</th>
<th>No.</th>
<th>GDM Groups</th>
<th>Mean±SD</th>
<th>No.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control Groups</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont-1</td>
<td>4.62±0.26</td>
<td>75</td>
<td>GDM-1</td>
<td>4.83±0.29</td>
<td>75</td>
<td>0.092</td>
</tr>
<tr>
<td>Cont-2</td>
<td>4.80±0.19</td>
<td></td>
<td>GDM-2</td>
<td>5.62±0.43</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>p-value</td>
<td>0.110</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

**Serum IL-1β Concentration:**

Table (5) illustrating a marked a rise in the levels of serum IL-1β in GDM-2 group in comparison with the Cont-2 group \( (p \leq 0.05) \). The present study shows no marked difference in the levels of serum IL-1β between GDM-1 group and Cont-1 group \( (p \leq 0.05) \). The results obtained in this study show a marked a rise in the levels of serum IL-1β in the GDM-2 group in comparison with the GDM-1 group \( (p \leq 0.05) \). Also the results show no marked difference in the levels of serum IL-1β between Cont-2 group and Cont-1 group \( (p \leq 0.05) \).

The investigation of IL-1β in pregnant women with risk factors for the development of GDM was prompted by a clinical interest in inflammatory markers in pregnancy. The results indicate a rise of serum IL-1β Levels in GDM compared to normal pregnancy. Similar
The placenta is an endocrine organ that produces hormones and cytokines and is involved in the processes of pregnancy and labor induction. In normal pregnancy, cytokines like adiponectin and IL-1β are produced in the placenta, according to previous research.

In this study, female pregnant with GDM had higher insulin resistance in the second trimester (a time when insulin resistance develops) than female pregnant without GDM with the same age and BMI.

Furthermore, female pregnant with GDM had higher IL-1β levels and lower serum adiponectin in the second trimester than female without GDM. As a result, it appears that GDM is linked with a rise production of pro-inflammatory cytokines including IL-1β and lower production of adiponectin, an insulin sensitivity-related adipocytokine. These results are consistent with modern research that has related GDM pregnancies to low adiponectin levels in the second trimester and a rise levels of TNF-α, IL-6 and high sensitivity (hs) CRP, all indicators of a pro-inflammatory status. The present specific metabolic can moderated the functional nature and inflammatory phenotype of immune cell. May be immune cell undergo bath a proinflammatory and metabolic, which in their function. May be inflammatory mediators, such as, IL-1β levels is induced by saturated fatty acid and disrupt insulin signaling.

In the current research, it was discovered that IL-1β levels in GDM pregnancies follow a pattern close to that of other pro-inflammatory cytokines. pregnancy with GDM is a state of insulin resistance linked with a rise IL-1β values. Both of these changes may be linked to placental involvement in pregnancies with GDM.

### Table (5): IL-1β concentration for studied groups

<table>
<thead>
<tr>
<th>Control Groups</th>
<th>IL-1β concentrations (pg/mL)</th>
<th>No.</th>
<th>GDM Groups</th>
<th>IL-1β concentrations (pg/mL)</th>
<th>No.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont-1</td>
<td>1.10±0.14</td>
<td>75</td>
<td>GDM-1</td>
<td>1.03±0.12</td>
<td>75</td>
<td>0.358</td>
</tr>
<tr>
<td>Cont-2</td>
<td>0.93±0.16</td>
<td></td>
<td>GDM-2</td>
<td>2.97±0.50</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>p-value</td>
<td>0.677</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Conclusion

In late second trimester and early third trimester of pregnancy, IL-1β concentrations were increase in female with GDM in compare with healthy pregnant, therefore, IL-1β as an early biomarker can assist in the prediction the development of GDM later in pregnancy. The presence of GDM in pregnant patients lead to disturbances in FBG, Insulin, HOMA-IR and HbA₁c.

### References


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24. Antosh S, and Meduri A. Use of methyl pyruvate to increase cellular energy production downstream of glycolysis for the PARP-1 ablation of HIV without necrotic cell death caused by continuous, chronic PARP-1 activation through the concomitant depletion of ATP and NAD. United States patent application. 2006;10/904,648.


Prevalence of Pediatric Gastroenteritis Disease in Al-Diwaniyah Governorate-Iraq

Abdulridha T. Sarhan 1, Enas K. Alkhazraji 1, Takwa S. Al-Meamar 1
1 Research Scholar, Dentistry Department, Hilla University College, Babylon, Iraq

Abstract

This study was conducted to evaluate the gastroenteritis disease among hospitalized children in Women and Children Hospital, Al-Diwaniyah Governorate-Iraq, between August 2020 and January 2021. Data about age, gender, feeding, clinical symptoms and the causal agents were collected. A total of 723 children were screened, 616 patients (85.2%) of the infants and children under five years old with gastroenteritis disease, including 332 patient males (53.9%) and 284 patient females (46.1%). The lowest infection was recorded among 60 infant patients (9.7%), while the highest infections were recorded among 392 patients (63.6%) of one year old as compared to 164 patients (26.6%) among children above one to five years old. Also, results revealed two types of diarrhea, acute and chronic diarrhea. There was no significant difference between patients gender. The acute diarrhea was revealed the highest percentage of all cases (78.4%) and the remaining cases were chronic diarrhea (21.6%). Concerning the relation between the types of feeding and diarrhea, higher percentage (52.2%) was recorded with the bottle feeding, while the lowest percentage (20.1%) was with breast feeding. The clinical symptoms among infected children had gastroenteritis were diarrhea, stomach pains, vomiting, fever, urinary tract infection and malnutrition. Also, results showed that diarrhea infections usually happen in the seasons with high-moderate temperatures. This study concluded that most significant factors that caused the incidence of gastroenteritis in children were type of water, feeding or complementary feeding practices and mothers’ lack of care to reduce the risk of diarrhea among children under five years old.

Keywords: gastroenteritis, pediatric diarrhea, acute diarrhea, chronic diarrhea.

Introduction

The gastrointestinal tract is a vulnerable organ for infections as there is constant contact with the outside, mainly via the oral route(1). Gastroenteritis is a very common problem for children, major cause of morbidity and remains a major cause of hospitalization(2). It can lead to dehydration, which alters the children natural balance of water to electrolytes(3). Diarrhea can be dangerous if not treated properly because it drains water and salts from the children, if these fluids are not put back quickly, children may become dehydrated and may need to be hospitalized(4). Sometimes children with diarrhea have other symptoms, such as fever, loss of appetite, nausea, vomiting, stomach pains, and blood and/or mucus in the bowel movement(5). Globally, diarrhea is one of the leading causes of death among children under five year old(6). Acute diarrhea lasts less than one week, while chronic diarrhea lasts for 14 days or more and these include patients who were presented at first with acute diarrhea. The most common cause of acute diarrhea is a viral, bacterial, fungal or parasitical...
infection\(^7\). The evaluation of diarrhea in children requires a careful review of medical history, a physical examination and occasion diagnostic testing\(^8\). Other causes include the side effects of antibiotics and infections not related to the gastrointestinal system, such as ear infection that can cause diarrhea\(^9\). Most children with bacterial infection do not require antibiotics and will improve with time and supportive measures, antibiotics can cause side effects and lead to development of antibiotic resistance\(^ {10,11}\). Signs and symptoms of mild dehydration include a slightly dry mouth, increased thirst, and slightly decreased urine output\(^ {12,13}\). This study was done to evaluate gastroenteritis disease among hospitalized children and determine the proportion of acute and chronic diarrhea in infants and children less than five years old and the associated risk factors.

Materials and Methods

This study was carried out in Women and Children Hospital, Al-Diwaniyah Governorate-Iraq, between September 2020 and January 2021. A total of 723 children were screened, 616 patients which presented gastroenteritis aged between 2 months to 5 years old were examined. The information of admitted children to the hospital during this period was obtained from the statistical department of the hospital. A questionnaire form were used for all patients with gastroenteritis, the following data were recorded: name, age, gender, date of infection, address, duration of diarrhea, type of feeding, previous hospitalization for diarrhea and clinical symptoms appeared on the patients. Laboratory data where ever available were included in the study, like: assessment of hemoglobin level, stool examination for parasite, leukocytes, red blood cells, pH and urine samples for microbial culture. The evaluation of gastroenteritis in children requires a careful review of them medical history, a physical examination, physical examination and occasion diagnostic testing. The clinician will perform a thorough examination because there are some infections unrelated to the bowels (such as an ear infection) that can cause diarrhea. Bloody diarrhea that contains mucus is somewhat more common with bacterial infection which persistent high fever. Diarrhea from microbial infections may last longer than two weeks. For infants with chronic diarrhea, the total days of hospital stay were calculated from time of admission (either less than or more than 5 days). Data about different therapeutic interventions for patients with chronic diarrhea were obtained. The data were collected and analyzed by the statistical method, analysis of variance (ANOVA).

Results and Discussion

During the period of this study, a total of 723 patients admitted to the hospital, 616 patients of the infants and children under five years old were with diarrheal diseases represent (85.2\%) of total hospitalization of children to the hospital, including 332 (53.9\%) patients males and 284(46.1\%) patients females. It was found that the incidence of infection in male is higher than in female, this is consistent with De Camp et al.\(^ {14}\), who indicated that the occurrence of infection is higher in males than females. Overall, lower infection 9.7 \% was in infants represent 60 patients and the higher infection 63.6 \% represents 392 patients was in children less than one year oldof all those less than five years old children admitted to the hospital with diarrhea compared to 164 patients which represent (26.7 \%) in those above one to five years old (Table 1). The acute diarrhea cases were revealed the highest percentage of all cases(78.4 \%) and (21.6 \%) is for chronic cases. These results were confirmed by Pérez-Gaxiola et al.\(^ {15}\).
Table 1: The age of hospitalized children with acute and chronic diarrhea.

<table>
<thead>
<tr>
<th>Type of diarrhea</th>
<th>Less than 2 months old</th>
<th>From 2 months – 1 year old</th>
<th>From 1 - 5 years old</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Acute diarrhea</td>
<td>42</td>
<td>6.8</td>
<td>313</td>
<td>50.8</td>
</tr>
<tr>
<td>Chronic diarrhea</td>
<td>18</td>
<td>2.9</td>
<td>79</td>
<td>12.8</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>9.7</td>
<td>392</td>
<td>63.6</td>
</tr>
</tbody>
</table>

LSD at $P = 0.05$ between types of diarrhea = 2.14 and gender = 2.81

The data in (Table 2) revealed the results of the two types of diarrhea, acute and chronic diarrhea according to gender, there was a significant difference between the males and females, and also between acute and chronic diarrhea. The percentages of children with acute diarrhea were (79.5% vs. 77.0%) and chronic diarrhea were (20.5% vs. 23.0%) respectively, Block et al.\(^{(16)}\).

Table 2: Distribution of children with acute and chronic diarrhea according to the gender.

<table>
<thead>
<tr>
<th>Type of diarrhea</th>
<th>Gender</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (No. = 342)</td>
<td>Female (No. = 274)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Acute diarrhea</td>
<td>272</td>
<td>79.5</td>
<td>211</td>
</tr>
<tr>
<td>Chronic diarrhea</td>
<td>70</td>
<td>20.5</td>
<td>63</td>
</tr>
</tbody>
</table>

LSD at $P = 0.05$ between types of diarrhea = 3.44 and gender = 1.81

Concerning type of feeding, nearly half of the patients with diarrhea whether acute or chronic who received bottle feeding only. A high percentages were (53.0% vs. 49.5) of infants and children of less than one year old with acute and chronic diarrhea respectively were bottle feeding compared to (25.9% vs. 34.0%) who used mixed feeding and (21.1% vs. 16.5%) of breast feeding (Table 3), This is consistent with the results of Zivichet al.\(^{(17)}\).
Table 3: Types of feeding among infants and children less than one year old with acute and chronic diarrhea.

<table>
<thead>
<tr>
<th>Type of feeding</th>
<th>Acute diarrhea</th>
<th>Chronic diarrhea</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Breast feeding</td>
<td>75</td>
<td>21.1</td>
<td>16</td>
</tr>
<tr>
<td>Bottle feeding</td>
<td>188</td>
<td>53.0</td>
<td>48</td>
</tr>
<tr>
<td>Mixed feeding</td>
<td>92</td>
<td>25.9</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>355</td>
<td>78.5</td>
<td>97</td>
</tr>
</tbody>
</table>

LSD at P = 0.05 between types of feeding = 5.59 and between types of diarrhea = 4.16

Results in Table 4 show the clinical symptoms among infants and children less than one year old with acute and chronic diarrhea, 107(23.7%) patients had stomach pains, 83(18.4%) patients had vomiting. Other significant symptoms include fever 75(16.6%) patients, urinary tract infections 86(19.0%) patients and ear infection 39(8.6%) patients, in addition to malnutrition 62(13.7%) patients. When sick children have diarrhea or vomiting, they can lose large amounts of salts and water from their bodies and can become dehydrated very quickly\(^{(18)}\). Dehydration can be very dangerous, especially for babies and toddlers. Children can even die if they are not treated\(^{(19,20)}\).

Table 4: Identification of the clinical symptoms among infants and children with acute and chronic diarrhea less than one year old.

<table>
<thead>
<tr>
<th>Type of symptoms</th>
<th>Children with acute and chronic diarrhea less than one year old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (Total = 452)</td>
</tr>
<tr>
<td>Stomach pains</td>
<td>107</td>
</tr>
<tr>
<td>Vomiting</td>
<td>83</td>
</tr>
<tr>
<td>Fever</td>
<td>75</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>86</td>
</tr>
<tr>
<td>Ear infection</td>
<td>39</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>62</td>
</tr>
</tbody>
</table>
Also, results in Table 5 are showed that diarrhea infections usually happen in the periods with high temperatures (August and September), this means that outbreak of diarrhea infections usually happen in the warm season which not consistent with the results of William et al.\cite{21} who reported that diarrhea usually happen in cold season. The clinical support for infected children with acute gastroenteritis is very important for recovering from infection.

### Table 5: Distribution of children with acute and chronic diarrhea according to the period of research.

<table>
<thead>
<tr>
<th>Period of research</th>
<th>Less than 2 months old</th>
<th>From 2 months to 1 year old</th>
<th>From 1 - 5 years old</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>August / 2020</td>
<td>8</td>
<td>6</td>
<td>58</td>
<td>52</td>
</tr>
<tr>
<td>September / 2020</td>
<td>4</td>
<td>2</td>
<td>50</td>
<td>43</td>
</tr>
<tr>
<td>October / 2020</td>
<td>8</td>
<td>5</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>November / 2020</td>
<td>6</td>
<td>3</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>December / 2020</td>
<td>5</td>
<td>2</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>January / 2021</td>
<td>6</td>
<td>4</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>22</td>
<td>203</td>
<td>177</td>
</tr>
</tbody>
</table>

LSD at $P = 0.05$ between months $= 1.78$ and between ages of children $= 4.22$

**Conclusions**

As conclusions from this study, it observed that diarrhea was a common infection in infants from two months to one year old and there were two types of diarrhea (acute and chronic). The higher appearance of diarrhea was happened at the season with high and moderate temperature (August and September). Concerning the geographic characteristics, the rural area showed higher percentage of infections than urban area. Also, the most predominant infections occurred with children received bottle feeding. There were other clinical symptoms among children with chronic diarrhea, urinary tract infection and ear infection. Our findings highlight the high prevalence of *C. albicans*, *E. coli* and *E. histolatica* infections as the major cause of pediatric gastroenteritis in hospitalized children.

**Acknowledgment:** We would like to thank the manager of the Women and Children Hospital, Al-Diwaniyah Governorate-Iraq and the staff in the statistics department for their assistance in carrying out this research.

**Ethical Clearance:** Taken from Hilla University College ethical committee

**Source of Funding:** Self

**Conflict of Interest:** Nil

**References**

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Patients’ Satisfaction about Quality of Health Care Services Provided in Maternity Wards at Al-Najaf City

Abeer Miri Abdullah¹, Rehab Lafta Mohammad¹, Zainab Neamat Jumaah Altaei¹
¹Research Scholar, University of Kufa, Faculty of Nursing, Iraq

Abstract

The study aimed to assess patients’ Satisfaction about Quality of Health Care Services Provided in maternity wards at Al-Zahraa teaching hospital at Al-Najaf city. A descriptive study was carried out through the present study in order to achieve the early stated objectives. The study was began from November, 2nd, 2019 to April, 22, 2019. The finding of this study reveals that that the patients are unsatisfied about quality of health care services provided at wards for most domain of health care services quality, while there is few patients satisfied about some health care services provided for them. The study concluded that most patients are unsatisfied about quality of health care services that provided at maternity wards. Recommendations The study recommends Assessing quality of health care services in the wards continuously, so that we can apply the quality improvement and/or quality assurance in wards, The domains and items related to the quality of health care services must be educate to health worker (physician, nurse … etc.) to teach them how they can apply it in the wards.

Keywords: Satisfaction, Quality, Health care services, Maternity Wards

Introduction

Patient satisfaction is deemed to be one of the important factors which determine the success of health care facility. The real challenge is not getting ready with mere requirements, but also delivers services ensuring good quality. Thus, there is a need to assess the health care systems regarding patient satisfaction as often as possible. The purpose of health care services is to improve the health status of the population. The scope of health services varies widely from country to country and influenced by general and ever changing national, state and local health problems, needs and attitudes as well as the available resources to provide these services. There is now broad agreement that health services should be comprehensive, accessible, acceptable, provide scope for community participation and available at a cost the community and country can afford(1). Patient satisfaction is deemed to be one of the important factors which determine the success of health care facility. It is easier to evaluate the patient’s satisfaction towards the services provided than to evaluate the quality of medical services that they receive(2). Therefore, a research on patient satisfaction can be an important tool to improve the quality of services. Health care consumers today, are more sophisticated than in the past and now demand increasingly more accurate and valid evidence of health plan quality. Health care organizations are operating in an extremely competitive environment, and patient satisfaction has become a key to gaining and maintaining market share. The health care system depends on availability, affordability, efficiency, feasibility and other factor (3)

Consumer satisfaction regarding medical care organization like our tertiary care hospital is important in the provision of services to patients. This study was therefore undertaken with the aim to find out the level
of patient satisfaction related to different parameters of quality of health care (4). Improving maternal health is one of the eight major developmental aims of the third millennium of World Health Organization (WHO) 2000 summit. Two important criteria for achieving this goal are reducing the maternal mortality ratio by three quarters compared to the year 1990, and improving women’s access to reproductive health services by 2015 (5). Today, maternal mortality is not only a health indicator but also one of the indicators used in developing communities, and shows the extent of care of the societies regarding maternal health (6). WHO has set its main activity goal as reducing the maternal and children mortality, and one of the key strategies for achieving this goal is providing parental care and care during and after delivery (7).

The main purpose of providing health care services is to improve public health through the provision of desirable and necessary health and treatment services. Improvement of quality of care requires evaluation of the quality of services and the level of clients’ satisfaction with the services provided (8) and (9). Patient satisfaction is a concept that is of a particular importance in today’s health care (10). Patient satisfaction depends on the health care that the patient receives, and this issue is very important. Satisfied clients compared with unsatisfied clients show different responses to the care services received. Satisfied clients adapt to the recommendations and follow them, and often invite other people to use these services. Therefore, satisfied clients not only continue using the healthcare services, but also promote their use (6).

Worldwide an estimated 515 000 women die of complications of pregnancy and childbirth every year, a rate of over 1400 maternal deaths each day (11). At least 7 million women who survive childbirth suffer serious health problems, and a further 50 million women suffer adverse health consequences after childbirth. The overwhelming majority of these deaths and complications occur in developing countries. Maternal mortality is the main factor that substantially lowers the life expectancy of women and it is a basic human right that pregnancy be made safe for all women (12), (13). Improving the quality at maternity care services is an effective strategy to reduce maternal mortality (14). The World Health Organization (WHO) emphasizes the importance of evaluating the structure, process and outcome of health services to improve the quality of care (15).

Methods

A descriptive study was carried out through the present study in order to achieve the early stated objectives. The study was began from November, 2nd , 2019 to April, 22, 2019. The study was conducted in Al-Najaf City / in maternity wards at Al-Zahraa teaching hospital. The study was carried out with 100 patients after excluding those who declined to participate. Data were collected by face-to-face interviews.

Results

Table (1) Statistical distribution of patients group by their Socio-Demographic Data

<table>
<thead>
<tr>
<th>Items</th>
<th>Sub-groups</th>
<th>Patients group Total = 200</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Percentage</td>
</tr>
<tr>
<td>Age / Years</td>
<td>14-19</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>20-25</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>26-31</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>32-37</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>38-43</td>
<td>4</td>
</tr>
</tbody>
</table>
Table (1) shows statistical distribution of patients group by their socio-demographic data, it explains that the majority of the patients subgroup are: patients with ages between (20-25) years old (38%), married patients (77%), those who live urban residents (74%), those who are illiterate (23%) , house wife patients (74%), and patients with adequate to some extent economic status (56%).

Table (2) shows that the patients’ unsatisfied toward Health Care Services in related to overall assessment, as the mean of scores is (2), above which each score is considered (unsatisfied), below which is considered as (Satisfied).

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>Single</th>
<th>6</th>
<th>6.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Married</td>
<td>77</td>
<td>77.0</td>
</tr>
<tr>
<td></td>
<td>Widowed</td>
<td>3</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Divorced</td>
<td>12</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Separated</td>
<td>2</td>
<td>2.0</td>
</tr>
<tr>
<td>Residency</td>
<td>Rural</td>
<td>26</td>
<td>26.0</td>
</tr>
<tr>
<td></td>
<td>Urban</td>
<td>74</td>
<td>74.0</td>
</tr>
<tr>
<td>Levels of Education</td>
<td>Illiterate</td>
<td>23</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>Able to read and write</td>
<td>22</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>Primary school graduated</td>
<td>22</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>Intermediate school graduated</td>
<td>11</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>preliminary school graduated</td>
<td>8</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>Institutes</td>
<td>6</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>College</td>
<td>8</td>
<td>8.0</td>
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<tr>
<td>Occupation Status</td>
<td>Employee</td>
<td>10</td>
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<td></td>
<td>Free business</td>
<td>9</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>Retired</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>House wife</td>
<td>74</td>
<td>74.0</td>
</tr>
<tr>
<td></td>
<td>Jobless</td>
<td>4</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>student</td>
<td>3</td>
<td>3.0</td>
</tr>
<tr>
<td>Economic Status</td>
<td>adequate</td>
<td>23</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>Adequate to Some Extent</td>
<td>56</td>
<td>56.0</td>
</tr>
<tr>
<td></td>
<td>Inadequate</td>
<td>21</td>
<td>21.0</td>
</tr>
</tbody>
</table>
Table (2) : Domain and overall Assessment of Patients’ Satisfaction about Health Care Services

<table>
<thead>
<tr>
<th>Items</th>
<th>MS</th>
<th>RS</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tangibility</td>
<td>2.18</td>
<td>72.57</td>
<td>Unsatisfied</td>
</tr>
<tr>
<td>Reliability</td>
<td>2.36</td>
<td>78.67</td>
<td>Unsatisfied</td>
</tr>
<tr>
<td>Response</td>
<td>2.51</td>
<td>83.67</td>
<td>Unsatisfied</td>
</tr>
<tr>
<td>Empathy</td>
<td>2.45</td>
<td>81.53</td>
<td>Unsatisfied</td>
</tr>
<tr>
<td>Assurance</td>
<td>2.38</td>
<td>79.33</td>
<td>Unsatisfied</td>
</tr>
<tr>
<td>Overall Assessment</td>
<td>2.38</td>
<td>79.15</td>
<td>Unsatisfied</td>
</tr>
</tbody>
</table>

**MS : Mean of Scores ; RS : MS : Ratio of Score**

Discussion

Concerning their age, the majority of study sample are at age group of (20-25) years. This result match with the result of (12) in their study “Quality of maternity care and satisfaction of patients attended teaching and non-teaching hospital” They conclude (most women at age (≥20 years). Regarding the marital status, the present study shows that the majority of sample in the study groups are married (16). They pointed in their study that the highest percent of study sample were married.

In addition, the study results indicate that the majority of the research sample are living in urban area (17), they revealed in their study that the majority of the study subjects are urban residents.

Concerning the socio-economic status, the highest percentage of study sample is Satisfied to some extent. High percent of the study sample does not read and write (18). In agreement with this result, they found that the majority of study subject were Illiterate. Regarding to the occupation status, the present study sample shows the high percentage (74.0%) were housewives. This result is supported by a study done by (4) as his result indicated that the higher percentage of study sample were housewives.

Regarding patients satisfaction concerning tangible domain of quality of health care services table (2), that based on the subjects’ responses, the results indicate that in the following items (In ward all amenities are provided such as continuous electricity, water, sanitation, ventilation and unpleasant odors) and (ward provides all required medication in the pharmacy) the responses of the study subjects are acceptable, while in the remaining responses for the services quality in the ward are insufficient to the patients’ requirements. This means that they are unacceptable for most of the subjects. The researcher suggest this result comes because the hospitals are provide an appropriate material and equipment that is enough for patients need. While not provided materials are not affect on patients satisfaction because this materials don’t directly related to the patients need. In addition, the subjects’ responses regarding reliability domain of the QHCS table (2), the study show that services of the ward are not adequate to the patient’s requirements at all items.

Also regarding responsiveness domain of the quality of health care services table (2), the study indicates that services are insufficient to meet the patients’ requirements at all items. Furthermore, the subjects’ responses regarding empathy and assurance domains of the quality of health care services respectively, the subjects responses are insufficient to their requirements at all items. This result disagree with the result of the study done by (19) who showed in his study that health care services that provided in public hospitals within acceptable and satisfying level. The researcher believe the reasoned is that most of nurses who work in maternity ward not graduated from institutes and with
poor knowledge and experience to caring patients. The study shows that patients are unsatisfied with the quality of health care services provided at the wards table (2), tangibility, reliability, responsiveness, empathy and assurance dimensions of the health care services quality, this results disagree with the results of the study done by (19) they mentioned that the majority of the patients are satisfied with health care services, but this result in agreement with study done by (20). they stated in their study that the quality of health services provided in health centers according to the dimensions of quality of health services (tangibility, reliability, power of responsiveness, empathy, trust and safety) are unsatisfied.

**Conclusion**

According to the present study findings, the researcher can mention the following conclusions: Most patients are unsatisfied with the services provided in the wards regarding all domain of healthcare services. There is satisfied from few of patients about the health care services provided in the wards regarding tangible domain of healthcare services related to (medication, continuous electricity, water, sanitation, ventilation). As general the study indicates that most of patients are unsatisfied toward all quality of health care services domains.

**Ethical Clearance**: Taken from University of Kufa ethical committee

**Source of Funding**: Self

**Conflict of Interest**: Nil

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An Epidemiological Study of Homicidal Cases Autopsied in the Mortuary of the Department of Forensic Medicine and Toxicology, North Bengal, Darjeeling

Achintya Biswas¹, Pappu Kumar², Sukanta Majumdar³, Soumeek Chowdhuri⁴, Somasish Ghosal⁵, Prabir Kumar Deb⁶

¹Associate Professor, Department of Forensic and State Medicine, Calcutta National Medical College & Hospital, Kolkata, ²Medical Officer, Raiganj Govt. Medical College & Hospital, Uttar Dinajpur, ³Assistant Professor, Department of Community Medicine, ⁴Assistant Professor, ⁵PhD Research Scholar, Department of Forensic and State Medicine, Calcutta National Medical College & Hospital, Kolkata, ⁶Professor, Department of Forensic Medicine & Toxicology, North Bengal Medical College, Darjeeling

Abstract

Homicide is regarded as a notorious crime against the society that causes intentional killing, aggravated assaults resulting in death. This brings a massive burden on national economies, law enforcement and lost productivity. The present study highlighted the epidemiological profile of homicidal deaths which accounted (3.9%) of all unnatural deaths autopsied. Overall trend shows high male predominance with low economic background where illiteracy, poverty and other social circumstances play a considerable role. Homicidal records have their importance in interpretation of socio-economic implications and overall administrative attributes on executing law and order in respect to time, place and conditions.

Keywords: Forensic medicine, homicidal cases, epidemiology.

Introduction

Homicides are heinous crime against the society and there has been a global increase in homicide that causes over 5000,000 deaths per year worldwide [1]. It includes premeditated murder, intentional killing, and aggravated assaults causing death. It may be a result of arguments between acquaintances, domestic violence, robberies, drug addiction and terrorism [2]. Homicides are regarded as one of the oldest crimes in human civilization. It is a common end point of many different behavioral pathways [3]. Grossly, death that occurs from violence also encompasses a wide range of physical, sexual, reproductive and mental health problems that imposes massive burden on national economics, law enforcement and lost productivity. In addition, homicide results in significant personal, social and economic loss [4].

As homicides comprise a major portion of medico-legal autopsies, they get special importance for general criminal profile of the society [5]. Medico-legal autopsies provide statistical significance related to legal incidents in the society along with the cause and manner of death [6]. In the present day world, violence is recognized as a global public health problem. It accounts for 9% of global mortality and 12% of all disability adjusted life years (DAILY) [7].

As per Global Burden of Armed Violence Report (2011), the average annual global violent death rate between 2004 and 2009 were 79 per million. Globally...
around 520,000 people die each year as a result of interpersonal violence which equates to 1400 deaths every single day [8]. Although official data from the National Crime Records Bureau (NCRB) shows that murder rate in India has been steadily declining over the past two decades [9], still it needed to be assessed from time to time.

There is a pressing need for socio-demographic studies on homicidal cases with rising trends of organized crimes being executed in a professional manner. Although quite a good number of work have already been done in different parts of India and in other countries, not much work have been reported from North Bengal regions. The present study shows the different aspects of homicides in relation to victims with special emphasis on its epidemiologic profile.

**Materials and Method**

In the present study all homicidal cases brought for medico-legal autopsies in the mortuary of North Bengal medical college and hospital (NBMCH) attached to the Department of Forensic Medicine and Toxicology, West Bengal, during the period of 1st May, 2018 to 30th April, 2019 were evaluated. This cross-sectional study included a total 94 cases of homicidal deaths. Autopsies on all cases of alleged homicidal deaths were included in the study, whereas, all cases of natural, suicidal deaths and either decomposed or unidentified bodies where autopsy findings were not suggested to homicidal mode of death even with the history of homicide were excluded from the study.

Parameters used in the study were police/ magistrate inquest report, history obtained from family members and autopsy findings. A descriptive study was designed to explore the epidemiologic profile of homicidal cases. Data was analyzed using SPSS software.

**Results**

In the present investigation of total 2423 unnatural deaths autopsied during the study period 94 cases (n=94) were homicidal (3.9%).

As per distribution of age (as shown in Table 1), it was found that 39.6% of victims were in the age group of 31-40 years. 23.4% victims were aged between 51-60 years. 18% victims were aged between 41 to 50 years. 7.4% victims were young adults aged between 21-30 years of age group. Only 3.1% victims were aged above 70 years.

As per distribution of sex (as shown in Table 2), it was observed that most of the victims (76.6%) were male and few (23.4%) were female. As per month and year wise distribution of victims (n= 94), the incidences of homicide were maximum (20.2 %) in the month of December 2018, followed January 2019 (17.1 %), March 2019 (10.6%), May 2018 and April 2019 (8.5%), October 2018 (6.4%), August 2018 (5.4%), November 2018 (5.3%), September 2018 (4.2%), June 2018 (3.2%) and July 2018 (2.1%).

Regarding seasonal variation, homicidal incidences were maximum in winter (51%) in the months of Nov-Feb, followed by summer (30.9%) and Monsoon (18.1%) as shown in Table 3. As per religion wise distribution, incidences of homicides were maximum in Hindus (69.14%) followed in Muslim (27.65%) followed Buddhist (2.12%) and Christian (1.06%). As per demographic distribution, rural cases comprises 66% and 34% belonged to urban area.

Educational status distribution, the maximum number observed (51.1%) of victims were illiterate followed by primary (34%), secondary (9.6%) and graduate (2.1%). Socio-economic status of the homicidal victims showed majority (70.2%) from low, followed by 25.5% middle class and 4.3% of high class. Of the distribution in marital status married victims were the major (80.9%), followed by unmarried (14.9%) and low among widower (4.2%). Among distribution of occupational status majority of victims were workers (34%), followed by businessman (22.3%), farmers (16%), housewife (12.8%), private employee (7.4%), retired persons (4.3%) and government employee only (3.2%).
As per distribution on time of incidence, most of the incident of homicide happened during late evening (33%) between 7pm to 10pm, followed by evening (23.4%), morning (12.8%), early morning (8.5%), midday (7.4%), afternoon and night (6.4%) and late night (2.1%). Distribution on place of occurrence showed majority of the incidence occurred in the public place (62%) and (32%) occurred at home. Majority of the victims (74.5%) did not get any medical care where only (25.5%) victims received it. As per methods of homicidal injury is concerned blunt weapon was used in maximum cases (45.7%), followed by heavy sharp weapon (23.4%), light sharp weapon and stab injury (9.6%), manual force (6.2%), firearm (3.2%) and strangulation (2.1%). Among the cause of death, instantaneous death (47.9%) was the most common followed by shock and hemorrhage (25.5%), coma (17%) and asphyxia (9.6%). By the distribution of the homicidal victims according to the region of injury, brain was the commonest organ to get injured (36.3%), followed by lungs (10.6%), stomach (7.5%), heart and intestines (6.4%), liver (4.2%), pancreas and kidneys (2.1%) and multiple organ involvement was observed in 22.3%.

Table 1: Age distribution of victims (n=94)

<table>
<thead>
<tr>
<th>Age in Year</th>
<th>No of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>11 – 20</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>21 – 30</td>
<td>07</td>
<td>7.4</td>
</tr>
<tr>
<td>31 – 40</td>
<td>37</td>
<td>39.6</td>
</tr>
<tr>
<td>41 – 50</td>
<td>17</td>
<td>18.0</td>
</tr>
<tr>
<td>51 – 60</td>
<td>22</td>
<td>23.4</td>
</tr>
<tr>
<td>61 – 70</td>
<td>08</td>
<td>8.5</td>
</tr>
<tr>
<td>&gt;70</td>
<td>03</td>
<td>3.1</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Sex distribution of victims (n=94)

<table>
<thead>
<tr>
<th>Sex</th>
<th>No of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>72</td>
<td>76.6</td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td>23.4</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 3: Season wise distribution of victims

<table>
<thead>
<tr>
<th>Season</th>
<th>No of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter (Nov-Feb)</td>
<td>48</td>
<td>51.0</td>
</tr>
<tr>
<td>Summer (March – June)</td>
<td>29</td>
<td>30.9</td>
</tr>
<tr>
<td>Monsoon (July – Oct)</td>
<td>17</td>
<td>18.1</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>100</td>
</tr>
</tbody>
</table>

Discussions

In the present investigation of total 2423 bodies brought for medico-legal autopsies in mortuary of NBMCH during the period of 1st May, 2018 to 30th April, 2019, 94 cases were sorted for homicidal which accounted (3.9%).

Prior study from Bangalore [10] showed that this age group was accounted for 61.50% cases of homicidal death. The factors responsible for highest incidents in the 18-40 years age groups (64.3%) were due to marital disputes, property disputes, infidelity, dowry death in females, gang rivalry, unemployment and heated arguments. Our study showed highest incidence (39.6%) in age group of 31 to 40 years.

Sex wise distribution showed 3.27 times more in male compared to female. The cause for male predominance may be because of their number, unemployment, jealousy, revenge, lack of patience, intake of alcohol and bad accomplices etc. the lower incidence in females is mainly attributed to custom, social values and preference of females to stay indoors.

In consistent to the study done by Sisti et al. (2012) [11] in Italy, with maximum homicidal death in a bimodal pattern, summer (July and August) and winter (December and January) seasons, our study showed maximum (20.2%) in the month of December with 51% in winter season (Nov-Feb).

The majority of victims (51.1%) were from illiterate backgrounds. This could be related to poverty, unemployment, prevalence of multicast system of the society and inability to solve the dispute by fruitful peaceful discussions.

Conclusion

However, trends of homicidal deaths differ from country to country, region to region and from time to time based on overall economic turbulences and social circumstances. Still deaths related to homicides are commonly found in low economic class with male predominance where illiteracy, poverty and mistrust definitely play a considerable role.

Therefore, along with the socio-economic improvement, the crime investigating agencies including medico-legal aspects and judiciary system has to be strengthened so that the law can be enforced stringently. Understanding the burden of issue on the socio-economical, cultural and multidirectional implications, continuous research in this field is necessary to validate data from different time, place and conditions.

Ethical Clearance: Taken from the Institutional Ethics Committee, North Bengal Medical College, Darjeeling.

Source of Funding: Nil

Conflict of Interest: Nil
References


Increased Corticosterone Levels due to Chronic Stress Reduces IGF-II Expression in *Rattus norvegicus*

Ade Septiari Rahman¹, Reny I’tishom², Ashon Sa’adi³

¹Master Degree of Reproductive Health Science Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia, ²Lecturer in Department of Medical Biology Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia, ³Lecturer and Consultant in Department of Obstetrics and Gynecology Faculty of Medicine Universitas Airlangga/ Dr. Soetomo General Hospital, Surabaya, Indonesia

Abstract

The optimal folliculogenesis process describes a good condition of the female reproductive system. One of the main growth factors in the folliculogenesis is IGF-II which is important for proliferation and differentiation of granulosa cells and also steroidogenesis. Chronic stress can cause ovulation failure and folliculogenesis disorders. Increased corticosterone levels due to chronic stress will cause disruption in the GnRH pulse, causing a decrease in the gonadotropin and lead to folliculogenesis disorders, and decrease IGF-II production. This study aims to prove the effect of increased corticosterone levels due to chronic stress on IGF-II expression in the ovaries of Rattus norvegicus. The research sample consisted of 34 Rattus norvegicus which were divided into 2 groups. The treatment group was given Chronic Unpredictable Mild Stress (CUMS) method for 20 days. Corticosterone levels were detected by the ELISA method as a marker of chronic stress and IGF-II expression was evaluated by immunohistochemical methods. The results of statistical tests showed that there were significant differences in corticosterone levels (p = 0.000) and IGF-II expression (p = 0.042) in the two groups (p <0.05). So the conclusion is increased corticosterone levels due to chronic stress can reduce IGF-II expression in the ovaries of Rattus norvegicus.

**Keywords:** Corticosterone, Chronic Stress, CUMS, IGF-II, Folliculogenesis

Introduction

Infertility problems are experienced in women as much as 35% of all cases(1). According to WHO, the most common causes of infertility in women are ovarian failure, which is 33% was caused by folliculogenesis disorders(2). Stress that that occurs continuously or chronic stress can cause ovulation failure and folliculogenesis disorders, and can subsequently lead to infertility.

**Corresponding Author:**
Dr. Ashon Sa’adi, MD, SpOG-K
Lecturer and Consultant
Department of Obstetrics and Gynecology Faculty of Medicine Universitas Airlangga/ Dr. Soetomo General Hospital; Tambaksari, Surabaya, East Java, Indonesia
E-mail: ashon.saadii@fk.unair.ac.id
Phone: +628170121121

Stress has been shown to inhibit oocyte maturation and produce many immature oocytes(3), and reduce the number of dominant follicles(4). Stress also causing a decrease in the number of fertilized oocytes, pregnancy rates, live births and birth weight in women undergoing in vitro fertilization (FIV)(5). Chronic stress has also been shown to reduce the thickness of the antral follicular granulosa cells, and as a result, folliculogenesis is disrupted(6).

Chronic stress can cause the distress condition, which causes the hypothalamus to produce Corticotrophin Releasing Hormone (CRH)(7), and then the anterior pituitary will produce Adenocorticotrophine Hormone (ACTH) which stimulates the adrenal glands to produce glucocorticoid hormones, namely cortisol(7,8). Glucocorticoid synthesis in response to chronic stress
will further impair reproductive function\textsuperscript{(7,9)}.

There are various kinds of growth factors that help the folliculogenesis process. One of them is the main and most abundant growth factor in the human ovarian system during folliculogenesis, namely Insulin-like Growth Factor-II (IGF-II). IGF-II plays a role in cell growth, proliferation, differentiation and acts as a regulator of the remodeling of the extracellular matrix of granulosa cells\textsuperscript{(10)}. In addition, IGF-II also increases the production of estradiol and progesterone by granulosa cells\textsuperscript{(11)}.

Increased levels of cortisol (corticosterone in rattus) due to chronic stress can indirectly interfere with GnRH pulses, causing a decrease in the amount of gonadotropin hormones. Because gonadotropins stimulate IGF production, the IGF-II formed will be reduced\textsuperscript{(7,9)}. Activation of IGF-II from the IGF-I and AKT receptors required FSH to stimulate CYP19A1 expression and granulosa cell proliferation. So with reduced IGF-II production, it will cause cell proliferation and steroidogenesis disrupted, followed by folliculogenesis disorder\textsuperscript{(11,12)}.

This study aims to prove that an increase in the hormone corticosterone due to chronic stress can result in a decrease in IGF-II expression in \textit{Rattus norvegicus}.

**Material and Method**

The design of this research is True Experimental with Post Test Only Control Group design. The study was conducted from April to June 2019 at the Animal Laboratory, Department of Biology, Faculty of Science and Technology, Airlangga University. The sample unit in this study was female rats (\textit{Rattus norvegicus}) 5-6 months old who had given birth, weighing 300-350 g and in good health. All sample units were adapted for 7 days and injected PGF2\alpha at a dose of 25 µg/gBW intraperitoneally to synchronize lust. This aims to get the diestrus phase at the time of surgery. Female rats were divided into 2 groups consisting of 17 individuals per group. The treatment was carried out using the Chronic Unpredictable Chronic Stress (CUMS) method, which is a model of giving minor intensity stressors that resemble stressors of daily life for several weeks\textsuperscript{(13)}. In this study, stress exposure was given for 20 days with 8 forms of treatment.

Ovarian tissue samples are taken surgically, and blood samples are taken intracardially. Corticosterone levels taken from the blood were detected by the ELISA method. IGF-II expression was detected by immunohistochemistry. The data obtained were then converted with the Remelle Scale Index. Statistical tests were performed using SPSS 25 software. The data obtained were first tested for normality by the Kolmogoroff-Smirnov and Shaporo-Wilk tests. Followed by a parametric statistical test, the Independent T-Test. If the data is not normally distributed, the Mann Whitney statistical test is used.

**Results and Discussion**

The sample unit in this study was 34 \textit{Rattus norvegicus} which were divided into two groups. The control (K1) and treatment (K2) groups each consisted of 17 \textit{Rattus norvegicus}. In the K1 group, there were two samples whose corticosterone level were undetectable, so they were excluded. In the K2 group, there was one sample unit that dropped out due to death. The death of \textit{Rattus norvegicus} occurred on the 16th day of the stressor treatment. Thus, the final number of sample units for each group is 15 in K1 and 16 in K2. This number is qualify the minimum sample size in this study (15).

**Table 1. Mean and SD of corticosterone levels in the blood serum of \textit{Rattus norvegicus}**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean ± SD (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>15</td>
<td>23.29 ± 8.42</td>
</tr>
<tr>
<td>K2</td>
<td>16</td>
<td>72.84 ± 64.03</td>
</tr>
</tbody>
</table>

The mean of corticosterone levels in the K2 group (72.84 ± 64.03) were higher than the K1 group (23.29 ± 8.42).
The results of the Mann Whitney test showed p=0.000 (p < 0.05), it means that there was a significant difference in corticosterone levels between the K1 and K2 groups.

The examination of IGF-II expression using immunohistochemical methods. Observation of IGF-II expression was carried out using a microscope connected to a computer with 64-bit Nikon NIS-Elements F-4.60.00 software. IGF-II expression can be seen in Figure 1.

![Figure 1](image)

**Figure 1.** Comparison of IGF-II expression in ovarian tissue of Rattus norvegicus. Detected with IGF-II monoclonal antibody with Immunohistochemical method, 200x magnification. Cells expressing IGF-II are indicated by a brown appearance indicated by red arrows. (A) Expression of IGF-II in the K1 group (B) Expression of IGF-II in the K2 group.

**Table 3.** Mean and SD of IGF-II expression in the ovary of Rattus norvegicus

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean ± SD (IRS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>15</td>
<td>3.73 ± 1.79</td>
</tr>
<tr>
<td>K2</td>
<td>16</td>
<td>2.44 ± 1.15</td>
</tr>
</tbody>
</table>

Based on the table, the mean of IGF-II expression in the K2 group was lower (2.44 ± 1.15) than the K1 group 3.73 ± 1.79.

**Table 4.** Mann Whitney analysis results of IGF-II expression in the ovary of Rattus norvegicus

<table>
<thead>
<tr>
<th>P Value</th>
<th>Different tes analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.042*</td>
<td>Mann Whitney test</td>
</tr>
</tbody>
</table>
The results of the Mann Whitney test showed the value of $p = 0.042$ ($p < \alpha$), it can be concluded that there was a significant difference in IGF-II expression of K1 and K2 groups.

The results showed that after 20 days treatment of stress, corticosterone levels in the treatment (K2) group increased significantly compared to the control (K1) group. And after giving CUMS method for 20 days, the IGF-II expression in the treatment group was lower than the control group (significantly different). This proves that chronic stress, which is characterized by an increase in corticosterone levels, can result in a decrease in IGF-II expression. Increased cortisol levels due to chronic stress will disrupt the GnRH pulsation, causing a decrease in gonadotropins. Because gonadotropins stimulate IGF production, the IGF-II formed will be reduced\(^7\)\(^9\). The increased cortisol hormone will lower the FSH hormone levels. FSH is also a strong enhancer of IGF-II expression, so that by decreasing FSH due to increased cortisol, IGF-II will also decrease, and consequently cell proliferation can be disrupted, and steroidogenesis as well\(^{11}\).

IGF-II interacts with IGF-1R, which causes activation of the PI3-kinase/Akt signaling pathway which impacts protein translation as well as cell proliferation and differentiation. IGF-II also interacts with IGF-2R which results in increasing steroidogenesis in human and mouse granulosa and theca cells. IGF-II increases the production of estradiol and progesterone by granulosa cells\(^{14}\). Studies on human ovarian follicle cultures proved that the addition of IGF-II to culture media stimulated primary follicle production and reduced the number of atretic follicles compared to the control group. This means that if IGF-II decreases, folliculogenesis can’t be optimal because IGF-II serve as a protector and stimulator for follicular development\(^{15}\).

**Conclusion**

The increase in corticosterone levels as a result of chronic stress can reduce IGF-II expression in *Rattus norvegicus*. 

**Ethical Clearance:** Ethical clearance of this study was taken from Ethical Committee of the Faculty of Medicine, Airlangga University, Surabaya, Indonesia.

**Source of Funding:** This study was fully funded by Indonesia Endowment Fund of Education (LPDP)

**Conflict of Interest:** None

**References**


Incense Smoke Exposure: An Appraisal of Organ Toxicity

Adebanji M. Akingbade¹, Samson A. Odukoya², Adeoye O. Oyewopo³, Nalini Govender⁴, Thajasvarie Naicker⁵

¹Lecturer, Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine Ekiti State University Ado-Ekiti, Nigeria PMB, 5363, ²Senior Lecturer, Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria PMB 13, ³Associate Professor, Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, University of Ilorin, Ilorin, Nigeria PMB 1515, ⁴Senior Lecturer, Department of Basic Medical Sciences, Durban University of Technology, P O Box 1334, Durban, South Africa, ⁵Professor, Optics and Imaging Centre, University of KwaZulu Natal, Doris Duke Medical Research Institute

Abstract

Background: This narrative type review aimed to address organ toxicity emanating from incense usage.

Methods: An online search using the following MeSH terms “Incense smoke,” “adverse effects,” “organotoxicity,” “oxidative stress” and “inflammation” was done to identify studies directly applicable to adverse effects of incense smoke exposure.

Findings: Exposure to incense smoke demonstrated various toxicity changes in the kidney, testes, lungs, liver and heart. Renal effects included a decrease in oxidative stress markers GSH and CAT, increase in MDA, serum creatinine uric acid and blood urea nitrogen (BUN). Testicular toxicity revealed significant disturbances in spermatogenetic patterns, testicular atrophy, germinal aplasia, hypospermia and damage to the basal seminiferous epithelia tissue. In the cardiac muscle, ultrastructural changes, increased oxidative stress, inflammation, and altered cardiac hypertrophic gene expression was noted. Thenegative impact of incense smoke emanating from free radical (ROS), lipid peroxidation and GSH destabilizes vascular homeostasis and initiates a hyperinflammatory response, warranting the need to understand the conceptual basis of the mode of action linked to incense exposure.

Conclusion: We highlight that incense generates ROS which initiates lipid peroxidation through ATP energy depletion and reduction in the natural antioxidants. This subsequently triggers oxidative stress, inflammation and endothelial dysfunction resulting in organotoxicity.

Keywords: Heart, incense, liver, lungs, organotoxicity, skin, testes

Introduction

Incense (from Latin incendere “to burn”) is composed of aromatic biotic materials and essential oils, which when burned release fragrant smoke (¹). The term “incense” refers to the substance itself rather than the odour produced (²). The use of incense dates back to Egyptian antiquity, Babylonians, Indus civilization and the early dynasties of China (Xia, Shang, Zhou and Song).

The presentation of incense is variable in that they may occur as sticks, joss sticks, cones, coils, powders, rope and rocks/charcoal (³). Incense usage differs with regard to culture, custom and the reason for usage.
with many using it for the simple appreciation of its aroma and as a deodorizer/fragrance within their homes\textsuperscript{(5, 6)}. It is used in religious ceremonies for ritual purification, to appease the Gods with its sweet smell and to deter demons\textsuperscript{(1)}, in aromatherapy\textsuperscript{(6)}, an insect repellant and at funeral ceremonies to mask the smell of decay\textsuperscript{(7, 8)}. In most South East Asian and Middle East countries, domestic incense burning is routine during daily religious rituals\textsuperscript{(9)}.

When incense is burnt, it emits smoke that contains CO, CO\textsubscript{2}, NO\textsubscript{2} and SO\textsubscript{2}, aldehydes, metallic elements, polycyclic aromatic hydrocarbons (PAHs) including benzo(a)pyrene (BaP), naphthalene, fluoranthene and volatile organic compounds (VOCs) such as benzene, toluene and xylene\textsuperscript{(10-12)}. Inhalation of these pollutants in a closed environment are detrimental to human health. In fact, particulate matter from incense burning is 45 mg/g compared a cigarette, which is 10 mg/g\textsuperscript{(13)}. Moreover, incense is associated with a weak mutagenic activity\textsuperscript{(14)}.

Nonetheless, despite its widespread availability and access, the mechanism of its adverse effect and toxicity remains a public health challenge\textsuperscript{(14)}. Several studies demonstrate that continuous exposure to incense smoke predisposes the development of asthma, dermatitis, respiratory complications and hypertension\textsuperscript{(15-17)}. Moreover, exposure to carcinogens emitted from incense burning increases the risk of cancer development\textsuperscript{(18-20)}. Furthermore, the pathological and pharmacological effect of incense smoke on organs have been reported in the lung, testis, skin, liver and kidney\textsuperscript{(19, 21-23)}.

The adverse effect of incense exposure on different organs is based on short-term and long-term exposure. In fact, long-term exposure to incense smoke increases inflammation of blood vessels thereby influencing blood flow\textsuperscript{(22)}. In light of the negative consequences associated with incense smoke exposure, this narrative type review aimed to address organ toxicity emanating from incense usage.

Pathogenesis of organotoxicity emanating from incense exposure

Although the underlying causal mechanisms of incense exposure remains unclear, changes to oxidative stress, reactive oxygen species (ROS), inflammation, endothelial dysfunction and lipid peroxidation pathways may synergistically and concurrently contribute to pathology.

i. Oxidative stress

Reactive oxygen species are chemically reactive molecules containing oxygen \textit{e.g.,} oxygen ions and peroxides. ROS is a natural by-product of the metabolism of oxygen and has an important role in cell signaling and homeostasis\textsuperscript{(24)}. The production of ROS such as peroxides and free radicals lead oxidative stress that causes considerable damage to proteins, lipids and DNA of a cell.

Oxidative stress contributes to NO depletion, emanating from excess superoxide anions generation\textsuperscript{(25)}. Also, the superoxide anions react with NO to produce peroxynitrate. This subsequently oxidizes tetrahydrobiopterin (BH4)\textsuperscript{(26)} which inactivates eNOS thereby exacerbating superoxide anions production\textsuperscript{(27)}. Oxidative stress also upregulates endothelin-1 expression and causes endothelial dysfunction\textsuperscript{(28)}.

Oxidative stress activates the specific adaptive stress response via enhanced protein expression of endogenous anti-oxidant enzymes\textsuperscript{(29)}. This stress response protects cells against the reactive oxygen species mediated toxicity whilst maintaining a tissue redox balance\textsuperscript{(29)}. The gene transcription of most anti-oxidant enzymes is regulated via transcription factors, nuclear factor-E2-related factor (Nrf2) and anti-oxidant response elements (ARE) in the genes encoding enzymatic anti-oxidants\textsuperscript{(30)}. Under physiological conditions, Nrf2 is linked to an actin-bound Kelch-like ECH-associated protein 1 (Keap1) that is located within the cytoplasm\textsuperscript{(31)}. However, under the influence of oxidative stress, Nrf2 dissociates from Keap1 and translocates to the nucleus, where it induces the transcription of ARE-regulated genes\textsuperscript{(30)}. Anti-oxidant enzymes such as superoxide dismutase, catalase and glutathione defend the host against the damaging effects
of the free radicals species \(^{(30, 32)}\). Several studies have also associated incense exposure to pathophysiological disturbances with increased generation of ROS and/or a depletion of anti-oxidants within the kidney, testes, liver, heart, skin and lungs \(^{(33-37)}\).

ii. Inflammation

Inflammation is a natural defense mechanism associated with microbial and viral infection, exposure to allergens, radiation and toxic chemicals, autoimmune, chronic diseases, obesity, alcohol consumption, tobacco use, and a high-calorie diet \(^{(38)}\). Notably, chronic diseases such as cancer, insulin resistance, diabetes mellitus, cardiovascular diseases, atherosclerosis, and aging are associated with an up-regulation of ROS, oxidative stress together with protein oxidation \(^{(39, 40)}\).

Inflammation is a major indicator of endothelial dysfunction as endothelial stress is associated with an increased endothelial secretion of chemokines and cytokines \(^{(41)}\). Persistent inflammation may further exacerbate the oxidative stress induced pathology \(^{(42)}\). Oxidative stress is also implicated in vasconstriction of vascular smooth muscle via upregulation of endothelin-1 expression within endothelial cells, with consequent abnormal vascular function \(^{(28)}\). Thus, oxidative stress is the key force that drives endothelial dysfunction by destabilizing vascular homeostasis via the down-regulation of NO together with an up-regulation of endothelin-1. A strong correlation between incense smoke and oxidative stress was demonstrated by Hussain et al \(^{(2019)}\). A significant reversal of oxidative stress was observed within 30 days of cessation of incense smoke exposure, with consequential up-regulation of endothelial function and inflammation, thus confirming that incense smoke may be responsible for the induction of the pathology \(^{(37)}\).

Furthermore, the induction of Cytochromes P450 (CYPs) by BaP or other constituents either alone or in combination in rats exposed to incense smoke, maybe responsible for the mechanistic event for the increase in oxidative stress and inflammation of kidney dysfunction and tissue degeneration \(^{(19)}\). Additionally, myocardial degeneration together with increased oxidative stress and inflammation may contribute to the increased risk of cardiovascular and endothelial dysfunction, as well as irregularity in heart rate amongst incense-smoke exposed subjects \(^{(43)}\). Similarly, rhodinol-based incense generates reactive oxygen species via ROS production that exceeds the body's own natural anti-oxidant defense thereby resulting in oxidative stress and consequent cellular damage \(^{(23, 44)}\). This may explain the decrease in enzymatic and non-enzymatic anti-oxidants such as CAT, SOD, GSH and GPx. Similarly, Ahmed et al., (2013) also reported a decrease in both enzymatic and non-enzymatic anti-oxidants after exposure to incense smoke \(^{(34)}\). Moreover, treatment of human alveolar epithelial cells, A549 with particle matter emanating from incense smoke correlates with increased oxidative stress \(^{(45)}\). Incense smoke combustion is also reported to contribute to reactive oxygen species generation and to increase oxidative stress and induce DNA damage \(^{(11)}\).

iii. Lipid peroxidation

Lipid oxidation is a naturally generated process in the body, occurring mainly as an effect of several reactive oxygen species (hydroxyl radical, hydrogen peroxide, etc.) or by the action of several phagocytes \(^{(46)}\). Since lipid peroxidation is a self-propagating chain-reaction, the initial oxidation of only a few lipid molecules may lead to significant tissue damage \(^{(47)}\). Lipid peroxidation has been implicated in diseases such as pre-eclampsia and in hepatoxicity, kidney damage, skin damage and testiculotoxicity \(^{(34, 48)}\). The mammalian spermatozoon is particularly vulnerable to lipid peroxidation because of the molecular anatomy of its plasma membrane \(^{(23)}\). Unlike somatic cells, mammalian sperm cells have a highly specific lipidic composition with a high content of polyunsaturated fatty acids (PUFA), plasmologens and sphingomyelins \(^{(23)}\). Their unusual plasmalemma content is responsible for its flexibility and the functional ability of sperm cells. The high PUFA content is the main substrate for peroxidation and may provoke severe sperm dysfunction \(^{(49, 50)}\). It is possible that the testicular oxidative status of rats exposed to varying weights of incense (2g and 3g) increases the
action of malondialdehyde (MDA), a product of lipid peroxidation and damages their structure (23, 34). An earlier study demonstrated that the incense smoke exposure significantly decreased the liver alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) glutathione (GSH) and the activities of SOD, CAT and GPx by significantly up-regulating lipid peroxidation (51).

In light of the above mechanistic actions of incense smoke, physiologic function of various organs may be compromised. These include:

a) Kidney

A significant increase in serum creatinine, uric acid, blood urea nitrogen (BUN), tissue MDA, tumor necrosis factor-alpha (TNF-α) and interleukin-4 (IL-4) level have been reported in rats exposed to incense smoke (19). They also reported a significant decline in tissue glutathione (GSH) and catalase activity together with associated ultrastructural kidney pathology. Furthermore, a significant increase in tissue gene expression of both CYP1A1 and CYP1A2 were noted in incense exposed rats (19). In Singapore, the long-term daily exposure of the Chinese population to domestic incense burning has been associated with an increased development of end stage renal disease (52).

b) Testes

Rats exposed daily to *Boswelliapapyrifera* and *Boswelliacartetii* - based incense show significant disturbances in sperm kinetics compared to unexposed rats (34). More specifically, incense smoke causes a secretory dysfunction of Leydig cells with a deficiency in sperm maturation and spermatogenesis (53). Similarly, over a ten-week period incense burning activates a secretory deficiency in Leydig and Sertoli cells with resultant impairment of epidymal sperm maturation and consequential diminished capacity of spermatozoa to penetrate oocytes (54). Furthermore, a deregulation of the oxidative pathway is believed to cause a derangement in testicular histology and sperm viability in Wistar rats exposed to rhodinol-based incense (23). Additionally, a histomorphometric and spermatogenic evaluation of musk-based incense induced testiculotoxicity in adult albino rats with concomitant decrease in sperm viability, testicular atrophy, germinal aplasia, hypospermatozoa formation and damage to the basal seminiferous epithelial tissue (23).

c) Lung

The continuous exposure to incense smoke initially causes physiological and cellular changes that directly affect the efficiency of respiratory organs especially the lung (55). Exposure to incense smoke correlates with moderate inflammation and lymphocyte infiltration in lungs of albino rats (56), while other studies have recorded pathology such as lung carcinoma (57). Alarifi et al., (2004b) reported ultra-structural changes of the alveolar pneumocytes of animals exposed to *tobakhour*-based incense (21). These changes affect the cell organelles and surfactant material of type II cells. Similarly, Alokail et al., (2004) showed that *bakhour* burning adversely affects respiratory health. Moreover, light microscopy evaluation of lung tissue revealed focal emphysema, rupture of alveolar walls, hemorrhage, congestion, edema and peri-bronchial lymphoid cells (36). In addition, chronic exposure elicits focal necrosis and degradation of epithelial bronchioles together with fibrosis of peribronchial tubes, thickening of alveolar walls and aggregation of lymphoid cells (58).

d) Liver

Using an animal model exposed to Arabian incense smoke a significant decrease in anti-oxidant enzyme activity with concomitant significant elevation in lipid peroxidation; MDA was noted, signifying its hepatotoxic effects (35). The impact of long-term exposure to incense induces the expression of CYP1A1, CYP1A2, and CYP1B1 mRNAs within the lung and liver (36). Incense smoke exposure also increases MDA, TNF-α and IL-4 levels with a concurrent reduction of glutathione thereby reducing catalase activity within the liver (36).
e) Heart

Particulate matter generated during incense burning is responsible for variability in heart rate and impaired endothelial function (43). Importantly, regular exposure to incense is correlated with increased cardiovascular dysfunction (18). Moreover, ultrastructural changes, increased oxidative stress, inflammation, and altered cardiac hypertrophic gene expression was noted in cardiac muscle exposed to incense smoke (56).

f) Skin

Incense smoke exposure is also associated with dermatological problems since it may elevate IgE levels, and cause allergic contact dermatitis (4, 15). An early study observed itchy depigmented macules on the dorsum manus, left shoulder and the abdomen (59). The same research group also reported cases of contact dermatitis due to long-term exposure to musk ambrette vaporized from incense burning (33).

g) Brain

Indoor incense burning is associated with vascular disease to predispose poor cognitive performance related to decreased brain connectivity (60). An earlier report indicates that incense smoke contains various N-nitroso compounds, which are powerful nervous system carcinogens (61).

1. Figure 1. Conceptual pathway of how incense exposure mediates organ toxicity. Incense generates free radicals (ROS) which initiates lipid peroxidation through ATP energy depletion and reduction in natural body antioxidant such as GSH that triggers oxidative stress, inflammation and endothelial dysfunction resulting in organotoxicity. Moreover, lipid peroxidation is a process generated naturally in small amounts in the body mainly by the effect of several phagocytes. Since lipid peroxidation is a self-propagating chain reaction, the initial oxidation of only a few lipid molecules can lead to significant tissue damage. In addition, oxidative stress can trigger the infiltration of inflammatory mediator which in turn can leads to inflammation of affected tissues. Also, oxidative stress appears to be a key force driving endothelial dysfunction via destabilizing vascular homeostatic by down-regulating NO levels as well as up-regulating endothelin-1 in vascular tissue (developed from Mukhtar et al., 2013; Alokai et al., 2014; Akingbade et al., 2015, Hussain et al., 2016).

![Figure 1. Conceptual pathway of how incense exposure mediates organ toxicity](image)
Conclusions and Recommendation

Incense burning remains an environmental agent of important public health concern, hence it is necessary to understand the conceptual basis of its mode of action. The deleterious capacity of incense smoke induces physiological and cellular oxidative stress that emanates from ROS. Consequently, a decline in lipid peroxidation and GSH destabilizes vascular homeostasis thereby initiating inflammatory elevation of mediators such as MDA, TNF-α and IL-4 levels. Finally, there is an urgent need to create public awareness of the potential side effects of these common household and religious products. Awareness will lead to a reduction in exposure time and increase ventilation of homes and temples to ameliorate the pathological side effects of incense smoke.

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Disclosure of Interest: The authors report no conflict of interest

Ethical Clearance: This is a narrative review, hence no ethical clearance was required.

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Covid-19: The Effects of Distance Learning in Indonesia based on a Commognitive Perspective

Adika Setyo Budi Lestari¹, Toto Nusantara², Susiswo³, Tjang Daniel Chandra⁴, Nonik Indrawatiningsih⁵

¹Doctoral Student, Mathematics Education, Universitas Negeri Malang, East Java, Indonesia, ²Professor, Corresponding Author, Mathematics Education, Universitas Negeri Malang, East Java, Indonesia, ³Associate Professor, Mathematics Education, Universitas Negeri Malang, East Java, Indonesia, ⁴Assistant Professor, Mathematics, Universitas Negeri Malang, East Java, Indonesia, ⁵Lecturer, Department of Mathematics Education, Universitas PGRI Wiranegara Pasuruan, East Java, Indonesia

Abstract

Distance learning is a learning system that does not take place in one room and there is no face-to-face interaction between the teacher and the learner. This study aims to determine the impact of implementing distance learning in Indonesia from a commognitive point of view. This research is a descriptive type of research with a total of 543 participants who come from high school students in Pasuruan district, Indonesia. Data collection using a questionnaire. After the questionnaire is collected, it is analyzed using the Miles and Huberman method through reduction, display data, and conclusion, then it will be studied based on the commognitive theory. The results show that based on commognitive studies, students are more likely to still need a visual mediator as a visible object to be used as a communication medium, its realization depends on the material context. Students need to communicate to ask questions related to material that has not been understood. So it can be said that visual mediator is important during distance learning.

Keywords – Distance Learning, Visual Mediator, Commognitive.

Introduction

The learning process is a two-way interaction process that involves face-to-face communication between teachers and students in a class. However, with the Covid-19 pandemic since March 2020, the face-to-face learning process in schools has to be temporarily halted. This is due to preventing massive transmission of Covid-19. The Ministry of Education and Culture provides instructions for schools to organize distance learning (online) and advises students to study from their homes. This condition forces educators to innovate and try digital platforms that are widely available to support the learning process as a solution to implementing learning from home policies (¹). Like it or not, ready or not, all elements in the world of education must be able to carry out distance learning.

Distance learning is a learning system that does not take place in one room and there is no face-to-face interaction between teacher and learner(²). Various kinds of platforms can be used in distance learning, both in the form of learning management systems and in the form of video conferencing. Learning management systems that are widely used include, google classrooms and E-learning portals owned by schools or colleges(¹). Meanwhile, video conferencing applications that are widely used during distance learning include the zoom meeting application, Google Meet and Visco Webex. Apart from these applications, Whatsapp Group is also

Corresponding author:
Toto Nusantara
Professor, Mathematics Education, Universitas Negeri Malang, East Java, Indonesia
andikalestari123@gmail.com
an alternative in implementing distance learning\(^{(3)(4)}\).

To succeed distance learning requires cooperation from various parties, not only from the educators but also from the students and families. Distance learning is a new challenge for educators because so far learning has been done directly.

Even though educators use digital platforms, communication is the main factor needed during the learning process using online. In solving a problem, communication and cognition skills are needed. Communication skills and cognition are combined with the term commognitive \(^{(5)}\). Commognitive is a method of analyzing how students solve a problem \(^{(6)}\). Solving problems is one of the abilities that must be possessed by students.

Distance learning is one way to continue education during a pandemic\(^{(7)}\). Distance learning is still designed in such a way as to be able to train students in solving problems. Distance learning is a new thing that must be applied in Indonesia during the pandemic period, so it still needs improvement. It can be said that distance learning is a transition period from conventional learning to digital learning \(^{(8)(9)}\). To be able to determine the impact of distance learning can be done by analyzing it in terms of communication and cognitive. Commognitive is a communicative cognitive process related to what is being thought, as a combination of communication and cognitive which consists of four components, including word use, routine, visual mediator, and narrative\(^{(5)(10)}\). Routine is the tendency of choosing terms or choosing representations in the process of pouring out ideas to solve the problems or problems faced so that they can support the narrative. Visual Mediators are visible objects that are used as communication media, the manifestation depends on the material context. Word use is the use of words that can reflect or represent the situation at hand. A narrative is a series of sentences that describe objects, relationships, and processes, such as definitions, theorems, and proofs.

Distance learning is closely related to the four commognitive components. The main question in this research is how is the impact of implementing distance learning based on a commognitive point of view?

**Research Methodology**

This research is qualitative research with a descriptive design conducted to determine the impact of the application of distance learning in Indonesia according to a commognitive view.

**Participant**

The population in this study were all junior high school students in Pasuruan Regency. The sample of this study was 543 students who came from junior high schools in Pasuruan district and had an average age of 13-15 years. The sample collection technique used is random sample sampling, which means that each member of the population has the same opportunity and opportunity to become a research sample.

**Procedure**

The procedure in this study is as follows: 543 junior high school students were given a questionnaire. The questionnaire was used to obtain preliminary data on the assessment of the application of distance learning carried out in Indonesia during the Covid 19 Pandemic, then after knowing the initial data, it will be studied about the impact of distance learning from a commognitive point of view. The questionnaire consisted of two parts, the first part related to the impact when implementing distance learning which consisted of 1 question with 5 answer choices. The second part deals with the impact after implementing distance learning which consists of 1 question with 3 answer choices.

The questionnaire instrument given to students is as follows:

1. What do you find burdensome in online learning? (choose only one answer that you think is the most burdensome).

   a. Duration online
   
   b. Pulse costs
2. After you have participated in online/online distance learning for almost 2 weeks, how do you feel?
   a. Troubled or restless
   b. Happy
   c. Ordinary
   d. Too many tasks
   e. Others

Data collection technique
The data collection technique was done by filling out a questionnaire online. The data obtained from the questionnaire were then analyzed using the Miles and Huberman concept through reduction, display data, and a conclusion.

Result and Discussion
Based on initial data obtained from a questionnaire distributed to 543 junior high school students, data is obtained in Table 1 and Table 2 below.

<table>
<thead>
<tr>
<th>Table 1. Student Response when Application of Distance Learning</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Answer Items</strong></td>
</tr>
<tr>
<td>It took a long time</td>
</tr>
<tr>
<td>High pulse costs</td>
</tr>
<tr>
<td>Students have difficulty learning the material</td>
</tr>
<tr>
<td>Students refused because of the large number of assignments</td>
</tr>
<tr>
<td>Etc.</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Table 1 above shows that when implementing distance learning (online), students still find it difficult to learn the subject matter provided by the teacher through online learning with a percentage of 37%. The second place is students objecting because students get more assignments when using online learning with a percentage of 36%. The third-place students objected because the credit was expensive with a percentage of 20%. The fourth place is that students answer other questions with a percentage of 5%. The fifth place is students objecting because the time is too long when using distance learning (online) with a percentage of 2%. The percentage is presented in Figure 1 below.
Based on the results in table 1, it shows that students, in terms of material content, still have difficulty understanding the material. This is because it is not common to learn online. Teachers as teachers and educators are also still not used to presenting material online. As a result, the material presented is not optimal for students because the ability to master technology is still not maximally mastered by the teacher. Learning is felt to be still not effective because of several influencing factors, including a lack of parental supervision\(^{(11)}\).

**Table 2. Student Response after Application of Distance Learning**

<table>
<thead>
<tr>
<th>Answer Items</th>
<th>Number of Respondents who answered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Students enjoy using distance learning</td>
<td>219</td>
</tr>
<tr>
<td>Students find it difficult or restless to use distance learning</td>
<td>139</td>
</tr>
<tr>
<td>Students are casual towards distance learning</td>
<td>185</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>543</strong></td>
</tr>
</tbody>
</table>
The percentage can be presented in Figure 2 below.

![Figure 2. Student Response after Application of Distance Learning](image)

Table 2 above shows that after implementing distance learning (online), students enjoy learning through distance learning with a percentage of 40%. Furthermore, students feel difficulty with distance learning with a percentage of 26%. Then students act normal if distance learning is applied with a percentage of 34%. The impact of distance learning is felt by students in various kinds of responses obtained from students, including boredom, mediocrity, frustration, and anxiety\(^\text{(12)}\). Based on table 1 distance learning most students feel happy, but also not a few students feel normal. Several factors affect, including because distance learning is a new thing for students, students learning online using cellphones feel freer to use cellphones than usual days.

Based on Table 1 and Table 2 above, if it is associated with the four commognitive components, namely word use, routine, visual mediator, and narrative. Students are more likely to still need a visual mediator as a visible object to be used as a communication medium, its realization depends on the material context\(^\text{(10)(13)}\). Students need to communicate to ask questions related to material that has not been understood. So it can be said that visual mediators are important during online learning. This is following the opinion of Sfard and Kieran\(^\text{(14)}\) that defines communication as effective when differences in the speech from the interlocutor generate responses that are in line with the speaker’s metadiscursive expectations\(^\text{(15)}\). Besides, communication will be more effective if students discuss in small groups to solve problems\(^\text{(16)(17)(18)}\). So it can be concluded that the use of words and visual mediators is very important in learning mathematics in distance learning.

Visual mediators are objects that are acted upon as part of the communication. Whereas everyday discourse
is mediated mainly by pictures of concrete objects that exist independently of a particular discourse, in mathematics most symbols and other mediators are created primarily for communication purposes. Visual mediators in mathematics learning include algebraic symbols that mediate ideas such as numbers and written graphics, or other symbols such as those representing variables, coefficients, and equations\(^\text{(19)}\). The mediator used in communication often influences what can be said about the ideas being discussed. To illustrate, while solving equations in algebra, students often use graphs as visual mediators. Also, a visual mediator is a means used by the participants as a discourse to identify the object of their conversation and coordinate their communication. Mathematical discourse often involves symbolics that is used as a form of certain communication\(^\text{(20)(21)}\). For students to be able to receive and understand the material presented by the teacher in the online learning process, creativity, and special skills from the teacher are needed to present the material.

In the learning process, both direct learning and using online media, communication is needed in providing an understanding of the material to students. Communication takes a broader view and also considers non-verbal aspects such as visual mediators\(^\text{(22)(23)}\). Commognitive with visual mediators focuses on cognitive frameworks about making routines explicit, supported narratives, words, and visual mediators such as lectures\(^\text{(24)}\). Meanwhile, commognition is a socio-cultural approach that aims to provide an understanding of what is learned during the learning process.

However, the problem with distance learning is that some teachers are still not tech-savvy, making online learning a new thing for most teachers in Indonesia. Resources, and the limitations of educational technology, as well as the skills and quality of the teachers, are not sufficient\(^\text{(1)(25)}\). Learning in schools is carried out by WA, zooming, and often by giving assignments, learning in the form of videos downloaded on YouTube. Whereas distance-learning studies according to a commognitive view state that students are more likely to still need a visual mediator as a visible object to be used as a communication medium, its realization depends on the material context. Students need to communicate to ask questions related to material that has not been understood. So it can be said that visual mediators are important during online learning.

Some of the recommendations suggested based on the results of this study are to carry out proper schedule control to guide students learning step by step and increase their understanding of the material that has been studied, motivate students to interact via digital platforms so that students can clearly express the problems they have faced, and The use of visual mediators is still applied so that students can improve their understanding.

**Acknowledgment:** This study supported by Universitas PGRI WIRANEGARA Pasuruan.

**Ethical Clearence:** Yes.

**Conflict of Interest:** No

**Source of Funding:** Authors

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Age Estimation by the Morphometric Analysis of Sternal End of Fourth Rib

Aditi Bhatnagar¹, Nirupma Gupta², Rachna Rohatgi¹
¹Assistant Professor, ²Professor & Head, Department of Anatomy, SMS&R, Sharda University, Greater Noida, U.P.

Abstract

Estimating the age at death in the adult skeleton is problematic owing to the biological variability in age indicators and the differential skeletal response to environmental factors over an individual’s life. While the pubic symphysis and intracortical morphometry have provided successful results in estimating age at death, other methods and sites in the skeleton are needed to improve the accuracy of age estimation. Present study is an attempt to develop a new method for estimation of age from sternal end of fourth rib. Currently there are different parameters available to determine the age of a person like study of teeth, ossification of bones and other ancillary data, but the accurate reliability of these measures is only limited to a particular age group i.e. 25± 5 years. For the age beyond this, many workers in different parts of the world have done their studies to accurately determine the age of a person from the skeleton. A random study of 100 cases for age estimation from sternal ends of the fourth ribs were carried out to estimate the age after death with minimal error.

Keywords: Fourth rib, Age estimation, Morphometric analysis.

Introduction

Identification of any individual is the first step for assessing the dead body, as in the cases of sudden or unexpected death or in any unclaimed bodies.

The Identity of a person is done by using various parameters as Sex, Age, Stature, Genetic constituents, etc. Age is an extremely vital parameter in the personal identity of living subjects as well as skeletonized dead bodies.(1)

Age estimation of unknown skeletal remains is very important in medico-legal practice. The procedures for age determination are complex and involve the consideration of many factors. Changes related to chronological age are seen in both hard and soft tissue. Amongst the hard tissues, bones are important as they undergo a series of changes from prenatal to postnatal life and changes in their composition and structure continue into old age and even after death. Hence, bones form a reliable source of information regarding growth and growth changes.

Morphologic methods are fast and easy to use for purpose of age determination. The sternal ends of the ribs are a reliable method of age estimation from late adolescence to old age.

Metamorphosis at the sternal extremity of the rib has already been established as a reliable indicator of age at death. It was shown that an accurate estimation of age can be made by direct examination of the bone itself.(2)

Therefore we have used the sternal end of the 4th ribs to determine the age of the Individual. The sternal
end of the rib is chosen because it has been shown from earlier study by Iscan et al\(^{(2,3,4)}\) to be a perfect bone to show the advancement of age and the age can also be estimated by this method if the partial skeleton is found.

This study aimed to develop regression formulae for estimating age at death using a macroscopic feature of bone.

**Materials and Method**

The present study was conducted in the Department of Anatomy, S. P.M.C, Bikaner & SMS&R, Sharda University, Greater Noida on 100 samples of 4th rib taken from dead bodies brought for postmortem examination. Its particulars were recorded and age was noted and it was cross-checked from relatives by authentic ID proof. The research work was approved by the ethical committee. Before taking the sample from the deceased the consent form was filled & signed by the kin of the deceased.

The specimens were separated from the body by cutting the fourth rib at two points i.e. three centimeters inner to and five centimeters outer to costochondral junction using a rib cutter without damaging the costochondral junction.

Macroscopic measurement had been taken after the extraction of bone. All of the measurements were taken with a sliding caliper calibrated to the nearest 0.1mm. The following measurements were used to determine age:

1. **Maximum Superior Inferior Height (SIH)**: the maximum distance between the most superior and inferior points at the end of the rib
2. **Maximum Anterior-Posterior Width (APW)**: the maximum distance between the most anterior and posterior points at the end of the bone
3. **Maximum Pit Depth (PD)**: the maximum depth of the pit was measured with a depth caliper keeping the caliper perpendicular to the base of the pit.

All the readings were taken thrice and an average of the three readings was taken for better accuracy. After calculating the above measurements the linear regression analysis was applied.

**Observations & Results**

In present study total 100 cases were examined. Out of 100 cases males constituted 67 while females constituted 33 of the samples.

**Table 1: Age distribution of study sample (N = number of individuals)**

<table>
<thead>
<tr>
<th>AGE RANGE</th>
<th>Groups coding</th>
<th>N</th>
<th>% distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-30</td>
<td>1</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>31-45</td>
<td>2</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>46-60</td>
<td>3</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>&lt;60</td>
<td>4</td>
<td>01</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>4</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
The age of all sample ranged between 15 and 62 years with a mean age of 32.73 years. The age range for males was between 15 to 60 years (mean age=34.38 years), while the females range was between 18 to 62 years (mean age=29.36 years).

For Age determination following parameters had been studied. Their descriptive statistics also calculated. (Table 2).

**Table 2:** Descriptive statistics of study samples according to their SIH, APW, PD (All values in cm)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>MEAN</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SIH (Maximum Superior-Inferior height)</td>
<td>1.379</td>
<td>0.233</td>
</tr>
<tr>
<td>2</td>
<td>APW(Maximum Antero-posterior Width)</td>
<td>0.445</td>
<td>0.121</td>
</tr>
<tr>
<td>3</td>
<td>PD(Maximum Pit depth)</td>
<td>0.18</td>
<td>0.74</td>
</tr>
</tbody>
</table>

For the calculated data for linear regression analysis SPSS 21.0 software has been used. Simple linear regression analysis was done. Data was charted in order to identify possible outliers. Regression analysis was undertaken in order to evaluate apparent trends for each of the three variables examined with actual age. For each variable the regression equation also has been derived after statistical analysis of data.

**Table 3:** Comparison between r value, r square, Standard error of estimate (SEE), t-value and significance level.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Variable</th>
<th>R value</th>
<th>R square</th>
<th>SEE (years)</th>
<th>t-value</th>
<th>Significance value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SIH</td>
<td>0.275</td>
<td>0.76</td>
<td>12.194</td>
<td>2.835</td>
<td>0.006</td>
</tr>
<tr>
<td>2</td>
<td>APW</td>
<td>0.412</td>
<td>0.170</td>
<td>11.559</td>
<td>4.47</td>
<td>0.0001</td>
</tr>
<tr>
<td>3</td>
<td>PD</td>
<td>0.493</td>
<td>0.243</td>
<td>11.037</td>
<td>5.608</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

According to the Table 3, the simple linear regression analysis that is best related to age for this pooled sexes sample is the Maximum Pit Depth (PD). The coefficient of determination ($r^2$) for this formula is 0.243 and the standard error of the estimate is ±11.03 years.

**Table 4:** Simple linear regression Equation (single variable only)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Variable</th>
<th>Regression Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SIH</td>
<td>Age Predicted = 12.046 + 14.898 (SIH)</td>
</tr>
<tr>
<td>2</td>
<td>APW</td>
<td>Age Predicted = 13.594 + 42.688 (APB)</td>
</tr>
<tr>
<td>3</td>
<td>PD</td>
<td>Age Predicted = 17.43 + 84.222 (PD)</td>
</tr>
</tbody>
</table>
From Analysis it is cleared that Maximum Superior-Inferior Height, Maximum Antero-Posterior Width and Maximum Pit Depth are all reliable factors. This indicates that these variables are easy to score and thus prove useful in age estimation techniques.

For better result Multivariate linear regression analysis has been done with same variables. In the multivariate analysis, the change in the coefficient of determination was noticed in comparison with the univariate analysis, with a slight improvement in the SEE.

Table 5: Multivariate Regression Analysis for Sexes Pooled

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Variables</th>
<th>R value</th>
<th>R square</th>
<th>SEE (Years)</th>
<th>Regression Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>SIH, APW, PD</td>
<td>0.562</td>
<td>0.294</td>
<td>10.603</td>
<td>Age (Predicted) = 9.844 – 2.988 (SIH) + 31.584 (APB) + 71.178 (PD)</td>
</tr>
</tbody>
</table>

Morphometric Study: Measuring SIH (Image 1) and APW (Image 2).

The highest coefficient of determination was 0.562. The best standard error of the estimate for the multivariate analysis was ± 10.603 years (Table 5). This indicates that there is a slight improvement in the standard error of the estimate and also improvement seen when the coefficient of determination is taken into account.

The question that remains is whether it is more practical to use the complex multiple regression formulae or the simpler, and less tedious linear regression formulae to estimate age at death.

Discussion

There are various methods and various bones were examined for estimation of age but very few methods are based upon morphometric or morphological analysis of bone. Morphometric Analysis are easy, Feasible and result can be achieved very fast.

In the present study morphometric analysis of Fourth rib has been done on 100 study sample and regression
equation for age prediction was derived. More of the previous studies on sternal end of fourth rib for age estimation are mainly based on histological, osteometric analysis and radiological techniques but morphometric analysis based studies are very less.

Results of the present study showed that the age of a subject can be estimated from metamorphic changes in the sterna end of 4th ribs in all the age groups, as the t-values computed in all the cases were found to be significant.

Iscan et al (3, 4) concluded that age at death can be estimated from a rib within about 2 years in 2nd decade to about 7 years in the 5th and 6th decades of life and Singh et al (5) revealed that age can be estimated from sternal end of 4th rib with an accuracy ranging between ±2 years up to 3rd decade and about ±8 years in the older age which is slightly invariance with our study. Pankaj Gupta et al (6) concluded that in the age group 17-30 years, age can be estimated from sternal end of 4th rib with an accuracy ranging between ±8 years, in the second age group 31 to 44 years ±15 years and in the third age group 45 years and above again ±8 years.

However, from the present study, for the sexes pooled and in all age group we could analyze that PD is most reliable among all factors and for this SEE is 11.037 years. for the better result in present study we had done the multivariate regression analysis and result showed SEE is 10.603 years.

So still in the field of Forensic Medicine, we remain handicapped to exactly pinpoint the accurate age from one single factor, as metamorphic changes in the sternal end of the 4th rib alone are not sufficient to assess the accurate age of a subject.

Conclusion

Positive identification involves matching of an “unknown” individual to a “known” individual. The identification of skeletal and other decomposed human remains is very important for legal and humanitarian reasons.

Most research on estimating age-at-death using human skeleton material suggested that degenerative processes are good indicators for age estimates. However, these processes can vary among populations, depending upon factors such as biological affinity and relate to growth and development.

The objective of this research was to estimate the age by using the sternal end of the fourth rib. We selected specimens of known age and sex. It was possible to observe the relationship between morphological changes and known age. For the fourth rib the morphometric features included in this study were SIH, APW and PD had a significant relationships with age.

The results of the analysis suggest that we can estimate age at death but for more precise and accurate results one must include we are also of the opinion that unifactorial parameter to assess the age from the sternal end of the ribs for practical purposes is not completely accurate. So multifactorial parameteric/ comprehensive approach should be the hallmark for arriving to conclusion as regard to the age of the subject.

We hope that these results will promote the evaluation of methods for estimation of age-at-death and a more critical consideration of factors that influence human variation.

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Ethical Clearance: Taken From Ethics committee, S.P. Medical College & associated Hospitals, Bikaner (Rajasthan)

Source of Funding: Self
**Conflicts of Interest:** None

**References**


Isolation and Identification of *Streptococcus pyogenes* from Patients and Objects in Hospital Environment in Thi-Qar City in Iraq

AdyanNafee Abbas¹, Qasim Hassan Wida²

¹Research Scholar, Corresponding Author, College of Science, University of Thi-Qar, Iraq, ²Professor, Biology Department, College of Nursing, University of Thi-Qar, Iraq

**Abstract**

This study aimed to screen *Streptococcus pyogenes* in the hospital and investigate the incidence of this bacteria. The genotypes of the *S. pyogenes* in the study samples will be determined using 16Sr RNA and some virulence factors (*emm, scpA, speA* genes). The majority of the samples (74.1%) were isolated from patients at the emergency room and (25.9%) were from hospital environment (objects). The current study showed that the total of 215 patients with pharyngitis were included 118 (55%) males higher than 97 (45%) females. The patients’ ages ranged from 2 to 62 years, which were divided into four categories. The majority of patients 65 (30.1%) of patients from 3 to 6 years old, 54 (25%) of patients less than 3 years, 51 (23.6%) from 7 to 11 years old, and 46 (21.3%) more than 12 years. Six isolates sent for sequencing were after that submission in NCBI-GenBank database. The results of nucleotide sequence alignment of the isolates revealed that there are multiple point mutations, four of them appeared as a single nucleotide polymorphism (SNP) and two appeared in duplicate. Polymorphic sites (Mutations) of 6 *S. pyogenes* isolates.

**Keywords**: GAS, 16S rRNA, Pharyngitis, *Streptococcus pyogenes*

**Introduction**

(Group A Streptococcus (GAS) is a gram-positive bacterium that grows in pairs or chains and causes complete, or B -hemolysis when cultured on sheep blood agar (1). *Streptococcus pyogenes* is an important extracellular species of gram-positive, spherical and non-spore species, *S. pyogenes* is a facultative anaerobic bacterium, which is present in chain form. This bacterium is a conditional and opportunistic pathogen, which is always in search for suitable environment to cause a disease, this bacterium built colonies in the skin or throat and causes a wide range of complexes (2,3). The most common site of GAS infection is the oropharynx, and this presence is usually considered a prerequisite for the development of rheumatic fever, the leading cause of preventable childhood heart disease in the developing world, while GAS is mainly an extracellular pathogen, data over the past decade have shown that the organism can invade epithelial cells and live within them, GAS proliferation in the oropharynx triggers the signs and symptoms of pharyngitis (4). A number of virulence factors that contribute to the infectious process are created by GAS. One of the major virulence factors is the M protein, encoded by the *emm* gene, which promotes evasion of phagocytic killing (5). In *S. pyogenes* pathogenicity M protein shows its effect by facilitating adhesion, providing opsonophagocytosis resistance and contributing to the overall burden of GAS infections (6). Up to 11 different secreted superantigens can be produced by the human pathogen *Streptococcus pyogenes* that contribute to the features of cytokine-induced toxic shock.

**Corresponding Author:**
AdyanNafee Abbas
Research Scholar, College of Science, University of Thi-Qar, Iraq,
E-Mail : adianalrekabyaa@gmail.com
during lethal, invasive infections such as necrotizing fasciitis. However, invasive diseases, manifested as pharyngitis, tonsillitis and childhood exanthem scarlet fever, are rare compared with symptomatic non-invasive disease that occurs in the nasopharynx, indeed, the throat and tonsils constitute the largest source of *S. pyogenes* in human populations carriage (7).

**Materials and Methods**

(Two hundred and ninety samples collected from hospital environment (75) and throat of patients(215) suffering from acute pharyngo-tonsillitis based on the symptoms and which the diagnosis by physicians with age range from 2 years to 60 years. The samples were collected in AL-Nassiriyah City. Selected samples were labeled and cultivated on blood agar and azide blood agar for 24 hours or 48 hours for better growth at 37°C in incubator. Any *S. pyogenes* suspected from samples make subculture on blood agar. Depending on morphological features of the B-haemolytic colonies and microscopically examination with gram’ stain, the pure cultures were prepared for biochemical tests and API-20 Strep to distinguish *S. pyogenes*. Polymerase Chain Reaction technique used for the amplification of specific genes belonging to *streptococcus* that is suspected to be present in both human pus. PCR products for 16S rRNA were sequenced by Macrogen Company (South Korea). The samples were sent through Iraq biotechnology and according to the requirements of the company including 15μl for each forward and reverse the PCR products and 50 μl (10pmoles/μl) for each forward and reverse prime. Each samples was labeled with a number and name identical to the number that sent to company and samples were sent in a cool box containing cool gel pack.

**Results**

(The total number of patients (215) who have symptoms were included 118 (55%) males and 97 (45%) females, where it was significant difference between them depending on frequency of different ages in males and females. The age ranged from 2 to 62 years old in a total 215 patients. The typical appearance of *S. pyogenes* colonies is dome – shaped with a smooth or moist surface and clear margins on blood agar after 24 hours of incubation at 37°C, and the most important feature is the hemolysis zone around the colonies. They display a white –greyish colour and have diameter of > 0.5 mm as shown in figure (1-A). Out of 61 beta haemolytic *Streptococci* isolates tested, 30 (49.2%) were bacitracin sensitive, the appearance of *Streptococcus pyogenes* produces inhibition zone around the disc on the blood agar, following 24 h of incubation under anaerobic conditions as shown in figure (1-B).

![Figure (1): (A) : Hemolysis of *S. pyogenes* ; (B): Bacitracin disc test →](image-url)
A total of twenty five isolates of *S.pyogenes* which identified by conventional methods such as; morphology of colonies, microscopic examination, biochemical tests and API 20 strep. Were subjected to DNA extraction and PCR assay for presence of *16S rRNA* gene. The results demonstrated that 25 (83.3%) of the isolates had *16SrRNA* gene with band 1500pb, as in figure (2). Twenty four isolates gave positive for each *emm* gene, *speA, scpA* gene as shown in figure (3), (4) and (5).

Figure (2): Conventional PCR for detection of *16S rRNA* gene (bp), in *Streptococcus pyogenes* isolates.

Figure (3): Conventional PCR for detection of (*emm*) gene.

Figure (4): Conventional PCR for detection of (*speA*) gene.
The results of nucleotide sequence alignment of the isolates revealed that there are multiple point mutations, four of them appeared as a single nucleotide polymorphism (SNP) and two appeared in duplicate, where a substitution of the nucleotide G instead of A occurred at site 262 as shown in, and AIX of G at site 849 of the sample gene segment with MW0425850 Accession number, also the nucleotide G was replaced by the A at site 363 of the links with MW0425846, there are double mutations that occurred at site 199 and 200 of sample MW0425847 where the nucleotides are CC instead of TT, and the same mutation was seen at site.

**Discussion**

*S. pyogenes* has remained a significant human pathogen for centuries, it causes a wide variety of infections in humans, which vary from mild upper respiratory and skin infections to non-suppuratives sequelae like Acute Rheumatic Fever and Rheumatic Heart Disease. The majority of patients were range (2-63 years) these results are similar to a study reported by (9), and also similar to another study reported by (10). The Samples were from (97) females (45%) and (118) males (55%), the males were predominant more than female, these results are agreement with results of study conducted by (11-13) and disagree with studies showed by (14,15), as shown in these result there is no big difference in the sex of patients. The results of the present study displayed that the occurrence of *S. pyogenes* was 25/290 isolates (6.8%), and this result disagree with a study in Thi-qar revealed by (16) who documented a high emergence of isolates from patients with tonsillitis, but similar to a study revealed by (17). Nosocomial transmission of GAS infection into patients in the hospital environment has been described in the medical literature, the range of transmission has been limited to small numbers of health care workers (18). The current study revealed that is no presence of *Streptococcus spp.* especially *S. pyogenes* and this result disagree with a study reported by (19) who showed that the rate of Streptococcus species is (2.75) and a high rate of *Staphylococcus aureus* (41.28). The results appeared 25 of the isolates were positive for 16S rRNA and 24 were positive for emm gene, these results are similar to a study reported by (6), who showed a similar DNA sequences of the 16S rRNA gene region of GAS isolates. The isolates appeared genetically close according to their sequence in the tree, as they are different in terms of the common ancestor, so we see them in the tree in the form singleton. Two isolates appeared in common with the ancestor, due to the presence of a high genetic similarity that appeared between them. In general, the isolates of the study were performed in the tree by their participation with one of the common ancestors, but it confirms the genetic changes that occurred in the isolates of the study, where a genetic bond was found with the isolates taken from the database NCBI. Phylogenetic tree analysis based on the partial 16S ribosomal RNA gene sequence that used for confirmative detection.
of *Streptococcus pyogenes* isolates, the analysis involved 6 nucleotide sequences, include (MW425845, MW425846, MW425847, MW425848, MW425849, MW425850). The resulting sequence were compared with worldwide reference sequence through NCBI BLAST, showed there is closely identical relationship between them (CP043530.1, CP049800.1, CP049799.1, CP047120.1, CP036531.1, CP031635.1) which were an isolates from USA (NCBI base). Sequence were aligned and edited using blast aligner.

**Conclusions**

High genetic variations were detected in *S.pyogenes* isolates. These genetics variations may make *S.pyogenes* more virulent and resistant to antibiotics.

**Ethical Clearance** : Taken from University of Thi-Qarethical committee

**Source of Funding** : Self

**Conflict of Interest** : Nil

**References**


Management of Pyogenic Granuloma (Clinicopathological Study)

Afrah A. Kh. Aldelaimi¹, Tahrir N. Aldelaimi²

¹Senior Lecturer, Oral & Maxillofacial Pathologist, Department of Oral Diagnosis, College of Dentistry, ²Professor & Consultant, Department of Oral & Maxillofacial Surgery, College of Dentistry, University of Anbar, Ramadi Teaching Hospital, Ramadi City, Anbar Province, Iraq

Abstract

Background: Pyogenic granuloma is a common oral cavity reactive lesion of. Injuries, calculus and hormonal changes are the common causative factors. It is often arises in the second decade of life as exophytic smooth lesion, mostly bleeds on simple probing. The study aimed to evaluate clinicopathological findings of pyogenic granuloma.

Materials: A total of 54 patients (including 40 male and 14 female) have been enrolled in this study subjected to surgical excision of pyogenic granuloma by diode laser 940nm. All the specimens were undergo a standard tissue processing procedure subsequently the paraffin embedded blocks were sliced in 5µm thickness into a clean glass slide and prepared to H & E staining and all slides were examined using light microspore. All cases in this study undergone surgical excision of pyogenic granuloma aged from 8 to 53 years (mean= 27 years) with the peak incidence of occurrence (44.5 %) in the (11-20 years) age group.

Conclusions: Pyogenic granulomas were most prevalent in male (74%) than female (26%). Oral pyogenic granuloma is a very common oral cavity occurring reactive lesion, mostly painless benign growth. Surgical excision with removal of etiological causes is the major treatment.

Keywords: pyogenic granuloma, oral pathology, oral lesion, oral tumors, benign tumors

Introduction

Pyogenic granulomas are common benign, non-neoplastic, localized, soft tissue lesions that misnomer as they neither represent a true granuloma nor infection with pyogenic micro-organisms¹. Anyhow, pyogenic granuloma has been retained in the literature since coined by Hartzell in 1904 because of its historic significance²,³. In pathological sciences pyogenic granulomas are known as granuloma gravidarum, granulation tissue-type haemangioma, lobular capillary haemangioma, eruptive haemangioma, or pregnancy tumor⁴,⁵. Pyogenic granulomas occur anywhere in oral cavity, head and neck region, trunk and extremities⁶. Scientifically many monographs proposed that low-grad injury from physical trauma or infection or chronic irritation from retained roots and/or dental calculus was the principle etiological factor in evolution of pyogenic granulomas⁷,⁸,⁹. Indeed, other studies offering certain drugs such as cyclosporine and hormonal alterations during puberty or pregnancy as causative factors in the development of the lesions¹⁰. Although numerous lesions resemble clinically with pyogenic granuloma arising in the oral
cavity but a detailed history reinforced with proper treatment plan and careful histopathological examination will be useful to accurately identifies the lesion 11. Pyogenic granuloma extirpation is correct treatment of choice, plenty alternative therapy such as lectrosurgery, sclerotherapy, cryosurgery, corticosteroid or ethanol injection and laser therapy have been declared to be effective 10,12. The abundant vascular nature of pyogenic granulomas required careful excision to avoid profuse bleeding. The excision by laser offer great advantage than conventional surgical excision in controlling bleeding through the surgical procedure 11,12. Pyogenic granuloma has an extremely increased recurrence rate with simple surgical ablation 13. Perfect treatment of pyogenic granuloma required correct removal of whole lesion to prevent its recurrence 12. The study aimed to evaluate clinicopathological findings of pyogenic granuloma in Iraq

Materials and Methods

A total of 54 patients (14 female and 40 male) have been enrolled in this study subjected to surgical excision of pyogenic granuloma in Ramadi Teaching Hospital, Razi Private Hospital and Private Dental Clinic in the Province of Anbar. This study was approved by Department of Scientific Affairs at College of Dentistry and was approved by Ethical Approval Committee at University of Anbar under Ref no. (6) On 24th of January, 2021. The patient consent form was obtained prior to surgery and the detailing of laser procedure was elucidated to the patients. Demographical information including patient`s name, age, sex, medical condition, past dental history and clinical examination of the features of the lesion (site, size, duration, color, texture of the lesion) were recorded and analyzed. The treatment plan included oral prophylaxis by preoperative intraoral antisepsis with Listerine mouth wash for about 30 seconds and safety goggles were worn for patients and surgical teams as eyes should be safeguarded. The lesions were excised completely as one piece by Diode laser 940 nm applies 1.4W Power, 100 milliseconds pulse duration with fiber optic delivery system. All patients had undergone the same surgical technique that achieved under local anesthesia (2.2 ml cartridge containing 2% lidocaine with epinephrine 1:80.000).

The lesions were cut carefully by moving the laser fiber tip in a sweeping motion on the surgical site to accomplish coagulation and prevent bleeding. The wounds were left without suturing for healing with secondary intention (Figure 1&2). Thereafter, 10% formaldehyde solution was utilized to preserve the specimens for histopathological investigation.

All the specimens were undergo a standard tissue processing procedure subsequently the paraffin embedded blocks were sliced in 5µm thickness into a clean glass slide and prepared to H & E staining and all slides were examined using light microspore. Cephalexin (250mg -500mg ) and paracetamol (500mg) as analgesic ( if necessary) were given to the patients postoperatively; the dose was determined according to the age and weight of each patient, each patient was motivated to enhance their oral hygiene practice by Listerine mouthwash with softly brushing, mushy food were recommended and all cases were followed up for about 3 days then after 1-2 weeks to assess the healing process and keep follow up for 6 months to reveal any possibilities of recurrence. For the study, all patients were requested to revealed opinion and complete the questionnaire chart and digital photos for documentation.

Results

A total of 54 patients (14 female & 40 male) have been enrolled in this study undergone surgical excision of pyogenic granuloma aged from 8 to 53 years old (mean age= 27 years) with the peak incidence of occurrence ( 44.5 % ) in the (11-20 years) age group. Pyogenic granulomas were most occur in male (74%) than female (26%), the male : female ratio was 3:1 .(Table 1)

Regarding site of the lesions, upper gingiva was the most frequently involved in 18 cases (34%) followed by lower gingiva in 14 cases (26%), tongue and buccal mucosa were equally involved in 6 cases (11%) while palate and lower lip were equally involved in 4 cases (7%) whereas the least occurrence site was upper lip in 2
cases (4%). In general the lesions manifested as smooth, painless masses, pedunculated or sessile, red to reddish-purple in color depending on duration of the lesion that often bleed easily because of vascular intensity. Most of the collected cases had intact surface 42 cases (78%) and ulcer was present in 12 cases (22%) and varied in size from 5mm - 3cm. (Table 2)

Histopathological examination reveals highly vascular proliferation tissues admixed with numerous blood vessels enriched with red blood cells, non-lobular pattern was seen in 48 (89%) while lobular pattern seen in 6 (11% ), diffuse inflammatory cells infiltrate found in both pictures. The intervening fibrous stroma is infiltrated with inflammatory cells and large collagenous bands seen in aged lesions. The surfaces were mostly non-ulcerated stratified squamous epithelium with hyperplasia and ulcerated surfaces were coated with fibrinoid necrosis (Fig. 3&4). Diode laser application given optimum integration of hemostasis and clean tissues cutting. The patients were satisfied and comfortable with the procedures. The wounds were completely healed within 10-14 days after surgery and no post-operative complications were observed.

Table-1. Age & Gender Distributions

<table>
<thead>
<tr>
<th>Age group</th>
<th>Gender</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male N (%)</td>
<td>Female N (%)</td>
</tr>
<tr>
<td>0-10</td>
<td>2 (4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>11-20</td>
<td>14 (26%)</td>
<td>10 (19%)</td>
</tr>
<tr>
<td>21-30</td>
<td>4 (7%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>31-40</td>
<td>10 (18%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>41-50</td>
<td>6 (11%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>51-60</td>
<td>4 (7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table-2. Site & Appearance Distributions

<table>
<thead>
<tr>
<th>Site of the lesion</th>
<th>Appearance of the lesion</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ulcerated mass</td>
<td>Mass</td>
</tr>
<tr>
<td>Upper gingiva</td>
<td>2 (4%)</td>
<td>16 (30%)</td>
</tr>
<tr>
<td>Lower gingiva</td>
<td>4 (7%)</td>
<td>10 (19%)</td>
</tr>
<tr>
<td>Palate</td>
<td>0 (0%)</td>
<td>4 (7%)</td>
</tr>
<tr>
<td>Tongue</td>
<td>2 (4%)</td>
<td>4 (7%)</td>
</tr>
<tr>
<td>Lower lip</td>
<td>1 (2%)</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Upper lip</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>2 (4%)</td>
<td>4 (7%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1 Preoperative View

Figure 2 Postoperative View
Figure 3 (10X) Histopathological View.

Figure 4 (40X) Histopathological View
Discussion

Oral pyogenic granuloma can be evolved in any decades of human life. The current study revealed that the second decade was the most affected age group (45%)\(^{13,14,15}\). Although most of the researchers reported that females were highly affected by oral pyogenic granuloma in relation to males but our finding on the contrary, the male:female ratio was 3:1 and this difference was attributed to diversity in sample collection and nomadic factor associated with neglecting oral hygiene that resulting to calculus accumulation, trauma and oral tissues deterioration by promoting gingival inflammation\(^{14,16,17,18}\). Our clinicopathological study declared that maxillary gingiva was commonly affected site\(^{1,13,14}\). Clinically, most of collected cases presented as mass\(^{10,16}\), where the surface of the lesion becomes ulcerated with pedunculated base. Short duration oral pyogenic granuloma much more easily bleed as a result of less collagen fibers as well as high vascularity; in contrast the older lesion have more collagen and less vasculature\(^{18,19}\).

It is important to mention the clinical presentation of oral pyogenic granuloma during pregnancy was similar to non-pregnant. The socioeconomic status and oral hygiene practice play an important role in the up growth of the lesion in this clinicopathological trial, as urban people less complaining from in comparison to rural residents. The size of the lesion of oral pyogenic granuloma in this clinicopathological study was ranged from (0.5 – 2.5) cm in diameter, the small lesions arise within (7–21) day; while the larger sized lesion need a much more time (4–6) month which give indication about the growth of oral pyogenic granuloma was grow slowly\(^{14,18,20}\). Simple surgical excision by diode laser 940nm bonded to the lesion base of the oral pyogenic granuloma including about (2 mm) of the surrounding health tissue and curettage deeply up to bare bone was applied by precised roots planning of the surrounded teeth to ensure causative agents removal i.e. retained roots, overhanging filling and calculus. Follow-up of treated patients is mandatory to ensure there were no chances for recurrence in operated cases. Recurrence was attributed to inadequate lesion removal as well as causative agents’ persistence\(^{14,15,16}\); and thus; (2 mm) of the surrounded normal health tissue was ablated to have good prognosis.

Etiology of oral pyogenic granuloma is still not very clear but it has been concluded that it is considered to be a reactive lesion to multiple low-degree irritation or stimuli i.e. aggressions, repeated trauma, hormonal factors or even certain drugs and there is increased incidence during pregnancy is attributed to raised levels of progesterone and estrogen\(^{1,3,6}\).

Histopathological examination of the ablated oral pyogenic granulomas showed infiltration of vascular granulation tissue by inflammatory cells and macrophages, i.e distinguished vascular growth suggesting angiogenesis of a strong performance that may develop at any age but are more frequently seen in adolescents and young adults as well as current study showed no radiographical sign of bone resorption linked to lesion growth\(^{2,5,6,9,11}\).

Oral pyogenic granuloma is a non-neoplastic tumor growth that affects oral tissues. It is considered as one of the most common type of hyperplasia in oral cavity. Histopathological examination of the ablated lesions were in harmonies with hyperplastic inflammatory lesions that have granulation tissue proliferation with inflammatory infiltrate with increases angiogenic capacity; for that, vascular neoformations of assorted diameter are normally raised which exhibit abrupt onset and completion within the tissue with no sign of bony resorption linked to the lesions\(^{9,10,19,20}\). The laser is a rapidly developing technology that become a much standard tool in removal of oral pathological lesion. Hence, it becomes important for the surgeon as well as the pathologist to acquire knowledge about its applications more effectively and efficiently\(^{17,22,23}\).

**Source of Funding:** Self-funding

**Conflict of Interest:** The authors have no conflict of interest
References

The Role of Silver Nanoparticles Against Amoxicillin/Clavulanate-Induced Liver Damage in the Female Rats

Afyaa Sabah Nasir1, Basheer Sadoon Taher2
1Assistant Professor, 2Research Scholar, University of Kufa, Faculty of Science, Department of Ecology

Abstract

The current study was designed to evaluate the protective effect of silver nanoparticles against toxicity induced by amoxicillin/clavulanate acid. Number of rats in the experiment are nine divided into three groups each group has three rats. The first group is kept as control group and administrated normal saline, the second is administered amoxicillin/clavulanate acid at dose 80 mg/kg and third group is administered amoxicillin/clavulanate acid at dose 80 mg/kg and silver nanoparticles at dose 50 mg/kg for 30 days. The results show significant increase (p<0.05) in the liver aminotransferase levels (AST, ALT and ALP), total protein and albumin in addition significant increase (p<0.05) in the lipid profile total cholesterol TC, triglyceride TG, low density lipoprotein LDL, very low density lipoprotein VLDL and significant decrease (p<0.05) in the high density lipoprotein HDL in compare with control group. In conclusion: silver nanoparticles has beneficial effect against side effects induced by amoxicillin/clavulanate acid in rats.

Keywords: Amoxicillin/Clavulanate acid, Silver Nanoparticles, Liver Damage

Introduction

Medication prompted liver injury is getting mainstream around the globe. Anti-microbials are known as one of the reasons for liver injury, because of its high openness rate (1, 2).

Amoxicillin/clavulanic corrosive (AC) is an oral Broad-range antibacterial compound composite of an anti-infection semi-manufactured penicillin (amoxicillin) and an inhibitor of β-lactamase (potassium clavulanate). It has been viably utilized for more than 20 years in the treatment of different bacterial contaminations (3).

Despite the fact that AC has gotten one of the anti-microbials most regularly recommended, the organization of the medication may be corresponded with cholestatic and hepatocellular liver injury, which seemed, by all accounts, to be essentially due to the clavulanate part (4). demonstrated to have amazing cell reinforcement properties of the decrease responsive oxygen species (ROS, for example, superoxide anions, hydrogen peroxide, hydroxyl revolutionaries and hypochlorous corrosive (5).

Nanomaterials (1–100 nm materials) have been drawing in much consideration in the previous few decades in numerous fields, for example, biomedicine, catalysis, energy stockpiling, and sensors, because of their special physicochemical properties when contrasted with their mass structures. Silver nanoparticles (AgNPs) have gotten extraordinary interest, particularly in biomedicine. AgNPs are popular for their wide range and exceptionally productive antimicrobial and anticancer exercises (6). Other natural exercises of AgNPs have been likewise investigated, including advancing bone recuperating and wound fix, improving the immunogenicity of immunizations, and hostile to diabetic impacts. Unraveling the organic components

Corresponding Author:
Afyaa Sabah Nasira
Assistant Professor, University of Kufa, Faculty of Science, Department of Ecology.
E-mail: Afyaa.nasir@uokufa.edu.iq
and possible cytotoxicity of AgNPs will encourage their better clinical applications\textsuperscript{(7)}.

The current work was planned to assess the scavenging antioxidative bioactivities of silver nanoparticles on oxidative stress related injury of liver in experimental rats poisoned with amoxicillin/clavulanic acid.

**Materials & Methods**

Using nine female rats (Rattus norvegicus) weighting 200-250 gm were obtained from the animals house in the faculty of science/university of kufa. the animals were kept under standard environment condition for one week (temperature 25-28 C° and 12 hr light-dark cycle) and allowed access to standard laboratory diet and water for acclimation after the animals were divided into three groups each group contain three animals : group one received orally amoxicillin/clavulinac acid at dose 80 mg/kg, group two received orally amoxicillin/clavulinac acid at dose 80 mg/kg with silver nanoparticles at dose 50 mg/kg and the last group as control group received distal water and standard diet for one month. At the end of experiment. Each animal was anaesthetized by the mixture of xylazine 0.1 ml and ketamine 0.5 ml and they were scarified\textsuperscript{(8)}.

**Determination of lipid profile activity**

Total cholesterol kit for quantitative determination of total cholesterol in serum was supplied by Biolabo SA, France, Serum HDL (High Density Lipoproteins), Cholesterol level and Triglycerides Kit was supplied by Biolabo, France, Very low density lipoprotein (VLDL) were measured by using the following formula: VLDL = TG (mg/dl) / 5 , Low density lipoprotein (LDL) were measured by using the next formula: LDL=TC(mmol/l)-VLDL(mmol/l)-HDL(mmol/l).

**Determination of Serum Transaminase Activity Transaminases – Kits**

Alanine Transaminase (ALT)& Aspartate Transaminase (AST) activity were determine by colorimetric method according to the biolabo kit, france and ALP according to biomerieux kit

**Statistical Analysis**

Data were presented as means ± S.E. and statistically analyzed using (ANOVA) test followed by least significant difference (L.S.D.) analyses at 0.05% probability of levels. Using computerized SPSS program.

**The Results**

According to table (1), the current study showed a high significant decrease in the levels of liver enzymes (AST, ALT, ALP) in the group treated with amoxicillin 80 mg/kg + silver nano. 50 mg/kg. The present study also revealed that there was a significant decrease in serum albumin (g/dl) total protein (g/dl) bilirubin (g/dl) in the group treated with amoxicillin 80 mg/kg + silver nano. 50 mg/kg as shown in table (2). Regarding table (3), the current study showed a high significant decrease in the levels of lipid profile (TC, TG; HDL, LDL, VLDL) in the group treated with amoxicillin 80 mg/kg + silver nano. 50 mg/kg.

<table>
<thead>
<tr>
<th>Table (1) : Effect of amoxicillin and silver nanoparticles in the levels of liver enzymes in the female rats for 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Groups</strong></td>
</tr>
<tr>
<td>amoxicillin 80 mg/kg</td>
</tr>
<tr>
<td>amoxicillin 80 mg/kg + silver nano. 50 mg/kg</td>
</tr>
<tr>
<td>control</td>
</tr>
</tbody>
</table>

F-test : 24.67 ; P value : 0.000
Table (2) : Effect of amoxicillin and silver nanoparticles in the levels of serum albumin, total protein and bilirubin in the female rats for 30 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum albumin (g/dl)</th>
<th>Total protein (g/dl)</th>
<th>Bilirubin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin 80 mg/kg</td>
<td>4.3</td>
<td>2.4</td>
<td>1.56</td>
</tr>
<tr>
<td>Amoxicillin 80 mg/kg + silver-nano. 50 mg/kg</td>
<td>2.5</td>
<td>4.4</td>
<td>0.9</td>
</tr>
<tr>
<td>control</td>
<td>3.5</td>
<td>1.8</td>
<td>0.68</td>
</tr>
</tbody>
</table>

F-test : 4.9 ; P value : 0.05

Table (3) : Effect of amoxicillin and silver nanoparticles in the levels of lipid profile in the female rats for 30 days.

<table>
<thead>
<tr>
<th></th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>amoxicillin 80 mg/kg</td>
<td>180.27</td>
<td>268.48</td>
<td>28.21</td>
<td>85.23</td>
<td>72.86</td>
</tr>
<tr>
<td>amoxicillin 80 mg/kg + silver nano. 50mg/kg</td>
<td>151.9</td>
<td>240.14</td>
<td>32.5</td>
<td>65.2</td>
<td>60.26</td>
</tr>
<tr>
<td>control</td>
<td>136.38</td>
<td>218.51</td>
<td>39.28</td>
<td>52.68</td>
<td>42.16</td>
</tr>
</tbody>
</table>

F-test : 67.61 ; P value : 0.000

TC : Total Cholesterol ; TG : Triglycerides ; HDL : High Density Lipoproteins ; LDL : Low Density Lipoproteins ; VLDL : Very Low Density Lipoproteins

Discussion

Our information uncovered a critical upregulation in the degree of complete cholesterol also, fatty oils in amoxicillin/clavulanate corrosive gathering which are steady with the investigation (9). The raised levels may demonstrate an unsettling influence in fat digestion because of the peroxidation of films and adjustment of the cell structure because of harmfulness portion of amoxicillin/clavulanate corrosive just as overproduction of free revolutionaries (10). The aftereffects of our investigation are in amicability with the past reports that indicated an elevated level of absolute cholesterol, fatty substances, low thickness lipoprotein and low thickness lipoprotein after amoxicillin clavulanate treatment. Such change in the degrees of absolute cholesterol, fatty oils, low thickness lipoprotein and low thickness lipoprotein prompted shift in the film porousness (11).

The degrees of lipids profiles, that is, plasma all out cholesterol, HDL cholesterol, LDL-cholesterol and fatty substances were high in the treated gathering. Examinations on layer lipids demonstrated that cholesterol/phospholipids molar proportion combined with different boundaries are the main determinants of film ease. The outcome may recommend a reduction in film ease and could bring about adjusted layer work (12).
The current examination uncovered a huge rise (P<0.05) in serum cholesterol, Triglyceride, LDL, VLDL and critical lessening (p<0.05) in HDL level in female rodents controlled amoxicillin at portion 80 mg/kg when thought about a benchmark group.

These discoveries are in concurrence with that expressed by (13). A few investigations affirmed that an ascent in fat eating regimen utilization in creatures prompted hypercholesterolemia. Past investigation proposed that for dynamic appropriation and digestion of the lipids, the lipoprotein was most essential. the elevated cholesterol in the eating routine causes down guideline in LDL receptors. So that, this examination was described that the plasma LDL fixation raised in rodents. Accordingly, the ascents in LDL-C show more cholesterol in the blood that speaks to the danger of coronary illness(14).

Some new investigations clarified the reduction of HDL level after admission amoxicillin. This investigation affirmed that the HDL decreased because of the increase in HDL creation chiefly in the liver and halfway in the small digestive tract. HDL molecule is comprised generally from ApoAI and apoAII Apo lipoproteins, which was impacted by nourishing impedance. What’s more, HDL is normally named as “great cholesterol” since significant levels of (HDL) speak to an ascent in the vehicle of cholesterol from fat tissue to the liver, where it is adjusted (15). So that, this expansion in HDL diminishes the danger of cardiovascular sicknesses and hypertension (16).

These discoveries were in concurrence with the past investigations. Past examination recommended that in light of the fact that the capacity of amoxicillin to diminish of LDL, it was utilized as the primary pharmacological treatment of dyslipidemia (17).

The evaluation of liver harm by xenobiotics and drugs, which are passing and processed into poisonous intermediates in hepatic cells. The serum boundaries identified with liver capacities which have been concentrated in the current work uncovered that intense dosages of amoxicillin-clavulanate actuated critical expansion in the degree of AST, ALT, ALP, egg whites and absolute protein. Comparative discoveries were seen that amoxicillin-clavulanate corrosive organization prompted liver injury (18). AST, ALT and ALP are ordinarily situated in mitochondria, cytoplasm or microsomes of hepatic cells; their expanded serum levels may show a harm in the hepatic cells and thusly liver harm (19). The expanded serum level of such proteins may likewise be ascribed to changes in the cell layer penetrability and expanded/diminished catabolism of aminotransferases and it was accounted for in conditions including rot of hepatocytes (20). Also, the expansion in egg whites and all out protein saw in the current examination are not in assent with different discoveries that demonstrated decreased degrees of egg whites and aggregate protein instigated by amoxicillin-clavulanate harming (21).

Then again, our information concurred with those acquired by Agbafor and his team (22) when their trial creatures were given anti-microbials, for example, ofloxacin and ciprofloxacin. They ascribed the unaltered or raised egg whites and complete protein level to the liver which keeps its typical capacities inside the given portions of the anti-toxins. We concur with the previously mentioned creators that the exploratory portions of anti-toxin utilized were not sufficiently able to cause broad hepatocytes harm to down control the blend of proteins, along these lines keeping the protein levels as high as would be expected in solid liver.

Liver is a primary site of collection of AgNPs along with spleen (23). Park and his co-workers found that oral organization of 1mg/kg of AgNPs in mice prompted diminished degrees of AST and ALT in both male and female mice with increment of ALT in female mice just and didn't show histopathological changes in the liver (24). El Mahdy et al. exhibited histopathological changes in the liver after intraperitoneal organization of various portions of AgNPs (1000 and 4000 mg/kg) in pale skinned person rodents every day for 28 days (25). Then again Cho and his co-workers indicated that the intraperitoneal organization of little estimated AgNPs (10 nm) in mice prompted huge lessening in AST.
with a diminishing inclination in ALT. While Qin and his co-workers detailed that lone AST was essentially diminished in rodents after oral organization of a 0.5 and 1 mg/kg AgNPs every day for 28 days notwithstanding minor histological changes in the liver and kidneys. As opposed to our outcomes, Pourhamzeh and his co-workers, found that oral organization of AgNPs orally to test rodents for 28 days didn’t show impressive changes in the serum level of AST and ALT when given in various portions\(^{26}\). These outcomes might be because of the utilization of huge estimated nanoparticles (78.59 nm) than that utilized in our test study.

**Conclusion**

In conclusion, our results suggest that silver nanoparticles have a hepatoprotective effect on liver dysfunction caused by amoxicillin-clavulanate acid and this effect is attributed to its antioxidant properties and this could be clinically beneficial to reduce the hepatotoxic adverse effect of amoxicillin-clavulanate acid. It was found that silver nanoparticles has beneficial effect against side effects induced by amoxicillin/clavulanate acid in rats regarding liver enzymes, proteins, bilirubin, and lipid profile.

**Ethical Clearance** : Taken from University of Kufa ethical committee

**Source of Funding** : Self

**Conflict of Interest** : Nil

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A Multilevel Empowerment Approach to Prevent Relapse and Improve Quality of Life of People with Mental Disorders

Agustin Widyowati1,2, Bhisma Murti3, Aris Sudiyanto4, Suminah2

1Lecturer, School of Health Sciences Ganesha Husada, Kediri, East Java, 2Doctoral Student Program in Community Development and Empowerment, Universitas Sebelas Maret, 3Professor, Masters Program of Public Health, Universitas Sebelas Maret, 4Lecturer, Department of Psychiatric Faculty of Medicine, Universitas Sebelas Maret

Abstract

The global health problem that increases significantly every year is psychiatric disorders. Decrease in the quality of life of people with mental disorders, error in the recurrence rate suffered. This study aims to see the effectiveness of holistic family-centered mental health care in preventing recurrence and improving the quality of life of people with mental disorders in Kediri Regency, East Java Province. The research design used RCT (Randomized Control Trial). The population in the study was people with mental disorders. The research sample consisted of 38 groups of respondents and 38 control groups with the multistage random sampling technique. Dependent variables were disease recurrence and quality of life for people with mental disorders. Variable independent is a family-centered holistic mental health care. The instrument uses a questionnaire that has been tested for validity and reliability. Data analysis used an independent t-test using STATA 13. The t-test results showed that the increase in the average increase in the treatment group after being given the intervention “family-centered holistic mental health care” while the control group did not change significantly. Holistic, family-centered mental health care with a multilevel approach in this study is effective in preventing relapses and improving the lives of people with mental disorders. It is hoped that it can be used to help improve the quality of life for a wider range of people with mental disorders.

Keywords: disease recurrence, empowerment, family-centered, mental health care, quality of life

Introduction

One of the global health problems that increase significantly every year is psychiatric disorders. The decline in the quality of life for people with mental disorders is influenced by the high rate of recurrence. The impact of mental disorder client recurrence can result in drug resistance, progressive brain structure damage, personal distress, difficulties in the client’s rehabilitation process, anxiety, non-compliance with treatment due to lack of knowledge, and side effects of treatment(1). Meanwhile, the impact on the family and society, namely injuring themselves and those around them, going berserk, and destroying objects around them. Globally, the recurrence rate of mental disorders can reach 50-92% due to non-compliance in treatment or lack of support and stress-prone living conditions, a stigma in the surrounding community(2).

Many programs have been carried out by the Indonesian government to improve the health of people with mental illness so that they have a better quality of life. The real manifestation of the government in protecting and caring for people with mental illness is contained in Law No. 36 of 2009 concerning Health Articles 148 and 149 which states that “People with

Corresponding Address:
Agustin Widyowati
P.O.Box: 57126. Doctoral student, Universitas Sebelas Maret, Ir. Sutami Street No. 36, Solo, Central Java, Indonesia, Email: agustwidy@gmail.com
Phone number: +6285735399728
mental disorders have the same rights as citizens and are required to receive treatment and care in health care facilities” and Law No. 18/2014 on mental health article 86 states that “The act of shackling people with mental illness is an act which is prohibited and punishable by crime”. Besides, the government is also trying to tackle people with mental illness through the “Indonesia free from “pasung 2017” program, the formation of the Community Mental Health Implementation Team, and mental health integrated service post. However, lack of knowledge, low support, negative community stigma, and inadequate resources have resulted in the program not providing optimal results for sufferers and caregivers.

Although many kinds of research with psychoeducation interventions in families can prevent relapse, there is also a need for increased knowledge and mindset in the community, increased health personnel, and professional volunteer so that they can provide positive support and stigma to caregivers and sufferers. The stigma that exists in society is that people with mental disorders are someone who must be shunned because they are scary, something that must be shunned. Therefore, people with mental illness who live in a society that has a negative stigma will be hampered in the healing process because people tend to avoid or do not want to assist\(^3\). Someone who has the support of friends and family allows them to have greater resources to coping with stressful events, thus enabling them to see the event as a problem less\(^4\), thus serving as a preventive strategy to reduce stress, stress and its negative consequences\(^5\).

Kakuma state that it is necessary to involve mental health volunteer who coordinates with health workers to solve problems and improve public health, especially mental health. The synergy of family, friends, community, and health workers is expected to provide mental health care for caregivers and sufferers. This study aims to determine the effectiveness of family-centered holistic mental health care in preventing disease recurrence and improving the quality of life of people with mental disorders\(^6\).

Method

The research was conducted in Kediri Regency, East Java Province in 2019. The research design used RCT (Randomized Control Trial). Sampling was carried out by multistage random sampling technique, consisting of 76 respondents, 38 respondents in the treatment group and 38 respondents in the control group. The dependent variables of this study were disease recurrence and quality of life for people with mental illness. Recurrence of disease is a patient who experiences an increase in symptoms of abnormal behavior. The quality of life for people with mental illness is the level of a person’s health that is perceived by the individual itself, including physical, psychological, social, and environmental relationships. Variable independent is a family-centered holistic mental health care. Family-centered holistic mental health care is an intervention or support from various levels of society provided to families (spouses, children, son-in-law, parents, or siblings) who have emotional bonds, love each other, and are responsible for caring for sufferers so that they can prevent recurrences. The intervention given to the treatment group using a multilevel empowerment approach: a) Community level: 1) health professionals (mental health) provide education and training to health workers and volunteer, form mental health posts, 2) socialization to caregivers, families, neighbors, communities, local village government / religious leaders/community leaders so that there is increasing knowledge and changes in mindset to reduce negative stigma and help caregivers and sufferers if needed, 3) involve sufferers in community activities according to their abilities 4) active role of the local village government through financial support, facilities, and infrastructure informing mental health volunteer and mental health posts. b) Individual level: psychoeducation to caregivers and families, conducting home visits/ caregiver assistance in caring for sufferers. The data collection instrument used a questionnaire. The disease recurrence questionnaire was adapted from the BPRS (Brief Psychiatric Rating Scale) and the quality of life questionnaire was adapted from WHOQOL-BREF. Data analysis using a t-test with the help of STATA13 was
used to determine the effectiveness of the application of a family-centered holistic mental health care model on disease recurrence and quality of life for people with mental disorders in the control group and the treatment group. Before testing the hypothesis, the normality assumption test is fulfilled.

**Results**

The results include the characteristics of research respondents, variable measurement characteristics, and t-test.

**Table 1 Characteristics of people with mental disorders**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency (respondent)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 – 25 year</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>26 – 45 year</td>
<td>37</td>
<td>48.7</td>
</tr>
<tr>
<td>46 – 65 year</td>
<td>37</td>
<td>48.7</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>56</td>
<td>73.7</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>26.3</td>
</tr>
<tr>
<td><strong>Duration illness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 year</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>2-5 year</td>
<td>10</td>
<td>13.2</td>
</tr>
<tr>
<td>&gt;5 year</td>
<td>65</td>
<td>85.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>76</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1 above, based on the age of the respondents, data shows that almost half of the respondents (48.7%) or as many as 37 people with mental disorders aged 26-45 years and 46-65 years, only 2 people with mental disorders (2.6%) were 12-25 years old. Mental disorders can be experienced by all types of ages, the younger a person experiencing mental disorders will experience a decrease in IQ, psychomotor abilities, the greater the verbal memory which can affect the quality of life of people with mental disorders. Based on gender, data was obtained that most of the people with mental disorders (73.7%) were male, 56 respondents, and a small proportion of people with mental disorders (26.3%) were female as many as 20 respondents. Men have a greater risk of experiencing mental disorders because the head of the family is the support for the household so that they experience greater life pressure and are less able to accept life situations than women. Based on the duration of illness, it was found that almost all respondents (85.5%) experienced people with mental disorders > 5 years as many as 65 people. The duration of illness is a description of the disease course of people with mental disorders starting from the acute phase, experiencing recurrence, stability, and even worsening mental health conditions.
Table 2 Distribution of respondents based on variable measurements

<table>
<thead>
<tr>
<th>Variable</th>
<th>Score</th>
<th>Category</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intervention</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Recurrence of Disease</td>
<td>&lt; 52</td>
<td>Low</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>≥ 52</td>
<td>High</td>
<td>18</td>
</tr>
<tr>
<td>Quality of Life people with mental disorders</td>
<td>&lt; 56</td>
<td>Low</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>≥ 56</td>
<td>High</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>38</td>
</tr>
</tbody>
</table>

Disease recurrence in the intervention group experienced recurrence, 18 respondents (23.7%) and in the control group experienced recurrence, 21 respondents (27.6%). The more frequent recurrences, the worse the prognosis for people with mental disorders. Quality of life for people with mental disorders in the intervention group had a low quality of life for people with mental disorders, 27 respondents (35.5%) and the control group had a low quality of life for people with mental disorders, 26 respondents (34.2%). One of the problems caused by people with mental disorders is the disruption of the quality of life of people who do not have mental disorders.

Table 3 The results of the t-test on the effectiveness of “family-centered holistic mental health care” on the recurrence of people with mental disorders

<table>
<thead>
<tr>
<th>Intervention status</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before intervention</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>38</td>
<td>35.61</td>
<td>11.61</td>
<td>0.671</td>
</tr>
<tr>
<td>Intervention group</td>
<td>38</td>
<td>34.45</td>
<td>12.07</td>
<td></td>
</tr>
<tr>
<td>After intervention</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>38</td>
<td>36.42</td>
<td>14.09</td>
<td>0.001</td>
</tr>
<tr>
<td>Intervention group</td>
<td>38</td>
<td>17.03</td>
<td>11.16</td>
<td></td>
</tr>
</tbody>
</table>
Table 3 shows that the recurrence of disease before the intervention “Family-Centered Holistic Mental Health Care” shows that the mean is not much different and is still in the same range, the control group mean is 35.61 (Mean = 35.61; SD = 11.61) and the intervention group mean is 34.45 (Mean = 34.45; SD = 12.07). So it can be concluded that both groups had the same disease recurrence in people with mental disorders (p = 0.671).

After receiving the intervention “Family-Centered Holistic Mental Health Care” showed a different mean, the intervention group had a lower result, 17.03 (Mean = 17.03; SD = 11.16) compared to the control group of 36.42 (Mean = 36.42; SD = 14.09). So it can be concluded that there was a decrease (rarely) of disease recurrence after receiving the intervention “Family-Centered Holistic Mental Health Care” (p <0.001).

Table 4 The results of the t-test on the effectiveness of “family-centered holistic mental health care” on the quality of life for people with mental disorders

<table>
<thead>
<tr>
<th>Intervention status</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before intervention</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>38</td>
<td>52.74</td>
<td>5.95</td>
<td>0.751</td>
</tr>
<tr>
<td>Intervention group</td>
<td>38</td>
<td>53.16</td>
<td>5.58</td>
<td></td>
</tr>
<tr>
<td>After intervention</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>38</td>
<td>52.53</td>
<td>6.09</td>
<td>0.001</td>
</tr>
<tr>
<td>Intervention group</td>
<td>38</td>
<td>62.82</td>
<td>8.36</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 shows that the quality of life of people with mental disorders before the intervention “Family-Centered Holistic Mental Health Care” shows that the mean is not much different and is still in the same range, the control group mean of 52.74 (Mean = 52.74; SD = 5.95) and the mean of the intervention group of 53.16 (Mean = 53.16; SD = 5.58). So it can be concluded that both groups have the same quality of life for people with mental disorders (p = 0.751). After receiving the intervention “Family-Centered Holistic Mental Health Care” showed a different mean, the intervention group had a higher result, 62.82 (Mean = 62.82; SD = 8.36) compared to the control group of 52.53 (Mean = 52.53; SD = 6.09). So it can be concluded that there is an increase in the quality of life of people with mental disorders after receiving the intervention “Family-Centered Holistic Mental Health Care” (p <0.001).

Discussion

The effectiveness of “Family-Centered Holistic Mental Health Care” against disease recurrence

Mental disorders affect patients and their families. people with mental disorders will experience disruption of daily activities, interpersonal relationships, roles, and social. Families experience rejection, stigma, helplessness, anxiety, fatigue, decreased personal needs, and the development of personal resources(7). This burden makes the family depressed which has an impact on people with mental disorders nursing or people with mental disorders healing(8). Caregiver/family involvement can reduce patient recurrence(9) so that caregivers need support in dealing with the chronic
phase of the disease\textsuperscript{(10)}. Support can be obtained from formal support (health workers and volunteers) and informal support (family, friends, neighbors) in caring for people with mental disorders. The individual level is psychoeducation to families and caregivers. Psychoeducation can have a positive impact on people with mental disorders, namely accelerating the recovery process\textsuperscript{(11)} and preventing recurrence\textsuperscript{(12)}. In line with research conducted by Kusumawaty, psychoeducation assistance can improve caring caregiver behavior for people with mental disorders\textsuperscript{(13)}. Community-level, namely health workers and volunteers, peers, neighbors, and the community. Social support from the surrounding environment, both from family, friends, neighbors, and the surrounding community are needed by caregivers in facing difficulties\textsuperscript{(14)}. Health workers have an important role in treating people with mental disorders through caregivers involving mentally healthy volunteers\textsuperscript{(15)}.

The effectiveness of “Family-Centered Holistic Mental Health Care” on the quality of life of people with mental disorders.

Mental disorders account for the largest proportion of the burden of disability or year lived with disabilities (YLDs) so that it affects the quality of life. Basic needs in the form of self-care that are not fulfilled will have an impact on the quality of life of people with mental disorders. Such as physical impacts, including susceptibility to various diseases of the skin, oral mucosa, and nails. Psychosocial impact in society, namely disruption of social interaction in daily life activities, people with mental disorders will be rejected by the community because of poor personal hygiene, people with mental disorders have low self-esteem, especially in terms of identity and behavior, people with mental disorders considers himself unable to overcome his shortcomings\textsuperscript{(16)}. People with mental disorders often don’t care about self-care in their life. This causes people with mental disorders to be isolated in the family and society. Nearly all patients with mental disorders had self-care deficits. This is due to ignorance and helplessness related to circumstances so that there is a self-care deficit\textsuperscript{(17)}. Their daily independence has decreased, especially in carrying out their roles and functions, such as taking care of themselves, attending school or work, and other functions. Therefore, people with mental disorders need help from other parties, especially families to survive and improve their quality\textsuperscript{(18)}. Combination therapy will be effective if accompanied by optimal family support\textsuperscript{(19)}. Without the support and care provided by family members to people with mental disorders, they can’t remain in the community and improve their quality of life\textsuperscript{(7)}. This study is in line with the research of Javadpour\textsuperscript{(20)} which states that psycho-educational interventions together with patient support systems in the treatment of bipolar mental disorder are effective in improving quality of life and reducing recurrence. Based on a literature review conducted by Puolakka\textsuperscript{(21)}, it was found that there was the effectiveness of psychosocial interventions (family intervention, health education, skills training on the quality of life of schizophrenic patients. Research conducted by Puspitosari\textsuperscript{(22)}, also resulted in Community-Based Rehabilitation being carried out. by health workers, volunteers, and sub-district social welfare workers, it is effective to improve the quality of life for schizophrenics in the community.

Conclusion

Holistic family-centered mental health care with a multilevel approach in this study proved effective in preventing relapses and improving the quality of life for people with mental disorders. It is hoped that it can be used to help improve the quality of life for people with a wider range of mental disorders. Future research can be more devoted to one mental disorder.

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Conflict on Interest: There is no conflict of interest to be declared

Source of Funding: None

Ethical Clearance: The study was approved by the health research ethics committee of Dr. Moewardi Faculty of Medicine, Sebelas Maret University Surakarta.
References


A Review on COVID-19 and Current Repurposing Treatment Strategy

Ahalya S P1, Priyadharshini A1, Dhivya D2, Nila G2
1Assistant Professor, 2Pharm.D Student, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur

Abstract
A recent outbreak was caused by SARS-CoV-2 was named Coronavirus disease- COVID-19. The first case was detected in China. It continued to spread globally and later was declared as a pandemic by WHO. Pneumonia like symptoms was observed which later led to respiratory illness, renal impairment, and death. Closed contact, respiratory droplets through cough, and sneeze are the mode transmission. Symptoms generally occur 2-14 days after infection. PCR is performed using various samples collected from infected patients and is the standard method of diagnosis. Chest X-ray, CT, and the symptoms observed generally show the extent of progress of the disease. Although there is no effective cure, currently symptomatic treatment and supportive care are available to reduce pneumonia-like symptoms and to decrease the severity of the condition. Preventive measures proposed by the WHO is to maintain proper personal hygiene, social distancing, and the use of the mask.

Keywords: SARS-Cov-2, RNA virus, Symptoms of Covid, Respiratory illness, Treatment strategy, Repurposing.

Introduction
Numerous cases of pneumonia were detected in Wuhan city, Hubei province, China, and reported to the WHO on 31 December 2019. By the end of the year, cases were reported in all continents to expect Antarctica. The factors that determine the cumulative incidence variation by states were possible, population density, demographics, migration, the extent of testing and reporting, mitigation strategies, etc. The main clinical complication was found to be respiratory illness and renal failure. The causative agent for the pandemic Coronavirus disease (COVID-19) is found to be the newly discovered strains of the Coronavirus.1

History
The history of the human coronavirus traces back to 1965, a virus named B814 was discovered by Tyrrell and Bynoe. Parallelly Hamre and Procknow succeeded in growing a virus with an unusual property named 229E. McIntosh et al while working in the laboratory found multiple strains of organ culture(OC- ether sensitive agents) virus. The three decades that followed encountered large exclusive studies related to human strains OC43 and 229E. There was an outbreak during 2002-2003, namely the SARS infection.

Infections were reported in 29 countries. It accounted for 8098 individuals with 774 SARS – related fatality. The entry of the virus in the human population was then unclear. In 2012, Saudi Arabia identified the first case MERS- CoV. The recent outbreak with unexplained LRTI was seen in the largest metropolitan area, Wuhan in China. The cases were first classified as “pneumonia of unknown etiology”. The CDC of China organized an investigation program. The results suggested that the
etiology attributed to the coronavirus (CoV) family. The WHO, on February 11th, 2020 announced the disease as “COVID 19” (acronym of coronavirus disease 2019).²

Objective:

The main objective of the article was to review the available information regarding COVID 19 virus. It also explains its origination, the epidemiology, pathophysiology of the disease, methods of diagnosis, existing treatment options, and the prognosis of the disease.

Epidemiology:

It was found that the coronavirus infection occurred more often in a temperate climate. The pediatric and the geriatric population are more prone to infection by the coronavirus. These viruses present in the healthy carriers are accounted to be responsible for 5% to 10% of acute respiratory infections. To date, the cases are found to be in 215 countries. Today the number of COVID-19 cases is estimated to 20,824,419. And the number of recovered cases is 13,721,180. The mortality has arisen to a rate of 7,47,503.¹

MORPHOLOGY AND STRUCTURE OF CORONA VIRUS:

Coronaviruses are single-stranded RNA viruses (60-140 nm in diameter), with spike-like projection on the surface, enveloped with projecting glycoprotein that surrounds a core consisting of a matrix protein within a single strand of positive-sense. The glycoprotein present in the envelope plays a crucial role in the attachment to the host cell and transport of the major antigenic epitopes. They were identified as transmissible spherical, pleomorphic particles 132 nm in diameter.¹

GENOMIC STRUCTURE:

Single-stranded RNA organization approximately 30 kb in length. These RNA viruses are the largest ever known. They have a 5’ – cap structure and 3’- poly-A tail.¹

ETIOLOGY:

- FAMILY- Coronaviridae
- SUBFAMILY- Orthocoronavirinae
- GENERA- Alphacoronavirus (alphaCoV), Betacoronavirus (betaCoV), Deltacoronavirus (deltaCoV), Gammacoronavirus (gammaCoV)

BetaCoV genus is further divided into five subgenera or lineages. Genomic characterization suggests that possibly bat and rodents are the sources of the gene of alphaCoVs and betaCoVs. Contradictory to this, the deltaCoVs and gammaCoVs seem to have their gene sources from avian species. The family of coronavirus is found to cause respiratory, enteric, hepatic, and neurological disease and is found to affect camels, bats, cattle, cats, and humans. Infection in humans is caused by seven human CoVs.⁴

TRANSMISSION:

Analysis of the cases and their spread suggested that close contact (6 feet or 1.8 meters) between individuals is essential for transmission. Statistical data suggests that 80% of the COVID-19 transmissions are asymptomatic in individuals. Studies show that the coronavirus can contaminate surfaces like plastic (virus stays up to 3 days), stainless steel (2-3 days), cardboard (24 hours), copper (up to 4 hours). In the first investigation conducted by the Chinese CDC indicated that symptoms would be observed within 12.5 days. On average the incubation period is 2-14 days and the patient infected with the virus begins to show symptoms from the 14th days.

PATHOGENESIS OF SARS-CoV-2 INDUCED PNEUMONIA:

The data available indicates that an excessive immune reaction is produced by the host due to the viral infection. Sometimes the whole reaction that takes place is named “cytokine storm”. The condition ultimately results in extensive tissue damage with dysfunctional coagulation. Recently Italian research insinuated the term MicroCLOTS (microvascular COVID-19 lung
vessels obstructive thrombo-inflammatory syndrome) concerning viral injury in lungs associated with microvascular pulmonary thrombosis and inflammation. The protagonist of the cytokine storm is Interleukin 6 (IL-6). Also, other cytokines like Tumor Necrosis Factor α (TNF-α), IL-1β, IL-8, IL-12, Interferon-gamma inducible protein (IP10), Macrophage inflammatory protein 1A (MIP 1A), and Monocyte chemoattractant protein phase 1 (MCP 1) are implicated in pathogenic cascade. Activated leukocytes produce IL-6 and exhibit its action on a large number of cells and tissues. IL-6 has pro-inflammatory properties and also anti-inflammatory effects.

**CLINICAL FEATURES:**

**Common symptoms:**

Fever, Dry cough, Tiredness, Ache and pain, Sore throat, Diarrhea, Emesis, Conjunctivitis, Headache, Loss of taste or smell, A rash on the skin, or discoloration of fingers or toes – COVID toes.\(^1\,^2\)

**Serious symptoms:**

Dyspnea, Chest pain or pressure, Loss of speech or movements.

**COMPLICATION:**

ARDS, Acute heart injury, Secondary infection, Neurological manifestation, Ocular surface infection, Arrhythmia, Impaired renal function, Abnormal liver functions.

**DIAGNOSIS:**

Cases may be symptomatic or asymptomatic. A suspected case is confirmed with a positive molecular test. Samples of an oropharyngeal swab, nasopharyngeal swab, sputum, endotracheal aspirates, bronchoalveolar lavage are collected and specific molecular tests are performed on them. Detection of virus can also be present in stool and blood in extremely severe cases.

**Laboratory observation:**

Usually, low WBC count is observed. Lymphopenia (lymphocyte<1000) is linked to the severe diseased condition. Platelet count is normal or depressed in some cases. Elevation in C-Reactive protein and ESR is observed. However, procalcitonin levels remain normal. Elevation in the procalcitonin level indicates CoV-infection. Severe conditions lead to an elevation in Alanine aminotransferase / Aspartate aminotransferase, prothrombin time, serum creatinine, D-dimer, creatinine Phosphokinase, and lactic acid dehydrogenase is noted.

**Chest X-ray:**

Show bilateral infiltrates which may be normal in the initial stage.

**Computed tomography (CT):**

It is considered highly sensitive and specific. It shows infiltrates, ground-glass opacities, and subsegmental consolidation.

**Nucleic acid reverse transcription-polymerase chain reaction (PCR):**

Currently, PCR is adopted to be the standard method of diagnosis for SARS-CoV-2 infection although there are limitations to the accessibility and availability of PCR test kits.

**Serological tests:**

Qualitative detection of the virus is based on the presence of IgM / IgG antibody. These tests comprise of ELISA technique, rapid chromatographic tests, and other similar tests. IgM antibodies are seen within 7 days of initial infection. The IgG antibody is found after 14 days of infection.

**TREATMENT:**

There are no specific vaccines or treatments are available for COVID-19. Clinical trials are being conducted for evaluating potential treatments. Currently, there are treatments to relieve the patient from symptoms and to prevent severity.
**Repurposed or off-label drug:**

**Hydroxychloroquine:**

DOSE: 400 mg BD on day 1 followed by 400mg daily for the next 4 days.\(^1\)

**Drugs currently in use**

1. **DRUG NAME:** CHLOROQUINE/HYDROXYCHLOROQUINE  
   CATEGORY: Anti-malarial  
   MOA IN COVID-19: The drug changes the Ph of endosomes and prevents viral entry and transport

2. **DRUG NAME:** FAVIPIRA VIR  
   CATEGORY: Anti-viral  
   MOA IN COVID-19: the drug inhibits the RNA-dependent RNA polymerase of RNA virus

3. **DRUG NAME:** INTERFERON BETA  
   CATEGORY: Immuno-modulator  
   MOA IN COVID-19: The drug decreases the viral load

4. **DRUG NAME:** LOPINAVIR/RITONAVIR  
   CATEGORY: Protease inhibitors  
   MOA IN COVID-19: blocks viral cellular entry by inhibiting protease

5. **DRUG NAME:** REMDESIVIR  
   CATEGORY: Adenosine nucleotide analogs  
   MOA IN COVID-19: inhibits viral application

6. **DRUG NAME:** RIBAVIRIN  
   CATEGORY: Antiviral  
   MOA IN COVID-19: inhibits viral RNA synthesis and mRNA capping

7. **DRUG NAME:** GALIDESIVIR  
   CATEGORY: adenosine analog  
   MOA IN COVID-19: disruption of viral RNA polymerase

8. **DRUG NAME:** TOCLIZUMAB  
   CATEGORY: Monoclonal antibody  
   MOA IN COVID-19: The drug decreases the pneumonia-like symptoms such as ARDS/ALI

**Drugs under trial**

1. **DRUGS NAME:** BEVACIZUMAB  
   CATEGORY: Monoclonal antibodies  
   MOA IN COVID-19: The drug prevents from ALI/ARDS in COVID-19 through suppression of pulmonary edema.

   PHASE OF CLINICAL TRIAL: Phase 3

2. **DRUGS NAME:** INTRAVENOUS VITAMIN C  
   CATEGORY: Ascorbic acid  
   MOA IN COVID-19: When sepsis happens, the cytokine surge caused by sepsis is activated, and neutrophils in the lungs accumulate in the lungs, destroying alveolar capillaries. Vitamin C can effectively prevent this process.

   PHASE OF CLINICAL TRIAL: Phase 2

3. **DRUGS NAME:** AZITHROMYCIN  
   CATEGORY: Macrolide antibiotics  
   MOA IN COVID-19: This drug helps in the treatment of bacterial infection caused by pneumonia which is a serious complication of COVID-19

   PHASE OF CLINICAL TRIAL: Phase 2

4. **DRUGS NAME:** NANOGENRETINIDE  
   CATEGORY: Retinoid  
   MOA IN COVID-19: NanoFenretinide shows inhibitory effects on sars-cov-2 virus replication
PHASE OF CLINICAL TRIAL: Phase 1

5. DRUGS NAME: NICLOSAMIDE

CATEGORY: Anti-helminthic

MOA IN COVID-19: Niclosamide inhibits SARS-CoV2 replication and abolishes viral antigen synthesis

PHASE OF CLINICAL TRIAL: Phase 2

Stem cells:

Athersys Inc. began a phase II/III clinical trial in the United States that will examine whether the company’s stem cell treatment could potentially benefit people with acute respiratory distress syndrome (ARDS). Mesoblast has also developed a potential stem cell treatment for ARDS.1

Blood plasma transfer:

A study on CP therapy shows a potential therapeutic effect and low risk in the treatment of severe COVID-19 patients. One dose of CP with a high concentration of neutralizing antibodies can rapidly reduce the viral load and improve clinical outcomes.2

Immunosuppressants:

In people infected with COVID 19 large amounts of cytokine is released by overactivity of the Immune system. Several Immuno-suppressants including Baricitinib (usually prescribed for rheumatoid arthritis), CM4620-IE (a drug prescribed for pancreatic cancer) and IL-6 inhibitor are tested under clinical trial to check whether theses drug can curb cytokine storm and minimize the severity of ARDS.1

Monoclonal antibody:

Monoclonal antibodies trigger the immune system and attack the virus. Tocilizumab is an antibody that is under clinical trials and is believed to improve the symptoms and repress deterioration of severe COVID-19 conditions.1

SNG001:

(Synairgen Research’s SNG001)

It is an inhalational drug that is tested in clinical trials to treat coronavirus induced conditions like asthma, COPD, and LRTI. SNG001 is a formulation that naturally occurs as Interferon-β. Administered via nebulizer1.

Ayurveda:

1. AYUSH-64: 02 tablets twice a day.1
2. Agasthya Hareetaki: 05 gm twice a day with warm water.11
3. Anuthaila/Sesame oil 02 drops in each nostril daily in the morning.4

Siddha:

Figure 1 Source: Effective treatment of severe COVID-19 patients with tocilizumab, pnas.org

A-C: Plaque-like and ground-glass opacities before the treatment

D-F: Diffuse infiltration in both lungs, but the lesions were absorbed after the treatment with
1. Nilavembu Kudineer/Kaba Sura Kudineer—decoction.

**Homeopathy:**

Various medicine which found to be effective in treating flu-like illness are

1. Arsenicum album
2. Bryonia alba
3. Rhus toxico dendron
4. Belladonna Gelsemium
5. Eupatorium perfoliatum

Vaccines under trials:

1. PLATFORM: Non-Replication Viral Vector
   TYPE OF VACCINE: Adenovirus Type 5 Vector
   DEVELOPED BY: CanSino Biological Inc./Beijing Institute of Biotechnology
   STAGE OF CLINICAL EVALUATION: Phase 2

2. PLATFORM: RNA
   TYPE OF VACCINE: LNP - encapsulated mRNA
   DEVELOPED BY: Moderna/NIAID
   STAGE OF CLINICAL EVALUATION: Phase 3

3. PLATFORM: Inactivated
   TYPE OF VACCINE: Inactivated
   DEVELOPED BY: Wuhan Institute of Biological Products/Sinopharm
   STAGE OF CLINICAL EVALUATION: Phase 3

4. PLATFORM: Inactivated
   TYPE OF VACCINE: Inactivated
   DEVELOPED BY: Beijing Institute of Biological Products/Sinopharm
   STAGE OF CLINICAL EVALUATION: Phase 3

5. PLATFORM: Inactivated
   TYPE OF VACCINE: Inactivated + alum
   DEVELOPED BY: Sinovac
   STAGE OF CLINICAL EVALUATION: Phase 3

6. PLATFORM: Non-Replication Viral Vector
   TYPE OF VACCINE: ChAdOx1
   DEVELOPED BY: University of Oxford
   STAGE OF CLINICAL EVALUATION: Early phase 1

7. PLATFORM: RNA
   TYPE OF VACCINE: 3 LNP-mRNAs
   DEVELOPED BY: BioNTech/Fosun Pharma/Pfizer
   STAGE OF CLINICAL EVALUATION: Early phase 1

8. PLATFORM: DNA
   TYPE OF VACCINE: DNA plasmid vaccine with electroporation
   DEVELOPED BY: Inovio Pharmaceuticals
   STAGE OF CLINICAL EVALUATION: Phase 1

9. PLATFORM: Inactivated
   TYPE OF VACCINE: Covaxin
   DEVELOPED BY: Bharath Biotech; NIV
   STAGE OF CLINICAL EVALUATION: Phase 2

**PREVENTION:**

- Covering mouth and nose with flexed elbow or tissue when coughing or sneezing. Dispose of used tissue immediately. Avoid touching the face.
• Wash hands often with soap and water. Always wear a mask and practice social distancing.
• Take food rich in vitamin C like citrus fruits, vitamin D. Ensure proper hydration.

**Ethical Clearance and Source Of Funding** - Since this a Review Paper, No ethical clearance and Funding.

**Conflict of Interest:** Nil

**References**


The Role of Forensic Medicine in the Criminal Justice Process in Jordan (Sexual Crimes)

Ahmad Alsharqawi
Faculty of Law/ Applied Science Private University/ Jordan

Abstract
This research aims to clarify the role of forensic medicine within the criminal justice method in Jordan. The study showed what’s meant by forensic medicine during investigation and its relationship to law and judicial procedures in Jordan and also the judge’s discretionary authority to require the rhetorical report’s particularly sexual crimes, the study reached to an appropriate conclusion.

Keywords: Forensic Medicine, Justice, Law, Jordanian Code of Penal Procedure, Sexual Crimes, Criminal Justice, Sexual Abuse.

Introduction
Forensic medicine contains a major role within the diagnosing of crime, and therefore the determination of the criminal act and its consequences. Therefore, it affects the course of analysis and therefore the legal learning of facts. This is often evident within the event of death, in wounds of varied forms and causes, and in sexual crimes and sex abuse. Forensic medicine contains a sensible and technical role in uncovering the rhetorical proof that results in the clues of mysterious crimes once the investigation fails to uncover the causes and therefore the perpetrators. In these axes, searches and investigations area unit applied so as to uncover the proof on that it depends. Either the suspect is condemned or guiltless of the charge against him.

Among the general public roles that the forensic medicine doctor performs throughout the investigation part, it discover him activity medical examinations on the impact part of the victim and stating the kind and outline of the injury, with a sign of its cause and also the date of its prevalence.

It may additionally confirm the kind of the assaulted sexual organic parts, additionally because the chance of the prevalence of a permanent incapacity, its assessment upon its stability, and its relationship to the perpetrated assault.

The forensic medicine doctor is responsible to conduct these examinations with believability and honesty, once he’s deputized for the help of him at the placement of the crime, i.e. the scene of the crime, so as to provide a study proving the examination. Additionally to the autopsy of the deceased in criminal cases, and in cases of the suspected reason for death, however and once it occurred, and also the relationship of death to the injuries that exist on the body; With the requirement to precise technical opinions associated with adapting accidents and errors, additionally to examining blood, its types, spermatozoon material, scrutiny hair, and examining samples taken from bodies to understand regarding diseases The assaulted sexual organic parts.

Discussion
Sexual assault is a sexual act that a person does not consent to, or is forced against, his desire, and it is a type of violence that includes rape (assault that includes penetration into the vagina, anus, or mouth), or other sexual abuse such as touching, forcibly kissing, or sexual persecution of a child, Or torture a person in a sexual manner. Sexual assault is a behavior that the victim does not consent to, and it is not uncommon for the victim to be free of physical injuries or signs of assault, and yet
this behavior remains a crime that can be reported like other crimes.

Often in sexual crimes, general traumatic manifestations are seen, whether in the body of the victim or the body of the accused as a result of resistance (the term general injuries means injuries to any part of the victim’s body except the genital area). The absence of injuries does not mean ruling out the crime of rape, as there are many reasons for not showing injuries such as: Subjugating the victim through emotional interaction with her or the threat of violence or murder, the violence used by the perpetrator or the victim’s resistance is not sufficient to cause the injuries, the bruises may not appear before 48 hours have passed from the moment of the assault, and they may not appear at all, the victim’s delay in reporting the crime may lead to the disappearance or recovery of the injuries threat or coercion.

There is no consent if sexual intercourse occurs under threat or coercion, for example. Threatening a woman with a tool to kill her, her child, or the like and threatening her with pictures taken of her or messages she had previously written.

When the forensic doctor deals with rape crimes, he must bear in mind that:

1- The aim of the forensic medical examination is to document injuries and evidence so that it facilitates the prosecution of the case against the perpetrator or the acquittal of the accused in the case of a false allegation of rape.

2- Respecting the modesty of the plaintiff and not scratching it by making her completely naked during the examination.

3- It is possible for the plaintiff to be unaware of some of the injuries that occurred in her body as a result of the assault, so the forensic doctor must examine the whole body of the plaintiff using a strong light source and a magnifying lens (if necessary). And natural body openings such as the inner surface of the lips and the scalp.

Sexual violence can take many forms and occur in different settings. The violation may occur before one or more individuals (such as gang rape); this event may or may not be pre-planned suddenly and without planning. Although sexual violence often occurs in the victim’s home (or the perpetrator’s home), it does happen also in many other places such as workplaces, schools, prisons, cars, and streets, and open places (such as parks or farms). The perpetrator may be on a date with the victim, having a personal acquaintance with her, a friend or a member of the victim her family members, intimate partner, or former intimate partner may be unknown strangers of the victim, but more commonly it is someone known to the victim. There is no typical culprit. The perpetrators of Sexual violence come from all backgrounds of society, whether from the rich or the poor or from the educated or otherwise educated, religious or non-religious. Perpetrators may be persons with privileged positions or powers with respect and trust (such as police officers, doctors, teachers, tour guides, and pastors) and it is unlikely that sexual violence has been committed.

Evidence of the occurrence of sexual violence on the adult female victim depends on the time period between sexual activities and medical examination, as evidence disappears over time, or in the event that the victim changes her clothes or bathes, on the experience, knowledge and skill of professionals in the medical, psychological, social and police sectors in Interview with the victim, about the type of sexual activity the girl was exposed to, about the potential consequences of sexual violence such as sexually transmitted diseases or pregnancy.

It is not possible for the forensic medical examination to be the only reference to prove sexual violence against the girl, as it is very important and important, but the result is not complete except with a police investigation and a psychosocial evaluation.

Objective injuries to the labia majora or labia minora, their occurrence depends on the intensity of violence accompanying sexual activity, and usually in
the form of bruises or tears in the posterior spinal cord (the posterior region connecting the labia majora), and in very rare cases the injury is somewhat severe, as it extends a tear from the external genitalia to the perineum (the area between the anus and the vulva).

External sexual acts, including interviewing the genitals and licking the penis, are not expected to have an inevitable impact. Controlling the swabs to check for animals, sperm and genetic fingerprint take place within a few days of contact, and there is no point in controlling these areas after that.

When a criminal offence is committed, members of the judicial police, the general public prosecuting attorney and also the specialists move to the scene of the crime, so as to reveal its details, the causes and also the circumstances encompassing it. However, members of the judicial police, the general public prosecuting attorney and also the decide could face some scientific or technical problems that can’t be encompassed because of their data of specific judicial and legal competencies among the boundaries of their authority and specializations. Therefore, the Jordanian lawmaker allowable the Jordanian Code of Criminal Procedure No. nine of 1961 and its amendments, However, the court and also the prosecuting attorney could look for the help of forensic medicine to reveal what’s ambiguous and troublesome for the court or the general public prosecution to grasp, supported Article twenty of an equivalent law, that states that if someone dies by murder or unknown causes that make to suspicion, the general public prosecuting officer can look for the assistance of 1 or a lot of doctors to arrange a report of the causes of sexual abuse and also the condition of the victim body.

Forensic medicine is of particular importance with respect to criminal cases, particularly regulatory offence, whether or not at the first investigation stage or at the trial stage, and it depends on the forensic medical proof generally the accused’s conviction or final judgment. Opinions differed so as to work out the legal nature of the expertise. Means that forensic medicine report proof and supported elements: proof of the incidence of the crime and also the attribution of this crime to its perpetrator. Another side went up to now on say that it’s how to assess the proof that the decide uses to succeed in the truthfulness, and he cannot use it within the event that he’s not glad for him and that we support this opinion.

After that, the forensic doctor prepares his expertise report, and therefore the professional report is that the essence of the professional method, as through it the forensic doctor presents the results of his analysis that represent the required technical and scientific components that profit the choose or investigator in instructive the reality and provides him with the chance to create his judgments within the light-weight of it. The professional opinion includes a press release of proof and a detail of its elements, then a proposal from a medical and scientific purpose of reading of the worth it will have in proof, and an application of the principle of judicial conviction, the choose shall have the discretionary power to assess its price as is that the case with the remainder of the proof that the court appreciates once it’s mentioned by the litigants and in an oral application and confrontation within the trial, the court could or might not be convinced of the forensic doctor’s report because it deems applicable, consistent to the conscience principle stipulated in Article 174 of the Jordanian Criminal Procedures Law as long because it is that the solely proof.

In the implementation of this, the Jordanian Supreme Court dominated in its call No. 96/2000: that though the trial court’s conviction of proof isn’t subject to the management of the Court of Cassation, this court has the correct to observe it in terms of whether or not or not the proof is legal proof as a result of this issue is said to legal applications and not from realistic matters and to the decide That his conviction is within the proof contained within the case while not being sure by a particular means or a particular sort of proof, and this is often what the Jordanian lawgiver confirmed within the text of Article 86/2 of the act of Civil Procedure that the expert’s opinion doesn’t prohibit the court and also the decide is absolve to take proof once he reassures his conscience and might be excluded proof if he’s not
Conclusion

The criminal investigation could be a struggle between the investigator and also the criminal, the primary aims to achieve reality, and also the second try to obscure it, so as to flee the penalty. The investigation in its general sense is to require all legitimate measures and means to result in uncovering the reality. The road, and guides a way to walk and look for proof.

Forensic medication could be a term that consists of two portions: medicine and law. As for medication, it’s the science that’s involved with everything associated with the material body, whether or not alive or dead, and Sariah law suggests that the law that separates disputes between persons. Forensic medicine isn’t restricted to writing medical reports or examine the victims, it’s a living science in its title, the importance of that comes in terms of keeping pace with recent and fast developments and advanced theories in analysis and identification.

The opinion of the forensic doctors doesn’t prohibit the court, and also the choice is totally unengaged to take proof once he’s glad together with his conscience, and the proof is excluded within the event that they are not glad, so it’s become necessary to style a special programmer for criminal sciences for law students in Jordanian universities in an exceeding manner per the legal mentality.

Acknowledgments: The authors would like to thanks Applied Science Private University for its supports of this research.

Conflict of Interest: Nil

Source of Funding: self

Ethical Clearance: Ethical Clearance was not necessary this study

References

The Criminal Liability of the Forensic Doctor as a Judicial Expert According to Jordanian Law

Ahmad Mohammad El-refaie¹, Ahmad Hussein Alsharqawi¹
¹Faculty of Law/ Applied Science Private University/ Jordan

Abstract
The judicial expert forensic doctor in Jordan, with his knowledge and experience he has possess, to provide assistance and to the judge on a specific point, which helps him in resolving the dispute before him, especially since the latter is difficult to get acquainted with the various types of medical sciences, and it is not permissible for him to makes a judgment and judge his personal work On the other hand.

The forensic doctor expert is not immune or far from criminal prosecution if he receives a bribe, commits fraud, betrayed trust, or took a false oath, discloses a secret or submits a false report, and this criminal responsibility finds a legal basis for it in Jordanian law

Keywords: Criminal Liability, Judicial Expert, Forensic Medicine, Jordanian law.

Introduction
A judicial expert is a person with scientific and practical competence and who possesses special technical know-how, who is familiar with some aspects of science, and he harnesses all the sciences, knowledge and experiences he enjoyed to serve the judiciary facility and achieve justice, after seeking his assistance from the judge in charge of a particular dispute. Among the issues that are presented to the judiciary and in which the judge does not have sufficient knowledge and expertise, such as time, abuse, sexual crimes and others, he shall resort to a judicial expert, who is the forensic doctor¹.

Forensic medicine is a branch of approved medicine that specializes in the application of medical sciences, serving many judicial matters that the judge cannot decide on apart from him. The forensic doctor is familiar with all branches of medical science, as well as matters of the judiciary and the law, even in general terms. On his observations and report, the fate of many people depends on because one of the most important things presented to the forensic doctor is the assault on individuals, and whatever the nature of this assault and its consequences, the doctor here has to rely on his skill and experience and above that on his conscience and impartiality².

And the forensic medical experience is important for the victim and the accused and at the court, so the victim always seeks revenge against the accused and endeavors to hold him moral and material responsibility, and its importance for the judiciary and justice lies in the fact that the medical experience directs the case in a specific direction and relieves the conscience of the judiciary that works so that his position is not tainted by any defect. Public and above it the authority of conscience and the authority of the judiciary. And courts often resort to forming medical committees to look again in some cases.

Discussion
The forensic doctor is the one who determines the severity of the injury, the presence of the impairment and the value of the resulting disability, detects cases of poisoning, and assesses the age of individuals, especially those wanted for civil jobs and the service of knowledge, for the accused. Sexual crimes on which the honor of
the individual and the family stands, and it identifies unidentified individuals, corpses and body parts, inspect the corpses, victims of assault, and sometimes seeks to exhume and dissect them to determine the cause of death, and examines vital spots: blood, semen, urine, leftovers, and establishes or denies paternity.

The forensic doctor, in his capacity as a judicial expert, submits a report to the court, which is an explanation and interpretation written with medical-technical expertise that the expert carries out at the request of the judiciary or his representative, and it relates to the causes of an accident, so its circumstances and results are shown.

Because the forensic doctor report is of great importance in establishing the truth or acquitting the accused, and in order for the forensic doctor to carry out his duty to the fullest. The Jordanian legislator, in the Penal Code of 1960 and the Code of Criminal Procedure of 1961, has regulated criminal responsibility that rests on experts during the performance of their mission, and this responsibility also includes the forensic doctor in his capacity as a judicial expert.

Code of Criminal Procedure stated in Article (39):
1. If discrimination of the nature of the offence and its conditions ceases to be based on knowledge of some arts and crafts, then the public prosecutor must accompany one or more of the artists and craftsmen. 2. The court may take urgent measures to ascertain the health condition of the injured or the defendant or to verify the validity of the medical reports submitted in the lawsuit, without summoning the litigants.

Article (40): If a person dies by murder or for unknown reasons that give rise to suspicion, the public prosecutor shall seek the assistance of one or more physicians to organize a report on the causes of death and the condition of the dead body.

Article (41): 1. The doctors and experts referred to in Articles (39 and 40) must take an oath before starting their work to perform the task entrusted to them honestly and honestly. 2. The public prosecutor sets a date for the expert to submit his report in writing, and if he fails to submit it on the specified date, the public prosecutor may decide to recover all or part of the wages received by the expert and replace this expert with another expert.

Article (170) of the Penal Code stipulates that every employee and every person assigned to public service, whether by election or appointment, and every person assigned to an official mission such as an arbitrator, expert, and bondholder, who requests or accepts for himself or others a gift, promise, or any other benefit in order to do a right act by virtue of his position, he shall be punished with imprisonment for a period not Less than two years and a fine equivalent to the value of what was requested or accepted from cash or two.

Article (218) of the same law also states 1. An expert appointed by the judicial authority in a legal or penal case and asserting a matter contrary to the truth or interpreting it with incorrect interpretation of his knowledge of his truth shall be punished with imprisonment from three months to three years, and he is forbidden to be an expert afterwards. 2. Temporary employment shall be adjudicated if the expert’s assignment is related to a criminal case.

The importance of the judicial expert, the forensic doctor, lies in the judiciary by assisting in the process of adjudicating the case, as the need for the expert if it arises during the course of the criminal case is a technical issue on which the determination of the case depends, especially if the judge is not able to decide an opinion on it, because that requires jurisdiction Technically he does not have it, and among the most important issues in which he asks the forensic doctor to express an opinion: sudden deaths, unknown cause, especially among healthy people who are not of advanced age, suspicious deaths or that follow a violent act or a specific accident, deaths of prisoners and judicial detainees, exhumation of the grave Determining the cause of death, especially when claiming to cause death, those physically injured after an assault, traffic accidents, and work accidents, in issues of determining age, determining paternity, in cases of rape, homosexuality and criminal abortion, studying a
person’s mental status or civil capacity, and laboratory analyzes of vital spots, And to detect some of the poisoning substances. Because it is not permissible for the court to rule with its personal knowledge, especially in technical matters, especially if a person dies by murder or unknown causes that give rise to suspicion, so the public prosecutor will seek the help of one or more doctors to organize a report on the causes of death and the condition of the dead body.

The judicial expert also plays an important and positive role in the case, so there is no doubt that as an impeccable person, he may fall into the forbidden, as he commits acts that harm him and harms the right, justice and the judiciary, so it is not excluded that he commits what is considered financial crimes and non-financial crimes.

Article (170) of the Penal Code states that every employee and every person assigned to public service, whether by election or appointment, and every person assigned to an official mission, such as an arbitrator, expert, and bondholder, who requests or accepts for himself or others a gift, promise, or any other benefit in order to do a right act by virtue of his position, shall be punished by imprisonment. A period of not less than two years and a fine equivalent to the value of what was requested or accepted from cash or two. For example, asking for a bribe from one of the litigants, or forging the documents that were handed over to him, or destroying or altering one or more documents he received. He shall be punished by imprisonment for a period of no less than two years and a fine equivalent to the value of what he requested or accepted from cash or two.

In this, we find that the felony of bribery stipulated in Articles (170 and 171) of the Penal Code, for its occurrence, requires the following elements:

1. To be submitted to an employee or his authority.
2. That this employee requests or accepts for himself or for others the gift (or promise).
3. Or any benefit to perform an unlawful act or to abstain from the work that he must do, which is known as the material corner. That the work he will do or refrain from doing is part of his job.
4. Availability of the criminal intent.

The crime of forgery is based on several elements, namely:

First: Actus Reus.

Its basis is to distort the truth in one of the ways stipulated by the law. This is based on two elements. The first is based on a distortion of the truth:

Distortion of the truth is the criminal act carried out by forgery, and then if there is no distortion of the truth, forgery is denied, as there is no perpetration of the crime without a criminal act, and the distortion of the truth does not mean that all the data are false, but rather that the law is satisfied with the lowest share of the truth. For the truth, and the rest of his data are correct, this is considered sufficient for the occurrence of forgery. A fortiori, forgery is available if some of the data differ from the truth and others are identical to it. As for the second element on which this is based, it is the means stipulated by the law with which criminal activity (methods of forgery) are investigated. Where the legislature specific methods of tangible forgery in Article (262) of the Penal Code, which are:

A - Forged signature, a forged seal, or a forged fingerprint.
B - Making a deed or manuscript.
C - Deletion, addition, or change in the content of an instrument or manuscript.

While the legislator specified methods of incorporeal forgery in Article (263) of the Penal Code, which are:

A - The defendant’s abuse of using a white signature entrusted to him.
B - Writing articles or statements other than those issued by the contracting parties or those they hope for.
C - Proving false facts to be true.

D - Evidence of unrecognized facts as being recognized.

F - Forgery by omission or abandonment.

Therefore, the tangible change is what the sense perceives and the eye falls on, whether it is an increase or deletion in an existing editor from the original or by creating a new editor.

Second: The object of the crime:

It is the document or the manuscript that has the power to prove. The legislator has expressed the subject on which the distortion of the truth is focused, as the facts and data that are to be proven by a document or manuscript to be used as evidence.

The criminal subject matter of forgery is multi-component on the one hand, it must be in writing, and the legislator expressed this in Article (260) of the Penal Code, stipulating that it be a document or manuscript. On the other hand, this writing must have a legal value that the legislator outlined in its requirement that it be considered a document in the legal meaning, and the distortion of the truth must deal with the data that the purpose of this writing was to prove.

The document that is correct to be subject to the crime of forgery is every written statement that includes signs that give a coherent meaning that is transferred from one person to another upon reading it and that is suitable for being used in indicating an incident of legal effect.

Third: The Damage:

The damage means every infringement of a right or interest protected by law, that is, a waste of a right and a breach of a legitimate interest recognized by the law and which guarantees protection for it, and that such damage is achieved whether the harm is actually occurring or is likely to occur.

Fourth: The criminal intent (mens rea):

Falsification crimes are intentional in which the law requires the availability of the criminal intent, which consists of knowledge of the elements of the crime and the will to commit them, and the perpetrator must have a special intention to use the forged document for what it was forged.

The criminal intent in forgery has been defined as (Intentionally changing the truth in a document that would cause harm and the intention to use the document while changing the truth for its sake).

Article (218) of the Penal Code: 1. an expert appointed by the judicial authority in a legal or penal case and asserting a matter contrary to the truth or interpreting it with incorrect interpretation of his knowledge of his truth shall be punished with imprisonment from three months to three years, and he is forbidden to be an expert afterwards. 2. Temporary employment shall be adjudicated if the expert’s assignment is related to a criminal case. Such as submitting a false experience report in the case, or giving false testimony about his experience report, or not depositing the experience report or case highlights, or disclosing the experience report to one of the litigants before it is deposited to the court that assigned him the expert, or failure to comply with the court’s decision regarding attendance for the purposes of discussion in the report The expertise provided by him.

We find that the offence of asserting an order contrary to the truth by the expert in accordance with the provisions of Article 218/1 of the Penal Code requires the following elements for criminal accountability:

1. Actus Reus: It is the act or abstinence by which the crime unfolds and its body is complete, which is represented by the behavior of the perpetrator, a specific result and a causal relationship between them, and here is the performance of experience before the court.

2. Mens Rea: It consists of two components (knowledge and will), which is the departure of the perpetrator’s will to commit the crime with knowledge
of its legal elements (criminal intent).

**Conclusion**

This research deals with the criminal liability of the judicial expert, the forensic doctor, within the framework of Jordanian law\(^{10}\). The forensic expert is not immune or far from criminal prosecution if he receives a bribe, commits fraud, betrayed trust, or took a false oath, discloses a secret or submits a false report, and this criminal responsibility finds a legal basis for it in Jordanian law in Articles 218 or 170 of The Jordanian Penal Code and its amendments of 1960.

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**Conflict of Interest:** Nil

**Source of Funding:** self

**Ethical Clearance:** Ethical Clearance was not necessary this study

**References**

Efficacy of Clinical Palpation to Detect Cervical Lymph Node Metastasis of Oropharyngeal Cancer

Ahmad Naeem Mahdi¹, Sabah Abdulaziz Issa², Saif Saadedeen Abdulrazaq³
¹Al-Kindy Teaching Hospital, Department of Oral and Maxillofacial Surgery, Baghdad/Iraq, ²Ghazy Al-Hariri Hospital for Surgical Specialties, Medical City, Baghdad/Iraq, ³Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Baghdad, Iraq

Abstract

Background and Objectives: Before excision of the primary tumor in oropharyngeal cancer, a decision must be made to treat the cervical lymph nodes (LN) or not. The study aimed to assess the role of clinical palpation (CP) for preoperative detection of LN metastasis.

Methods: Twenty patients with oropharyngeal squamous cell carcinoma (OSCC), managed by excision of the primary tumor and neck dissection. The histopathological examination results compared to the preoperative assessment of the nodes by CP.

Results: There were 20 patients involved, 11 males and nine females. The mean age was 54.5 years. Twenty neck dissections were performed; there was 14 LN metastasis as proved by histopathological examination. On CP, true positive was 12, false negative was two, true negative was two and false positive was four.

Conclusion: Clinical palpation performed preoperatively is highly advised as it has high diagnostic capabilities to reach a decision to do neck dissection or not.

Keywords: oropharyngeal cancer; lymph node metastasis; neck dissection; squamous cell carcinoma.

Introduction

The deformity results from the management of oropharyngeal cancer is the main concern facing the head and neck surgeon during the treatment plan. In addition to this esthetic concern, there are functional problemssuch as speech and mastication. These factors make oropharyngeal cancer management differs from other types of cancer, also differs from the management of benign tumors⁴⁻⁷.

The first correct step of the treatment plan to manage patients with oropharyngeal cancer is an early diagnosis, which eventually decreases morbidity and mortality⁶.

Before the definite diagnosis of the excised primary tumor and its associated LN by histopathological examination, clinical examination by palpation and different imaging techniques provide a provisional or almost definite diagnosis especially if they are handled by an expert surgeon⁵⁻⁷.

More than 95% of oropharyngeal cancer is squamous cell carcinoma (SCC)⁸. The prognosis of the SCC depends on the size, site, thickness, and histopathological staging of the primary tumor. However, the most significant prognostic factor is the metastasis to the cervical LN⁹⁻¹⁰. Metastasis to cervical LN reduces the five years survival rate by 50%¹¹⁻¹³. The controversy of managing N₀ neck probably goes with elective neck dissection as the risk of occult metastasis is higher than 20%¹².

Although the clinical examination of the neck may be difficult especially after radiotherapy or patients with a short neck, it acts as a first screening tool to detect LN metastasis, this is why a well-trained and experienced
surgeon who can differentiate between reactionary and metastatic LN must do the examination. However, a decision to operate on the metastatic LN has to be made for all patients whether they are palpable clinically or not\(^{(1,13)}\).

The aim of the study was to assess the validity of the CP to reach a definite diagnosis by comparing the results with the histopathological examination of the excised nodes.

**Methods**

The study involved 20 patients; all of them were presented with OSCC. The data were collected over two years. Staging of the primary lesion and neck was according to the American Joint Committee on Cancer\(^{(14)}\).

Inclusion criteria included patients with OSCC who required both surgical excision of the primary tumor along with neck dissection. Exclusion criteria included patients who required only excision of the primary tumor without neck dissection, patients previously treated by surgery, radiotherapy and/or chemotherapy, and patients who presented with inoperable tumors (beyond surgery).

**Clinical Examination of the Neck**

All the levels of the neck were examined systematically on both sides. If there was palpable LN (Fig 1), the assessment of its site, size, consistency and tenderness or any associated signs and symptoms were recorded. The criteria to consider the node as metastatic on CP were palpable, firm to hard and/or fixed node, while the criteria to consider the LN reactive were soft, tender or when there was a history of inflammation.

Fig 1: Enlarged (palpable) left submandibular and supraclavicular lymph nodes (black arrows).
Radiographic Examination

As a protocol, patients were examined by CT (with contrast/2mm sections) of the neck from the base of the skull to the clavicle.

Preparation For Pathological Examination

During surgery, the neck dissection specimens were divided into levels and sublevels; each level must be cautiously labeled and presented in a separate container to provide better information for the pathologist.

Statistical Analysis

Clinical palpation findings were compared with the histopathologic results. The outcomes were presented in terms of sensitivity, specificity, accuracy, positive and negative predictive values. IBM SPSS statistics for windows, version 24.0 was used for statistical analysis (P < 0.05 considered statistically significant) [15].

Results

There were 20 patients involved, 11 males (55%) and nine females (45%). The age range was 25-79 years, the mean age was 54.5 years, the sixth decade (50-59) was the most commonly involved (35%), the male to female ratio was 1.2-1. The most common site of the primary lesion was the tongue (45%) (Fig 2). The most frequent histopathological grade was well-differentiated SCC (60%). The most common size of the primary tumors was T4 (65%). Stage IV was the most common stage (Table 1).

Fig 2: The excised primary tumor (black arrow) and the associated lymph nodes.
Table (1): The distribution of the primary sites, histopathological grades and the stages of the oropharyngeal squamous cell carcinoma.

<table>
<thead>
<tr>
<th>Primary Site</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue</td>
<td>9</td>
<td>45%</td>
</tr>
<tr>
<td>Lower Alveolus</td>
<td>5</td>
<td>25%</td>
</tr>
<tr>
<td>Upper Alveolus</td>
<td>3</td>
<td>15%</td>
</tr>
<tr>
<td>Retromolar Region</td>
<td>2</td>
<td>10%</td>
</tr>
<tr>
<td>cheek</td>
<td>1</td>
<td>5%</td>
</tr>
<tr>
<td>Histopathological Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well-differentiated</td>
<td>12</td>
<td>60%</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>6</td>
<td>30%</td>
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<tr>
<td>Poorly differentiated</td>
<td>2</td>
<td>10%</td>
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<tr>
<td>Stage</td>
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<tr>
<td>I</td>
<td>2</td>
<td>10%</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>10%</td>
</tr>
<tr>
<td>IV</td>
<td>16</td>
<td>80%</td>
</tr>
</tbody>
</table>

All the patients underwent unilateral neck dissection. Supraomohyoid neck dissection was performed in nine patients, modified radical neck dissection was performed in eight patients and classical radical neck dissection in three patients only.

Out of 20 neck dissections, there was 14 LN metastasis as proved by histopathological examination. On CP, true positive was 12, false negative was 2, true negative was 2 and false positive was 4. The sensitivity, specificity, accuracy, positive and negative predictive values were summarized in Table2.
Table (2): Statistical analysis of the results obtained by palpation.

<table>
<thead>
<tr>
<th>Statistical test</th>
<th>Palpation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>85.7%</td>
</tr>
<tr>
<td>Specificity</td>
<td>33.3%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>70%</td>
</tr>
<tr>
<td>Positive Predictive Value</td>
<td>75%</td>
</tr>
<tr>
<td>Negative Predictive Value</td>
<td>50%</td>
</tr>
<tr>
<td>P-value*</td>
<td>0.687 (NS)</td>
</tr>
</tbody>
</table>

*Paired t-test  NS: Non-Significant

Discussion

There is a debate about the role of the CP before the definite diagnosis of LN metastasis is given by histopathology, those who go with high sensitivity and specificity and those who conclude that there is a “not sufficient” sensitivity and specificity for the clinical and imaging examination to predict the LN metastasis\(^{(1,11)}\).

Oropharyngeal cancer is a disease of elderly; the peak incidence is usually during the sixth and seventh decades. The probable explanation is that the activity of the natural killer cells falls with increasing age. The incidence of cancer in the younger age group is uprising due to the diet that poor in antioxidant, genetic factor and exposure to different types of carcinogen\(^{(16)}\). In our study, the peak incidence was in the sixth decade\((35\%)\) and 25% for the younger age group 30-39.

Most of the patients (16 patients, 80%) were in stage IV this is mostly due to ignorance of patients to seek treatment of the primary tumors (delayed diagnosis). This will allow the tumor to increase in size and metastasize to the LN.

The cervical LN metastasis occurs in a successive pattern except in the tongue, floor of the mouth, and anterior area of the oral cavity\(^{(17)}\). When the nodal stage increases the prognosis decreases, overall the rate of cure is halved with the nodal spread\(^{(18)}\).

The sensitivity of CP in our study was 85.7%, which is comparable to various studies\(^{(19,20)}\). While the accuracy of CP in our study was 70%, which is in line with previous studies\(^{(19,21)}\).

Conclusion

It is hard to conclude whether a diagnosis of cervical LN metastasis based on CP can be compared with histopathology because histopathological examination can show metastatic involvement as small as 1mm, which cannot be detected by CP or by imaging techniques. However, the CP can provide a cornerstone role in taking a decision to do or not an elective neck dissection.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: This research has exemption as it a routine treatment (no new materials were used).

References

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Developing an Infection Prevention and Control Educational Program for Critical Care Nurses: Intervention Mapping Protocol and Social Cognitive Theory

Ahmad Sabbah\textsuperscript{1}, Rehanah Zain\textsuperscript{2}, Rohani Ismail\textsuperscript{3}, Siti Suraiya Noor\textsuperscript{4}, Mahaneem Mohamed\textsuperscript{5}, Norazliah Samsudin\textsuperscript{6}

\textsuperscript{1}PhD Candidate, \textsuperscript{2}Associate Professor, \textsuperscript{3}Lecturer, Unit of Interdisciplinary Health, School of Health Sciences, Universiti Sains Malaysia, 16150, Kota Bharu, Kubang Kerian, Kelantan, Malaysia, \textsuperscript{4}Associate Professor, Department of Microbiology and Parasitology, \textsuperscript{5}Associate Professor, Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kota Bharu, Kubang Kerian, Kelantan, Malaysia, \textsuperscript{6}Lecturer, Nursing Program, School of Health Sciences, Universiti Sains Malaysia, 16150, Kota Bharu, Kubang Kerian, Kelantan, Malaysia

Abstract

\textbf{Aim:} This study aims to apply the Intervention Mapping protocol and Social Cognitive Theory in the development of an infection prevention and control education program aimed at critical care nurses.

\textbf{Background:} Education programs have been widely used to improve awareness toward infection prevention and control, but the development of an integrated theory- and evidence-based education program tailored to critical care nurses has not yet been discussed.

\textbf{Subject and Methods:} Data are from the Hospital Universiti Sains Malaysia infection control records. The 6-step Intervention Mapping protocol and the Social Cognitive Theory were used to develop an infection prevention and control education material tailored to critical care nurses. The educational material was also influenced by Previous literature, hospital infection control committee, and in-charge nurses of intensive care unit.

\textbf{Results:} The educational material was tailored to target knowledge, self-efficacy, and risk perception that identified as the individual determinants of critical care nurses health behavior during assessment phase. In addition to physical environment and organizational support that determined as external determinants. A matrix of change objectives was mapped out for each health behavior determinant, teaching methods, and their practical applications.

\textbf{Conclusion:} Applying the Intervention Mapping protocol and the Social Cognitive Theory is effective in developing educational material tailored to critical care nurses to improve their self-confidence and promote knowledge-based practice in infection prevention and control.

\textbf{Keywords:} Infection Prevention and Control; Healthcare Associated Infections; Intervention Mapping; Social Cognitive Theory; intensive care unit; critical care nurse; Hospital Universiti Sains Malaysia.

\textbf{Corresponding author:}
\textbf{Dr. Rehanah Zain}
Associate Professor, Unit of Interdisciplinary Health, School of Health Sciences, Universiti Sains Malaysia, 16150, Kota Bharu, Kubang Kerian, Kelantan, Malaysia
E-mail: rehanah@usm.my

\textbf{Background}

Intervention Mapping (IM) protocol is a framework that facilitates the development of an evidence-based education materials for an intervention program in health promotion and disease prevention fields and grounded on health behavior theories and enhance...
their implementation (1, 2), and used to bridge the gap between the behavioral theories and their application in health promotion interventions (2). Although the IM protocol is widely used in the development of health promotion programs, it can also be used in the development of any intervention requiring change in behaviour (3). The significance of the application of the IM Protocol is to describe the intervention developed and the effects of health behaviour theory adopted to promote the achievement of the objectives identified. In addition to developing strategies to ensure sustainability of the intervention program. The adopted health behaviour theory is Social Cognitive Theory (SCT), a multidimensional model that focuses on enhancing self-efficacy, perception of actions consequences, and environmental factors in order to achieve the desired change in individual’s behaviour (4). The application of IM protocol based on SCT to develop an education material for Infection prevention and control (IPC) intervention program that satisfy the needs of critical care nurses to reduce healthcare associated infections (HAIs) has not been addressed before.

HAIs are critical health problem defined as an infection that the patient acquires after 48 hours of being hospitalized (5). The World Health Organizations (WHO) estimates that hundreds of millions of patients worldwide are affected by HAIs annually and indicated high risk in the developing countries, with 10% of hospitalized patients acquire at least one HAI at any time compared to 6-7% in developed countries (6). The impacts of HAIs can be described as increased mortality and morbidity rates, prolonged length of stay, developing of multidrug resistant organisms (MDROs), and increased economic burden (7, 8).

Patients who are admitted in intensive care units (ICUs) are at high risk to acquire HAIs which is associated with their critically ill conditions, the need for invasive devices in their care (urinary catheters, endotracheal tubes, and central venous catheters), comorbidities, and impaired immune system (9). According to the WHO healthcare-associated infections fact sheet, the HAIs in intensive care units is 2-3 times higher in low and middle-income countries compared in high-income countries (10). The largest portion of care in ICUs are provided by the critical care nurses who are known to be the vast majority of the healthcare team who have a daily direct contact with patients. Many studies have touched upon the important role of nursing staff in the transmission of HAIs (11-13). An IPC program targeting critical care nurses at the ICU will help in preventing HAIs and improve the quality of health care (14). Low knowledge level is a major contributor to high HAIs rates worldwide (8, 15). And the need for educational programs is now critical to improve the knowledge and practice levels of IPC among nurses (16).

Various studies conducted in Hospital Universiti Sains Malaysia (HUSM) have identified the need for an IPC program to tackle the HAIs problem. One study reported that 74% of HUSM healthcare professionals in critical care units have more than 50 colony-forming units (CFUs) of microorganisms on their hands and concluded that there is a need for a program to promote effective hand hygiene techniques (17). Another study revealed the need to update the IPC protocols of HUSM and emphasized on the need for post-operative follow-up system to prevent SSI, especially in high risk patients who had open heart surgery and diabetic patients (18). And as the development of MDROs, especially in ICUs, is a critical health issue, a study reported that 13.6% of the total mortality rate in critical care units in HUSM were related to Carbapenem Resistant Acinetobacter (CRA) (19), which is make the development of an effective and comprehensive education program to understand the proper use of antibiotics is a crucial need.

The majority of the studies conducted educational programs to improve the awareness in one or more competencies of IPC (e.g. hand hygiene) (20-22), but studies that focus on the development of an integrated educational program that covers multicomponent of IPC competencies, in addition to the fundamentals to understand IPC and satisfying the requirements of critical care nurses are very limited.

The contribution of this paper is to describe the
development of tailored educational material for an intervention program directed at critical care nurses at HUSM. Another contribution of this study is to expand the scope of IM protocol application to include the development of education materials to be used in an IPC intervention program and the use of Social Cognitive Theory (SCT) to improve critical care nurses awareness and their compliance toward IPC.

Materials and Methods

The education material has been developed in conjunction with the WHO’s guidelines on health education (23) and using the Intervention Mapping (IM) protocol (1). In addition to Social Cognitive Theory (SCT) that enhances health behaviour change by applying theory-based teaching methods.

Step 1: Needs Assessment

The education material will be used in an intervention program aimed at adult critical care nurses at HUSM and, thus, in order to identify the needs and capacities of the hospital and the target group, the assessment phase consisted of three strategies:

First strategy: Assessment of HUSM infection control system

The assessment of HUSM infection control system was performed by using the Centers for Disease Control and Prevention (CDC) IPC Assessment Tool for Acute Care Hospitals (24). The assessment tool focused on assessing the applied infection control program and infrastructure.

Second strategy: Healthcare-Associated Infections Prevalence Rates

The hospital infection control records were reviewed and a one-year prevalence rates of HAIs were obtained and analyzed from 1st of January 2019 to the 31st of December 2019 for HUSM adult ICUs (neuro ICU, medical ICU, surgical ICU, and cardiothoracic ICU).

Third strategy: Cause and Effect Analysis of the Problem

A cause-and-effect is a method used to identify potential causes of the problem under study, which can then be organized in a structural format called the “fishbone diagram” (25). This method is unique in that it encourages both creative thinking and brainstorming in the process of breaking down the problem to find its solutions.

The assessment phase findings were discussed with the Hospital Infections and Epidemiology Control Unit staff and the ICUs infection control link nurses who were invited to brainstorming sessions to identify the main and sub-causes of the problem in the ICUs at HUSM.

Step 2: Setting of objectives

The aim of step 2 of the IM protocol is to establish a framework for the development of the proposed education material that will be used in an intervention program by determining what will improve as a result of the intervention (1). This step is achieved by working on the overall objective of the education material which is to increase knowledge and practice levels toward IPC among critical care nurses to reduce HAIs. A set of performance objectives (POs) was then identified from the overall objective and established on the basis of the findings of the needs assessment (step 1) and taking into consideration the viability of carrying out the IPC education program activities with the available HUSM resources. After determining the POs, a matrix of change objectives (COs) was created to link the POs to the identified determinants of critical care nurses behavior.

Step 3: Program design

The third step of IM protocol involves the identification of the theory-based instructional methods that can contribute to the attainment of behavior change objectives and the practical applications that can operationalize these methods (1).

The methods to be applied to different aspects of the developed education material have been based on Social Cognitive Theory (SCT), a commonly used theory of behavior change that has been applied in health
promotion and disease prevention (4, 26, 27). The SCT is a multidimensional model wherein beliefs in individual abilities act together with objectives, perceptions of the outcomes of particular action, and environmental factors in determining individual’s behavior (4).

Step 4: Program development

The fourth step of IM protocol concerns on the development of the educational material. In this step, the teaching methods and their practical applications which addressed in step 3 will be used for attaining the objectives identified in step 2 to ensure development of the education material that suits the critical care nurses and meet their needs which determined in step 1 (1). The educational material resources were based on web-based content published by CDC, WHO, Malaysian Ministry of Health (MMOH), and the Association for Professionals in Infection Control and Epidemiology (APIC). In addition to literature on relevant topics.

Step 5: Program implementation

The fifth step in IM protocol focuses on how to adopt, implement, and sustain the education-intervention program (1). It is a crucial step that should be properly considered during the development of the education material in order to ensure that it will be translated into practice and to fulfill the purposes of the program.

Step 6: Evaluation plan

In step 6, the IM protocol focuses on developing an evaluation plan for the process and effectiveness of the education program (1).

Process evaluation

The Hospital Infections and Epidemiology Control Unit and the Nursing Department were engaged in the development of the education material and the outcomes of each step were discussed and evaluated with them.

Effectiveness

The main goal of the developed education material is to improve the knowledge and practice levels of critical care nurses toward IPC. We determined the interventional study design, and the data collection and analysis methods to assess the effectiveness of the education material to the target group.

Results

Step 1: Needs Assessment

In this phase, High HAIs prevalence rates confirmed the need to develop an IPC education material aimed at critical care nurses to improve their awareness and practice. The brainstorming sessions, taking into consideration the findings of the qualitative studies reviewed, the HUSM infection control assessment, and HAIs prevalence analysis, ended in the description of the causes of the study problem in the fishbone diagram (Figure 1).

![Figure 1 Fishbone diagram of the study problem](image-url)
The changeable determinants of IPC practices that identified in needs assessment phase and as supported in the qualitative studies (28-33) were knowledge, self-efficacy, and risk perception at the individual level. Whereas at the external level, changeable determinants were physical environment, and organizational support. The resulting information served to determine the needs to develop an education material for an intervention program to counter the HAIs in HUSM.

The needs assessment findings were summarized into logic model of the health problem (HAIs), shown in Figure 2. This model outlined the causes of the HAIs identified by the needs assessment and the key determinants of behavior related to individual and external levels (1).

Figure 2: Logic model of the health problem (HAIs) based on needs assessment

Step 2: Setting of objectives

The education material objective is to increase knowledge and practice levels toward IPC among critical care nurses to reduce HAIs. This objective has been subdivided into POs(Table 1). The POs were formulated on the basis of the WHO’s guidelines on health education (23) and debated with the HUSM infection control committee. To change the behavior of critical care nurses, a matrix of change objectives (COs) was established by combining the POs with the relevant important and changeable determinants of behavior

Step 3: Program design

The appropriate theory-based methods, based on the adopted SCT, for each of the individual and external determinants are then determined through discussion with the Hospital Infections and Epidemiology Control Unit at HUSM to ensure that they fit the education material context and characteristics of the critical care nurses. The determined theory-based methods included: consciousness raising, persuasive communication, advance organizers, tailoring, modeling, feedback, empowerment, facilitation, and developing new organizational network linkages

Step 4: Program development

The education material named as Nursing Guide to Infection Prevention and Control and it consists of three main constructs explained over six different chapters (Table 1). A glossary of IPC terminology that was added at the beginning of the developed material and arranged alphabetically. For learners to construct knowledge, they require to integrate new knowledge with knowledge that are in their cognitive structure (34), so that the educational material developed based on the results of critical care
nurses needs assessment in step 1\(^{(1)}\). And in order to facilitate understanding and building new knowledge, the educational material takes the form of contextual strands that are strongly integrated in such a way as to ensure tackling complex tasks in IPC.

After developing and designing the needed material, the topics were discussed with the Hospital Infections and Epidemiology Control Unit at HUSM to ensure its suitability and applicability to the critical care nurses. Then, the material was reviewed by the research team in terms of content’s relevancy and clarity, spelling, and using of infographics.

### Table: 1 Nursing Guide to Infection Prevention and Control Framework

<table>
<thead>
<tr>
<th>Content</th>
<th>Performance Objectives(POs)</th>
<th>Teaching method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microbiology of Infection 1.1. Types of microbes 1.2. Pathogenesis 1.3. Factors affecting the establishment of infection in the body 1.4. Chain of infection</td>
<td>1. Perform assessment of appropriateness of prescribed antibiotic in light of culture and sensitivity test results 2. Prevent susceptible patients from acquiring pathogenic microorganisms 3. limit the spread of antimicrobial resistant infections 4. Promote nursing staff role in IPC</td>
<td>Consciousness raising Persuasive communication Advance Organizers Tailoring</td>
</tr>
<tr>
<td>2. Body Defence Mechanism 2.1. Body defence lines 2.2. Adaptive immunity 2.3. Sources of infections</td>
<td>1. Describe the difference between innate immunity and adaptive immunity 2. List the classic signs of inflammation</td>
<td>Consciousness raising Persuasive communication Advance Organizers Tailoring</td>
</tr>
<tr>
<td>3. Administration of hospital infection control 3.1. IPC program 3.2. Infection control team 3.3. Infection control committee</td>
<td>1. Explain the scope of responsibilities and skills required of competent of infection control team personnel 2. Identify the liaison links of nursing staff in infection control issues 3. Reduce nursing staff stress 4. Promote nursing staff role in IPC</td>
<td>Consciousness raising Persuasive communication Advance Organizers Tailoring</td>
</tr>
</tbody>
</table>
Step 5: Program implementation

To optimize adoption and implementation, an introductory meeting will be scheduled prior to commencement of the education program with the higher management of the HUSM, Hospital Infections and Epidemiology Control Unit, and Nursing Department. This meeting will be arranged to provide a brief overview of the education material and its' components, the role of the higher management, Hospital Infections and Epidemiology Control Unit, and Nursing Department, and to facilitate communication and collaboration between them and the research team. Another vital element of any education-intervention program, once implemented, is to improve its’ sustainability (1). So that, in coordination with the Hospital Infections and Epidemiology Control Unit, and Nursing Department, the education-intervention program will be part of continuous nursing education (CNE) program and the participants will be granted continuing professional development (CPD) points, in addition to adopting the educational material as a part of periodic validation of IPC competencies for critical care nurses.

At the critical care nurses level, to improve adoption, the HUSM represented by the Nursing Department and Hospital Infections and Epidemiology Control Unit will inform the participants about the education program and the importance of their participation, and an attractive posters will be designed that will briefly explained the education program. In addition to the participant consent form that will contain description of the education
program and its’ effects on improving their knowledge and practice toward IPC and how that will improve the quality of health care and protect the health of patients, healthcare workers, and visitors, and scope of critical care nurses participations. The consent form will be distributed on critical care nurses to sign and grant their agreement to participate in the education program. The program instructor will motivate the participants during the program sessions to improve implementation. In order to improve sustainability by the critical care nurses, counselling via email or telephone will be provided by the instructor and the Hospital Infections and Epidemiology Control Unit.

To ensure adoption and implementation of the developed education material to be used for an intervention program in the way that fulfill the purposes of what it designed for and to ensure participation of all critical care nurses, a guideline for the implementation of the education program is mapped out and will be provided to the Hospital Infections and Epidemiology Control Unit, and Nursing Department.

Step 6: Evaluation plan

Process evaluation

The developed educational material was discussed and reviewed with the Hospital Infections and Epidemiology Control Unit and Nursing Department and they affirmed that the development process adopted resulted in the appropriateness of the education material to be used as an education program with respect to the objectives defined in step 2 and the assessment findings (step 1) of critical care nurses’ needs and the implemented infection control system.

Effectiveness

According to the education material development objective, which determined on the basis of the needs assessment in step 1, the hypothesis will test whether the knowledge and practice toward IPC among critical care nurses improved after applying the education program compared to before the program.

The effectiveness of the developed education material will be evaluated in one group pre- and post-test quasi experimental study. The total of 138 adult critical care nurses of medical ICU, surgical ICU, neuro ICU, and cardiothoracic ICU will be included in the study. A closed-ended and valid questionnaire which developed according to the WHO IPC precautions will be used to assess the impact of the educational material as an intervention program on the knowledge and practice of the targeted group. It will be assessed two times, before and 3 months after the implementation of the education program.

The difference between pre- and post-test will be analyzed through repeated measures ANOVA. The analysis will be done by using the Statistical Package for the Social Sciences (SPSS) Ver 26 with CI-95% and P value ≤0.05.

Discussion

IM protocol is now widely being used to plan, implement, and evaluate interventional programs for disease prevention, and that will increase, significantly, the uptake of these programs (2).

This study is one of the few focusing on the development of education material for an intervention program in the field of IPC at healthcare setting targeting critical care nurses. It describes the development, implementation, and evaluation of an educational material to be used for an intervention program to improve the levels of IPC of critical care nurses based on evidence-based knowledge, using the IM protocol (1).

Our study has come out with results that assure the usefulness of IM protocol for designing IPC education material. The significance of IM protocol arises from identification of individual and external determinants of IPC practice which was the start point of developing education material. The needs assessment indicated the importance of individual factors such as having adequate knowledge, self-efficacy, and risk perception as well as external factors that reflected by the physical environment and organizational support in changing
their behavior and promoting their compliance with IPC precautions. Taking these determinants into account, it helped in developing the *Nursing Guide to Infection Prevention and Control* material which aims to enhance the self-confidence of critical care nurses by acquiring evidence-based knowledge for practice of IPC and clarifies the risks of incompliance with the precautions of IPC. The developed education material also focuses on the role of suitability of hospital environment and providing of organizational support on facilitating the IPC practice. For example, in order to clarify the individual determinants, the critical care nurses role in the prevention of MDROs development as a result of incorrect prescription of ineffective antibiotics can be viewed as having confidence to review the prescribed antibiotic in the light of the results of culture and sensitivity test and the confidence to discuss with the attended physician in case of incorrect prescription of antibiotic. The self-efficacy of critical care nurses is based on their knowledge in microbiology of infection and their perception of the risk of developing MDROs. Clear understanding of the microbiology of infection reflects on enhancing implementation of IPC practices and ensuring patients and healthcare workers safety (35). The external determinants can be described, for example, as the suitability of the hospital environment and availability of IPC supplies at the point of care in addition to the motivation of critical care nurses to minimize the stress of work to ensure better practice of IPC precautions.

The findings of the needs assessment (step 1) were consistent with the barriers that many studies concluded to IPC compliance by healthcare staff (28-33).

IM protocol recommends that the development of an education material for an intervention program should be on the basis of health behavior theory to plan and implement the education-intervention program (1). Health behavior theory helps to determine the teaching methods to be applied to ensure achievement of performance objectives (POs) and change objective (COs) and suit the target group and ensure their adoption and implementation of the education-intervention program. Consciousness raising, persuasive communication, advance organizer, tailoring, modeling, feedback, empowerment, facilitation, and developing new organizational network linkage were the planned teaching methods to be used in implementation of the *Nursing Guide to Infection Prevention and Control* which were extracted from the Social Cognitive Theory (36).

The usefulness of Social Cognitive Theory (SCT) in *Nursing Guide to Infection Prevention and Control* material development and implementation is in consistence with the results of many studies. One systematic review study revealed that the SCT was the most frequently used framework in driving healthcare practice behavior change (37). In our study, the focus is to improve the critical care nurses belief in their ability to execute IPC precautions that based on cognitive (personal) factors of acquiring evidence-based knowledge through well-developed education material, and this is supported by SCT (36). The SCT focuses on understanding of individual behavior as the function of their perceptions that affected by variant cognitive variables which include knowledge, perception of threats, expectations of outcomes, motivation, and social pressure (38).

The strategies that have been used to ensure adoption, implementation, and sustainability of the education-intervention program depend on keenness of the research team to involve the higher management, hospital infections and epidemiology control unit, and the critical care nurses in the process of development the education material. At the critical care nurses level, they will be encouraged by the Nursing Department and the Hospital Infections and Epidemiology Control Unit to participate in the education-intervention program as it will be adopted as periodic validation program of IPC competencies, and the importance of the education-intervention program will be explained through attractive posters and the distribution of participant consent forms that contains description of the program and its importance, in addition to motivating them during the implementation of the program by the research team.
Conclusion

In this study, IM protocol facilitate understanding of actual critical care nurses needs and the barriers to compliance with IPC precautions. And It is effectively enhancing the development of an integrated educational material that satisfy the needs of critical care nurses across three interlinked contextual strands, including: fundamentals to understand IPC, principles of IPC, and specific IPC.

The teaching methods that extracted from the adopted SCT, facilitate the development of education content that will enhance achievement of the desired individual’s behaviour change. The application of IM protocol based on SCT was successfully guide the development of critical care nurse-oriented, feasible, and an integrated education material to be used in an intervention program to improve awareness and increase compliance with IPC to reduce the HAIs.

Ethical Approval

The study has been approved by the Human Research Ethics Committee of HUSM (approval code: HUSM/JEPeM/19070440) and carried out in compliance with the research ethics guidelines outlined by the 1964 Helsinki Declaration.

Acknowledgments

The authors gratefully acknowledge the higher management of HUSM and the health care staff of HUSM represented by Hospital Infections and Epidemiology Control Unit and Nursing Department, and the academic staff of health campus of HUSM represented by microbiology and parasitology and medical education departments at school of medical sciences and interdisciplinary health department at school of health sciences for their collaboration and rich contribution in making the success of this study.

Competing Interest

The authors declare no competing interest. This study was funded by Research University (Individual) Grant (RUI grant No.: 1001.PPSK.8012373) of USM. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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The Correlation Study between Some Biochemical Parameters of 256 Covid-19 Cases Considering Diabetes

Ahmed Ab. Jabbar
Lecturer, Medical Laboratory Techniques Department, Erbil Technical Health College, Erbil Polytechnic University, Erbil, 44001, Iraq

Abstract

Background: Diabetic Mellitus is an independent risk factor in patients with coronavirus disease 2019 (COVID-19), but data on the association of diabetes, acute kidney injury, and COVID-19 severity are limited to Middle East populations.

Materials and Methods: This cohort study aimed to investigate these associations in 256 patients sequentially admitted between June 2020 and November 2020, at Emirati Hospital/Erbil. The investigation included some main biochemical parameters including liver and kidney function tests. Data analysis was processed by SPSS and the Pearson correlation pathway using log-converted data.

Results: Diabetic patients showed the highest correlations between measured parameters, of which glucose level showed a positive correlation coefficient (0.13**) with C-reactive protein and other biochemical parameters. Based on the glycemic status, COVID-19 patients were found in 3 groups, euglycemia group with an abundance of 148 (57.81%), hyperglycemia patients were 67 (26.17%), and 41 of them were diabetics (16.01%).

The Diabetic group showed a significantly (0.0002) increased level of C-reactive Protein and Glucose in comparison with hyperglycemia [CRP 15.38 mg/dl vs 10.09mg/dl, glucose 429.75 mg/dl vs 160.62mg/dl] with a Significantly (0.00374) higher mortality rate (29.26%) than that (10.44%), and (6.75%) of hyperglycemia, and euglycemia, respectively.

Conclusion: Male COVID-19 cases showed higher correlations between the estimated parameters than female cases. Diabetes was significantly associated with elevation of almost all kidney and liver function parameters. Finally, diabetes followed by COVID-19 disease was related to severe complications and higher mortality rates than nondiabetic COVID-19 cases.

Keywords: Biochemistry, COVID-19, Diabetes, Kidney and liver injury, correlations

Introduction

A new viral infection known by the coronavirus (COVID-19) has invaded Wuhan, China, in December 2019. Zoonotic exposure led to the transmission of coronavirus (COVID-19), resulting in the current pandemic disease. After one year, since the first case announcement, the total COVID-19 cases in December 2020, has reached 75 million reported cases and 1.6 million deaths of Novel COVID-19 disease.

Diabetic disease is considered a chronic disease that results from either complete lack of insulin (type 1 diabetes) or resistance to insulin (type 2 diabetes). Diabetics are more vulnerable to multiple infectious diseases, eventually, damaging to their immune system.

Corresponding author: Ahmed Ab. Jabbar
ahmed.abuljabbar@epu.edu.iq
and even causing organ failure, resulting in higher mortality rates as in COVID-19 patients. In addition, the death incidence of 27955 Italian diabetes by COVID-19 was 31.1%. This has risen in another survey in the UK, the prevalence death of 23804 British diabetes by COVID-19 was 32%. While another study showed 22% of death occurrence of diabetes with confirmed COVID-19 cases among 52 intensive care unit patients in China.

Acute kidney injury (AKI) incidence in COVID-19 disease was varied as evidenced by Chinese, invasion of COVID-19 virus into kidney tissue, clinical epidemiology of COVID-19–associated acute kidney injury (AKI) across our health system later also has been reported. A rapid increased serum urea and creatinine levels caused a hypercatabolic state in which understanding the causes will help as a marker for disease severity, predicting pulmonary or kidney outcomes. Previous study published different incidence rates of AKI from only 0.5% among 1099 patients, to 22.2% in 3908 patients with a 75% mortality rate. A recent cohort study on 3200 patients with COVID-19, showed that closely half of them had AKI and acute dialysis. Therefore, exploring the biochemistry of COVID-19 patients with diabetes will aid the physicians to reduce the incidence of severity. A retrospective study on 256 COVID-19 after confirmation by Real-time polymerase chain reaction (PCR) in one hospital was made and the correlations between estimated biochemical parameters were discussed.

**Materials and method**

**Data collection**

A total of 256 Admitted patients between June 2020 and November 2020, at Emirati hospital/Erbil, were enrolled in this study. After confirmation of Real-time polymerase chain reaction positivity for COVID-19 infection, the patients were separated into males (157) and females (99). Based on the glycemic status, COVID-19 cases were divided into 3 groups, euglycemia group (had normal blood sugar level before and during hospitalization), hyperglycemia group (increased blood glucose during hospitalization with no history of diabetes but their blood sugar normalized after hospital discharge), and Diabetes group had Elevated blood glucose level with a history of using diabetic treatments. Blood Samples were taken to the laboratory for examination of high sensitive C-reactive protein (hs-CRP), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin (TBILL), Direct bilirubin (DBIL), Urea, and Creatinine by the biochemical auto analyzer (Cobas - Roche C 311, Hitachi company, Japan).

**Statistical Analysis**

Data processing was made by SPSS (0).21 (IBM, Armonk, NY, USA). The Undistributed data were converted into a log 10 scale. The relation between the parameters was found by the Pearson correlation pathway using log-converted data.

**Results and Discussion**

The Data analysis in Table 1. shows that among the 256 included cases of COVID-19, 157 of them were males (63.33%) with an average age of 56.13 years, and 99 of them were female (38.67%) with an average age of 53.62 years. As illustrated in (table 2), from a total of 256 COVID-19 patients, 148 of them were euglycemia (57.81%), 67 of them were hyperglycemia (26.17%), and 41 of them were diabetics (16.01 %). the average age was higher in diabetic patients (55.68 years) than those of euglycemia (48.12years) and hyperglycemia (51.08years), respectively. Males have outnumbered females with no significant differences on the estimated parameters. Diabetes was associated with significantly higher mortality rates (29.26%) than that of hyperglycemia (10.44%), and euglycemia(6.75%), respectively (Table 2), (Figure 1). The displaced laboratory results in (Table 1) show that the levels of Urea, creatinine, AST, ALT, TBILL, DBILL, ALP, and CRP were higher nonsignificantly in male COVID-19 cases than that of female cases.
Table 1. Statistical findings of the Age and biochemical parameters based on gender.

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<tr>
<th>Parameter</th>
<th>Gender</th>
<th>N</th>
<th>Mean</th>
<th>SEM</th>
<th>STDev</th>
<th>Min</th>
<th>Max</th>
<th>p (Log10)</th>
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<td>TBILL (mg/dl)</td>
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<td>DBILL (mg/dl)</td>
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<td>5.02</td>
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<td>CRP (mg/dl)</td>
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<td>10.53</td>
<td>0.39</td>
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Note: Mean ± standard deviation in COVID-19–positive patients.

Data analysis in Table 2. showed significantly (p-value =0.002) higher urea values in diabetes 58.75 (27.3-132.4)mg/dl than that of 49.03(23-151) mg/dl, 37.09(17-104)mg/dl, for Hyperglycemia and euglycemia, respectively. Creatinine was significantly (p-value=0.00236) higher in the diabetic group 0.807 (0.5-3.3)mg/dl than that of 0.60 (0.3-2.9)mg/dl, 0.57 (0.3-2.7)mg/dl for Hyperglycemia, and euglycemia, respectively. The Diabetic group showed significant (0.0002 and 0.001) differences in the level of CRP and Glucose, respectively, in comparison with hyperglycemia [ CRP 15.38 (3.1-25.8) vs 10.09 (1.8-17.1), glucose 429.75(276-580) vs 160.62(120-213)] mg/dl and euglycemia group [ CRP 15.38 (3.1-25.8) vs 8.33 (1.6-16.3), glucose 429.75(276-580) vs 91.49 (54-115)]mg/dl. Insignificant changes existed in other tested parameters, such as AST, ALT, TBILL, DBIL, and ALP levels.
Table 2. Laboratory findings of three COVID-19 groups based on the glycemic status.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Euglycaemic (n= 148)</th>
<th>Hyperglycemia (n= 67)</th>
<th>Diabetics (n= 41)</th>
<th>P-value</th>
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<td>Age</td>
<td>48.12 (37-56)</td>
<td>51.08 (44-63)</td>
<td>55.68 (47-68)</td>
<td>0.26545</td>
</tr>
<tr>
<td>Gender</td>
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</tr>
<tr>
<td>Male (n, %)</td>
<td>82 (55.40%)</td>
<td>38 (56.71%)</td>
<td>23 (56.09%)</td>
<td>0.18743</td>
</tr>
<tr>
<td>Female (n, %)</td>
<td>66 (44.59%)</td>
<td>29 (43.28%)</td>
<td>18 (43.90%)</td>
<td>0.17659</td>
</tr>
<tr>
<td>Mortality rate (n, %)</td>
<td>10 (6.75%)</td>
<td>7 (10.44%)</td>
<td>12 (29.26%)</td>
<td>0.00374</td>
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<tr>
<td>Urea (mg/dl)</td>
<td>37.09 (8-104)</td>
<td>49.03 (23-125)</td>
<td>58.75 (27.3-132.4)</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>0.57 (0.3-2.7)</td>
<td>0.60 (0.3-2.9)</td>
<td>0.807 (0.5-3.3)</td>
<td>0.00236</td>
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<tr>
<td>AST (mg/dl)</td>
<td>38.91 (17.9-154)</td>
<td>45.23 (23-137.7)</td>
<td>39.91 (17.8-94.7)</td>
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<td>ALT (mg/dl)</td>
<td>40.33 (20.9-184)</td>
<td>42.94 (26-300)</td>
<td>41.11 (21-128.6)</td>
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<td>TBILL (mg/dl)</td>
<td>0.98 (0.3-8.4)</td>
<td>1.21 (0.3-2.1)</td>
<td>1.114 (0.4-3.4)</td>
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<tr>
<td>DBIL (mg/dl)</td>
<td>0.33 (0.12-0.79)</td>
<td>0.45 (0.24-1.22)</td>
<td>0.301 (0.13-0.45)</td>
<td>0.79437</td>
</tr>
<tr>
<td>ALP (mg/dl)</td>
<td>112.5 (59.7-163)</td>
<td>104.95 (38-223)</td>
<td>106.31 (80-394.9)</td>
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<tr>
<td>CRP (mg/dl)</td>
<td>8.33 (1.6-16.3)</td>
<td>10.09 (1.8-17.1)</td>
<td>15.38 (3.1-25.8)</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>91.49 (54-115)</td>
<td>160.62 (120-213)</td>
<td>429.75 (276-580)</td>
<td>0.0001</td>
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</table>

Data are shown as medians and interquartile ranges.

In male COVID-19 patients, the analyzed data anticipated the positive correlation coefficient (r-value) between urea (0.69), AST (0.37), DBIL (0.22), and CRP (0.039). While DBIL had a positive correlation with Urea (0.22), creatinine (0.21), and TBILL (0.28), and negatively correlated with the AST (-0.02) and ALP (-0.03). CRP values showed significant relations with the Urea (0.39) and creatinine (0.40) numbers, Table 3. The measured parameters showed fewer correlations in the female cases, which only AST had a significant correlation coefficient (r-value) with the Urea (0.21) and creatinine (0.21) numbers. And, ALT was significantly correlated with ALP (0.22) and AST (0.64) as shown in table 4.
<table>
<thead>
<tr>
<th>Parameter (mg/dl)</th>
<th>Urea</th>
<th>Creatinine</th>
<th>AST</th>
<th>ALT</th>
<th>TBILL</th>
<th>DBIL</th>
<th>ALP</th>
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<tr>
<td>Creatinine</td>
<td>0.69**</td>
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<tr>
<td></td>
<td>0.37**</td>
<td><strong>0.001</strong></td>
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<tr>
<td>AST</td>
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<td>157</td>
<td>0.60**</td>
<td>0.001</td>
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*< 0.05; **< 0.01. The Bold values represent statistically significant findings. The first line shows the r values, the second line for p values, and the third line is for patient numbers in all correlations included.
Table 4. Correlation findings detected in the female patients.

<table>
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<th>GPT</th>
<th>TBILL</th>
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*< 0.05; **< 0.01. The Bold values represent statistically significant findings. The first line shows the r values, the second line for p values, and the third line is for patient numbers in all correlations included.

Diabetic patients showed the highest correlations between measured parameters, of which glucose value had positive correlation coefficient (r value) with urea (0.22**), creatinine (0.17**), AST (0.19**), ALT (0.03*), DBIL (0.02**), ALP (0.05**), and CRP (0.13**) levels. CRP values showed significant correlations with the Urea (0.32**), Creatinine (0.27**), AST (0.31**), and ALT (0.15*) levels. ALP was significantly associated with creatinine and Urea (0.24**), AST (0.23**), and ALT (0.19*). No significant relationship was detected between TBILL and other chemical parameters (except for DBILL). And finally, Creatinine values were found to be negatively correlated with the TBILL values (-016*), Table 5.
Table 5. Correlation findings detected in the Diabetic patients.

<table>
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<tr>
<th>Parameter (mg/dl)</th>
<th>Urea</th>
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<th>AST</th>
<th>ALT</th>
<th>TBILL</th>
<th>DBIL</th>
<th>ALP</th>
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*< 0.05; **< 0.01. The Bold values represent statistically significant findings. Gender discrimination did not consider, the first line shows the r values, the second line for p values, and the third line is for patient numbers in all correlations included.
Coronavirus is a multiorgan inflammatory disease, specialized from another virus of their family, by its rapid spreading, highly contagious, and more vital to the respiratory system with higher mortality rate than previous virus outbreak. Our data showed that the biochemical parameters did not change significantly based on gender. The same was found by other researchers.

Men were more common in all three glycemic groups. Our results showed that men COVID-19 patients had higher correlations between their biochemical parameters, specifically urea and creatinine values, which in severe cases may lead to acute kidney injury. By our outcome, previous reports also concluded that regardless of age, men are at higher risk for more severe results with a higher incidence of death than women.

Based on the glycemic status, the current work showed significant differences in the levels of CRP, glucose, and kidney function parameters (Urea and creatinine) between the three tested groups, in which the values were significantly higher in groups with higher sugar levels (Hyperglycemia and diabetics) in compare with euglycemia COVID-19 patients. This may explain the elevated mortality (29.26%) in diabetic group in our data analysis. Such an increased mortality rate in diabetic patients is following a previous study, which proposed a mortality rate of diabetes with COVID-19 disease between 20-32% in different countries according to COVID-19 severity and poverty status.

Studies showed that increased glycemic status is an independent risk factor of mortality and in the case of COVID-19 patients, causing severe complications because of increased releasing of glucocorticoids and catecholamines into circulation. also led to an increase in the glycation end products and worsened prognosis. Another study showed that increased ACE2 (Angiotensin-converting enzyme2) receptors in hyperglycemia and diabetic patients facilitate COVID-19 virus adherence to the pancreatic cells leading to decreased insulin secretion by β-cells and thus raising

Figure 1. Shows the mortality rate in the COVID-19 group according to the glycemic levels.
the severity of COVID-19 infection in those patients 24,25. Another reason behind the high mortality rate in diabetes may be due to impaired leukocyte function of phagocytosis leading to increased viral infections. In addition, increased cellular binding affinity, decreased viral clearance, reduced T-cell function, and increased susceptibility to hyper-inflammation, and renin-angiotensin-aldosterone system (RAAS) activation are other causes behind the high mortality rate of diabetes following COVID-19 disease 25. The abundance of Dipeptidyl Peptidase-4 which degrades incretin 25 and furin 26 in diabetic patients, is another entry enhancement for coronavirus. Diabetes also has different lung volumes and pulmonary diffusing capacity that also affects their severity level of COVID-19 disease 27.

Our data analysis showed that the level of urea and creatinine positively correlated with the CRP and almost all other estimated parameters without gender discrimination. Diabetes had significantly higher levels of urea and creatinine than those of the euglycemia and hyperglycemia groups. The same result was found by the previous researchers 28. Previous studies also showed kidney dysfunction and acute kidney injury (AKI) occurrence in a range of 3-9% with 3-fold higher odds of death than COVID-19 without AKI 29. Studies on hospitalized patients with COVID-19 disease reported that 44%, 30 and 63% 31, of them had proteinuria and significantly increased serum creatinine and blood urea nitrogen levels. Thus, AKI is considered as an independent risk factor of death with or without COVID-19 30. A recent study considered the kidney as a target organ of the COVID-19 virus after successful isolation of the virus in the urine sample of an infected patient 32. Pathogenesis between the current virus and AKI may be also due to sepsis occurrence heading to cytokine storm syndrome 33.

**Conclusion**

The COVID-19 pandemic still has many unraveled sites that need to be undertaken. As shown from our results that the gender has no significant effect on the severity and progression of COVID-19 disease, although more correlations between biochemical parameters were found in males than in females. The presence of Diabetic Mellitus followed by COVID-19 disease will increase the odds to possess severe complications of liver and kidney organs. C-reactive protein and glucose levels of diabetic COVID-19 patients were positively correlated with almost all estimated biochemical tests, which could be the reason behind the higher mortality rates of diabetes than those of euglycemia and Hyperglycemia COVID-19 patients. The severity of COVID-19 and increased levels of CRP was associated with increased levels of urea and creatinine regardless of gender or glycemic status, which may be referred to an important indications of acute kidney injury. Due to data limitations, some laboratory tests were not included. More elaborate research trials are still required to anticipate other postulates related to correlations between COVID-19, diabetes, and acute kidney injury.

**Ethical Clearance:** Taken from the ethics committee of Erbil technical health college, Erbil Polytechnic University.

**Source of support:** Self.

**Conflict of Interest:** Nil.

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lung, an underrated complication from restrictive functional pattern to pulmonary hypertension. *Diabetes/Metabolism Research and Reviews*. 2019; **35**(6).


Psychological Distress among Caregivers of Children with Down Syndrome at Al Najaf Province

Ahmed Burhan Abdulameer¹, Arafat Hussein Al-Dujaili²

¹Research Scholar, Faculty of Nursing, University of Kufa, Iraq, ²Professor, Faculty of Medicine, University of Kufa, Iraq

Abstract

Psychological Distress has recently been shown to have a negative impact on caregivers, but there has been little longitudinal research on this subject. A sample (61) caregivers of children with Down Syndrome have been selected Al-Najaf Province centers. The researcher adapts and modifies the questionnaire to fulfill the study’s objectives. The results of the study revealed that caregivers of children with DS have severe level of psychological distress (38.7%). There is a significant relationship (P < 0.05) between Psychological Distress and some of the socio-demographic characteristics (child age, family member and trainings about down syndrome). The present study recommends providing families with Educational programs to increase parents knowledge about etiology, signs and symptom and treatment of stress and Providing scientific booklet, publication and journal about stress. In addition to increasing schools and institutes specialized in educating children with Down syndrome, as they are few in the province of Najaf, and some of them are far from their places of residence.

Keywords - Psychological Distress, Caregivers, Children, Down Syndrome

Introduction

Globally, mental health disorders have emerged as major public health concerns; disabilities, physical mortality and morbidity, and poor social functioning can all result from psychiatric disorders; furthermore, psychological issues can exacerbate the social burden and economic insecurity in a variety of societies around the world (¹). Psychological distress (PD) is a form of emotional turmoil marked by a blending of depression and anxiety symptoms (²). In Europe and the United States, the prevalence of psychological distress among parents of children with chronic diseases is as high as 50% (³), and there are 8.27 individuals with down syndrome for every 10,000 people in the United States (90 % UI, 6.14-10.62) (⁴). Psychological distress is broadly defined as an emotional state characterized by symptoms of depression (e.g., loss of interest; sadness; hopelessness) and anxiety (e.g., restlessness; tenseness); these symptoms may be linked to somatic symptoms (such as insomnia, headaches, and a lack of energy), PD is viewed as an emotional disturbance that may impact on the social functioning and day-to-day living of individuals (⁵). Down syndrome (DS) is the most common chromosomal anomaly which is associated with intellectual disability (ID), typical physical features, and health problems. The incidence of DS is about 1–1.5 of every 1000 live births. DS is the most frequent genetic cause of mental retardation (MR). People with MR have behavioral, emotional, and psychiatric problems more often than the general population. DS exhibits distinctive neurodevelopmental, neurocognitive, and psychopathological patterns when compared to other genetic syndromes leading to ID, albeit higher than the general population. A 28.9% of the children with DS have psychiatric comorbidity (⁶). Caregivers of children
with DS are often distressed, many of the children have unmet needs that are not being addressed by services, and the degree of unmet need is related to caregiver distress; these high levels of anxiety are likely to have an effect on the parents’ health and ability to care for their children (7). Due to a child’s disability, parents may be required to pursue educational and recreational accommodations to ensure that the child has the same opportunities as a child who is not disabled, caring for a child with a chronic condition (such as a child with Down syndrome or DS) causes caregivers to experience additional stress, which may lead to caregiver distress (8). The main person with complete or greater responsibility for the care given to the person being cared for is the family caregiver, and there is no financial reimbursement for the care provided (9,10). Parents are the most common caregivers, since they look after their children in all aspects of their lives (11). The majority of family caregivers are women who perform time-consuming and demanding activities such as personal care, aside from the physical, emotional, and financial effect, there are also limitations in social and recreational activities (12).

Materials and Methods

A descriptive (correlational) quantitative design study was carried throughout the present study to identify the psychological distress among caregivers of children with Down Syndrome. During the period from 15th September 2020 to 15th April 2021. A non-probability sampling technique (purposive sample) of (61) caregivers of children with Down Syndrome are included in the study. The General Health Questionnaire-12 (GHQ-12) is a tool for assessing current mental health that has been used in the current study.

The Kessler Psychological Distress Scale (K10) was used as indicator of psychological symptoms.

Results

The caregivers in this study are within ages of 37 and 46 (39.3%), female caregivers (75.4%), mother’s age (49.2%), child age (57.4%), those are graduated in institute and college (27.9%), those with barely sufficient monthly income (54.1%), those who are married (86.9%); those who are housewives (52.5%); those who live in urban areas (85.2%); those who are freehold owning their houses (78.7%); those who were not trained for down Syndrome (78.7%); those with no physical disorder (73.8%); those with no psychiatric disorder (93.4%).

Table (1) : Descriptive statistics of caregiver’s subgroups according to their total mean of score of GHQ-12

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<th>GHQ-12 Assessment</th>
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<tr>
<td>Frequency</td>
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<tr>
<td>Percentage</td>
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</tr>
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Good : MS = 0-0.99 ; Moderate : MS = 1-1.99 ; Poor : MS ≥ 2

Table (1) illustrate the descriptive statistics of caregiver’s subgroups according to their total score of GHQ -12. They reveal that the majority of caregiver have (moderate) level of general health assessment (60.66%); while (37.7%) of them have (good) level of general health assessment; finally only (1.64%) of them have (poor) level of general health assessment.
Table (2) : Descriptive statistics of caregiver’s subgroups according to their total score (K10) of Kessler Psychological Distress Scale

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<td>Freq.</td>
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<tr>
<td>%</td>
<td>12.9</td>
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Good : K10 Score = 10-19 ; Mild disorder : K10 Score = 20-29 ; Moderate disorder : K10 Score: = 30-39 2 ; Severe disorder : K10 Score = 30 – 50

Table (2) advance the descriptive statistics of caregiver’s subgroups according to their total score of Kessler Psychological Distress Scale .They reveal that the majority of caregiver have (Severe Disorder) level of Kessler Psychological Distress Scale assessment (38.7%) ; while (12.9 %) of them have (good) level of Kessler Psychological Distress Scale assessment ; only (21 %) of them have (Mild Disorder) level of Kessler Psychological Distress Scale assessment ; and finally (27.4 %) of them have (Moderate Disorder) level of Kessler Psychological Distress Scale assessment .

Table (3) illustrates the relationship between overall assessment of Kessler Psychological Distress Scale for caregivers and their demographic data. It reveals that there is no significant relationship between overall assessment of Kessler Psychological Distress Scale for caregivers and their demographic data (P>0.05).

Table (3) : Relationship between total score assessment of Kessler Psychological Distress Scale for caregivers and their demographic data

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df : degree of freedom
Discussion

Psychological distress (PD) is a popular community mental health problem,\(^{(14)}\). The influence of caregiver stress, which can lead to distress, on caregivers’ mental wellbeing has been well recorded, caregivers of people with a range of physical and psychological disabilities have higher rates of anxiety and depression than noncaregivers, and caregivers are twice as inclined to seek mental health treatment emulated with noncaregivers\(^{(15)}\). Globally, mental health problems accounted for 13% of the overall burden of disease, and 31% of all years lived with disability.

The analysis of findings revealed that most of sample were married mothers of children with down syndrome, fall in the age group (37- 46) years old, graduated from institute or college(27.9%) with barely sufficient monthly income . These findings have revealed that parenting of DS children fell overwhelmingly on the hands of mothers more than fathers; these mothers are responsible for their children’s care. Fathers generally play a secondary role in the care for children or sometimes no role at all, leaving the primary caregiver responsibility to the mother. This finding was backed up by Rodrigue and his colleagues, who found that mothers accounted for 74% of all parents. Peishi Wang and his colleagues discovered that 59.2 percent of parents were mothers, which provided additional support. Also this approves with study of\(^{(16)}\), The study results indicate that the more caregiver is the mother’s(74%), \(^{(17)}\) mention that (53.8%) of caregivers were mothers.

The results showed that the majority of the subjects’ job (52.5%) are house wife , In the point of view that is in our culture, fathers spend most of their time in work and had provider responsibility, and mothers have the responsibility for household and child care, so mothers spent all her time for their children . This finding comes along with \(^{(18)}\) who found that (70%) of samples are housewives. Supplementary support was found by \(^{(19)}\) the study that show (71.4%) of samples are housewives .

Concerning the training cycle, the results show that caregivers without a training cycle account for the largest percentage of those with training. This result agrees with \(^{(16)}\). Table (1) reveal that the majority of caregiver have (moderate) level of general health assessment (60.66%) ; while (37.7%) of them have (good) level of general health assessment.

This result accords with Dijkstra-de Neijs& his colleagues (2020) whose studies show that most of parents of children with chronic disabilities suffer from disturbance in mental health and well-being .Furthermore, the results were in line with those of Sangeetha, et al. (2017), who found that (40 %) caregivers are experienced moderate to severe level of general health assessment , Depression was detected in (63%)and (53.8%) of The caregivers’ psychological well-being was clinically disturbed.

Table (2) and Figure(4.3) assert that the majority of caregivers have severe disorder (24) of K10 Scale assessment(38.7%). It is clear that some of individual who participated in the study have severe disorder, this may be due a history of anxiety and depression as a result of stressful situations .This result is consistent with previous research that indicates chronic illness or a negative self-evaluation of one’s health is related to increased psychological distress. This result is agree with \(^{(17)}\) the study assures that (57.7%) of caregivers have psychological distress, \(^{(20)}\) who found that (39.6%) of caregivers had GHQ score suggesting severe level of psychological distress. Additionally supporting was done by \(^{(21)}\) who reported that (60.5 %) of caregivers experience elevated level of psychological distress .

Tables (3) display that there is a significant relationship between psychological distress among caregivers of DS children and child’s age. This result may be due to the fact that children of this age (8-13 years) have more mobility and their needs are more than the youngest and older children. This result almost agree a study by Johnson and his colleagues, (2013) who found that the majority of children(7.7 to 8.8 years ) with chronic conditions contribute to increase distress of their parents . The current result agrees with Gilbert, (2009) he revealed that there is a significant association between
age of the child and psychological burden. They are positively correlated and the child’s age increases with the increase in respondent’s burden.

Conclusions

The researcher has reached the following conclusions based on the discussion and analysis of the study findings: The study concluded that most of the caregivers were to have moderate psychological distress. Psychological distress was statistically significant among females. Family member, child age and Training about Down Syndrome were statistically significant while other parameters regarding socio-demographic characteristics of caregivers were not significant. The caregivers were under the age of fifty, and more than three quarters of the caregivers’ children were females (mothers). The majority of the subjects were married, had a high degree of education, and had a meager income.

Ethical Clearance: Taken from University of Kufa ethical committee

Source of Funding: Self

Conflict of Interest: Nil

References


Evaluation of the Antioxidant and Antibiofilm Activities of *Rosmarinus Officinalis* Essential Oil Extract

Ahmed H. Al-Azawi¹, Kais Kassim Ghaima², Noor Saad Latteef³, Alaa Aziz Abdulhassan⁴

¹Associate Professor, ²Professor, ³Lecturer, ⁴Research Scholar, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq

Abstract

Biological activities of essential oils from various plants, including Rosemary, have been attributed to the presence of specific chemical compounds with antimicrobial, anti-inflammatory and antioxidant activities. The aim of this study is to estimate the antioxidant and antifungal activity of *Rosmarinus officinalis* essential oil extract. The study included the extraction of essential oil using a Clevenger apparatus. The chemical compositions were evaluated by GC-MS and High Performance Liquid Chromatography (HPLC). The rosemary essential oil extract was tested with regard to antioxidant utilizing 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Microtiter plate Assay used to determine the antifungal and antibiofilm activity. The result showed that the GC-MS analysis revealed that the major components determined in *R. officinalis* essential oil were linalool (17.09 %), L- Borneol (11.92 %), Verbenone (8.52 %), camphor (5.30 %), Eucalyptol (4.79 %), while the chemical compositions identified by HPLC shows four phenolic acids were identified in the essential oil, Rosmarinic acid, Caffeic acid, p-Coumaric acid, 4-hydroxybenzoic acid, and lignans (medioresinol), while Isorhamnetin was the only flavonol detected. The free radicals scavenging activity increased gradually with the increase in the concentration of essential oil which was 81.59 % when compared with BHT and V.C (92.34 and 97.42) respectively. The results of the antifungal activity revealed that Minimum Inhibitory Concentration of *C. albicans* and *C. krusei* was 3.125%, while the MIC of *C. glabrata* was 12.5% in contrast with the highest MIC which recorded for *C. tropicalis* at 25% of rosemary essential oil. The current results revealed that the reduction of biofilm formation among *C. albicans* and *C. krusei* was obvious at the lower concentration (1.56%), where the percentage of biofilm formation in *C. albicans* was (91.25%) and *C. krusei* was (84.25%), while *C. tropicalis* exhibit (86.32%) for biofilm reduction at the concentration (12.5%) of rosemary essential oil, also it was found that the effect of essential oil on *C. glabrata* biofilm formation was at the concentrations 3.125% and 6.25%. The findings of this study indicated to the significant effect of rosemary essential oil against the growth and biofilm formation of the important pathogenic yeast *C. albicans* at low concentrations.

Keywords: *Rosmarinus officinalis*, Essential oil, GC-MS, HPLC, Antioxidant, Antibiofilm

Introduction

The use of traditional herbs and medicinal plants has recently become very popular because they contain large amounts of natural products with biological properties. Medicinal plants extracts possessed strong antioxidant and antimicrobial activities against pathogenic organisms. Medicinal and aromatic plants have been used for their bioactive compounds with potential applications in food industry, nutraceuticals, cosmetics and perfumes. Also, some plants from the Lamiaceae family are very rich in phenolic compounds, such as flavonoids, phenolic acids and phenolic diterpenes, and possess high antioxidant activities. These compounds...
can delay or inhibit the oxidative damage caused by free radicals and can protect us against major diseases such as coronary heart disease and cancer in human (4, 5). Also it was found that the extracts and essential oils of Rosmarinus officinalis show inhibitory effects against cyanobacteria (6).

Rosemary (Rosmarinus officinalis L.) is a spice and medicinal herb widely used around the world. They are also used as flavouring agents in foods (7). Additionally, various pharmacological studies have demonstrated the analgesic, anti-inflammatory, and anti-ulcerogenic properties of R. officinalis (8, 9). Many compounds have been isolated from rosemary, including flavones, diterpenes, steroids, and triterpenes. Of these, the antioxidant activity of rosemary extracts has been primarily related to two phenolic diterpenes: carnosic acid and carnosol. The main compounds responsible for the antimicrobial activity are α-pinene, bornyl acetate, camphor and 1, 8-cineole (10, 11). Thus, the purpose of this research is to estimate the antioxidant, antifungal, and antibiofilm activities of Rosmarinus officinalis essential oil extract

Material and Methods

Chemical reagents

The chemical reagents DPPH (2,2-diphenyl-1-picrylhydrazyl), Butylated hydroxytoluene (BHT), ascorbic acid, gallic acid monohydrate (3,4,5-trihydroxybenzoic acid) and sodium carbonate were purchased from Sigma aldrich chemicals (St. Louis, USA). Folin Ciocalteu reagent was purchased from Merck (Darmstadt, Germany), Resazurin dye (Himedia, India), Sabouraud’s dextrose broth (Himedia, India) and crystal violet (Pro-Lab, Canada).

Candida spp. isolates

The isolates of Candida spp. (C. albicans, C. glabrata, C. tropicalis and C. krusei) were obtained from specialist hospital in Baghdad, Iraq, from female patients with Vulvovaginitis and identified by Vitek2 system.

Plant material

Fresh leaves of Rosmarinus officinalis plant were procured from the greenhouse in Baghdad, Iraq. Authentication and identification of the plant were carried out by the specialist, Department of Biology, College of Science, University of Baghdad.

Extraction of the essential oil

Air-dried leaves of the Rosmarinus officinalis plant was subjected to steam distillation for 4 h using a Clevenger apparatus. The essential oil was kept at −4°C in an amber glass airtight container.

Fourier transform infrared (FTIR) assay

FTIR stands for Fourier Transform Infrared, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted), the resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum, this makes infrared spectroscopy useful for several types of analysis. The FTIR spectrum was recorded between 4000 and 400 cm−1 (12).

Gas chromatography mass spectrophotometer analysis

Analysis of the Rosmarinus officinalis essential oil was carried out on GC-MS equipment. The experimental conditions of the equipment are: HP-5MS ultra inert capillary non-polar column, dimensions: 30 mm × 0.25 mm; ID: 0.25 mm, film thickness: 0.25 μm. The flow rate of mobile gas: 1.0 ml/min. The oven temperature for the gas chromatographic part was 50°C raised to 300°C at 7oC/min for 10 min. The nature and structure of compounds were identified by the mass spectrometer. The spectrum of unidentified components was compared with the spectrum of identified components stored in the national institute standard and technology (NIST) library (13).
High-Performance Liquid Chromatography (HPLC) Analyses

HPLC analysis was performed for analysis of Rosmarinus officinalis essential oil extract. The HPLC analysis was carried out according to Adham (2015) (14) the separation was performed in reversed-phase ODS-C18 column (250 mm×4.6 mm i.d); the mobile phase consisting of 80% methanol as solvent (a) and 20% (water with 0.1% acetic acid) as solvent (b). A flow rate was set at 1 ml/min for 10 min, detected by UV at 320 nm.

Determination of total phenolic contents

Total phenolic content of Rosmarinus officinalis essential oil extract was determined spectrophotometrically using the Folin-Ciocalteu method described by Jayaprakasha et al. (2001) (15), 2 ml of Folin-Ciocalteu reagent (diluted 10 times) was mixed with 1.6 ml of 7.5% sodium carbonate solution and 0.4 ml of the essential oil extracts. The volume was completed to 5 ml by adding distilled water. The tubes were covered with parafilm for 30 min. at room temperature, and then the absorbance was read at 760 nm spectrophotometrically. The total phenolic content was calibrated against gallic acid standards and expressed the results as mg gallic acid equivalents (GAE)/g extract. The test was performed in triplicate.

DPPH assay

The DPPH free radical scavenging activity of Rosmarinus officinalis essential oil extract was determined following the method described by Kedare and Singh, (2011) (16), 5ml of a freshly prepared 0.004% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was mixed with 50 μl of different concentrations (0.312, 0.625, 1.25, 2.5 and 5) μg/ml of the essential oil, then the mixture was left to stand for 30 min. The absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) and ascorbic acid were used as a positive control. All tests were performed in triplicate. The percentage of DPPH reduction was calculated as:

% Reduction = (Abs DPPH – Abs Dil.) / Abs DPPH × 100

Where: Abs DPPH = average absorption of the DPPH solution, Abs Dil. = average absorption of the three absorption values of each dilution.

With the obtained values, a graphic was made using Microsoft Excel. The EC50 of each extract (concentration of extract or compound at which reduced 50% of DPPH) was taken from the graphic.

Determination of the antifungal activity

Antifungal activities of the rosemary essential oil towards Candida spp. (C. albicans, C. glabrata, C. tropicalis and C. krusei) were determined by the Resazurin Microtiter plate Assay (broth dilution method) (17). Fifty microliters of the essential oil was two-fold serially diluted (0.19-100%) with Sabouraud’s dextrose broth in a microtiter plate. Fifty microliters of the Candida suspension was added and mixed with the oil. Candida cultured in the broth without the tested agents served as a positive control and the mixture of broth and the tested agents without microorganism served as a negative control. The plates were incubated for 24 h, at 37 °C. Then Candida growth was examined and the lowest concentration of the tested agents which inhibited the visible growth of the yeast was recorded as the minimum growth inhibitory concentration (MIC). All experiments were repeated on two of each species of Candida and three separate occasions, with triplicate determinations on each occasion.

Biofilm formation of Candida spp.

Biofilm formation was determined by crystal violet assay (18). After the growth of Candida spp. (C. albicans, C. glabrata, C. tropicalis and C. krusei) with serial dilutions of sub-inhibitory concentrations of Rosemary essential oil (0.78-25%) in the wells of microtiter plate, the content of each well was carefully removed and the plate was washed five times with sterile saline solution to remove any unattached cells. Pure methanol (200 μL) was added to each well and incubated for 15 min.
Methanol was removed, the plates were thoroughly dried at room temperature, and 200 μL of 0.5% crystal violet was added for 15 min. Then, the stain was removed and the wells were washed in tap water and dried. Two hundred microliters of 95% ethanol was added to each well. Finally, the absorbance (OD450) was read using a microplate reader.

Inhibition of biofilm was determined from the formula described by Jadhav et al. (2013) (19).

\[
\% \text{ Inhibition} = 100 - \left( \frac{\text{OD450 nm sample}}{\text{OD450 nm growth control}} \times 100 \right)
\]

**Results and Discussion**

**Fourier Transform Infra-Red (FTIR)**

Figure (1) shows the infrared spectra of the *Rosmarinus officinalis* essential oil, the results revealed that the presence of different functional groups such as phenolic–OH group stretching, C-H stretching, C≡C stretch, Aromatic C=C, P=O stretch, N-O stretch and Aliphatic C–O (Table 1).

![Infrared spectrum of the Rosmarinus officinalis essential oil](image)
Table 1: The IR Frequencies region for the functional groups of the *Rosmarinus officinalis* essential oil

<table>
<thead>
<tr>
<th>The Functional Groups</th>
<th>I.R Frequencies Standard Groups (cm⁻¹)</th>
<th>I.R. Frequencies of essential oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic–OH group stretching</td>
<td>3650-2500</td>
<td>3356.83</td>
</tr>
<tr>
<td>C-H stretching</td>
<td>3100-2850</td>
<td>2956.95</td>
</tr>
<tr>
<td>C≡C stretch</td>
<td>2230-2100</td>
<td>2117.75</td>
</tr>
<tr>
<td>Aromatic C=C</td>
<td>1680-1600</td>
<td>1671.28</td>
</tr>
<tr>
<td>P=O stretch</td>
<td>1260-1230</td>
<td>1232.54</td>
</tr>
<tr>
<td>N-O stretch</td>
<td>1390-1300</td>
<td>1372.60</td>
</tr>
<tr>
<td>Aliphatic C-O</td>
<td>1300-1000</td>
<td>1025.20</td>
</tr>
</tbody>
</table>

Gas chromatography mass spectrophotometer

The phytochemical constituents present in the essential oil extract of *Rosmarinus officinalis* showed twenty four constituents (Figure 2), the major components such as Eucalyptol, Linalool, Camphor, Borneol, Verbenone, Caryophyllene and molecular formula with retention time are shown in Table 2.

![Figure 2: GC-MS Chromatogram of *Rosmarinus officinalis* essential oil](image-url)
Table 2: GC-MS report of *Rosmarinus officinalis* essential oil

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of Compound</th>
<th>Ret. time (min)</th>
<th>Area (%)</th>
<th>M. weight (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-Linalool</td>
<td>4.200</td>
<td>17.09</td>
<td>154</td>
</tr>
<tr>
<td>2</td>
<td>Eucalyptol</td>
<td>5.215</td>
<td>4.79</td>
<td>154</td>
</tr>
<tr>
<td>3</td>
<td>Camphor</td>
<td>5.847</td>
<td>5.30</td>
<td>152</td>
</tr>
<tr>
<td>4</td>
<td>L- Borneol</td>
<td>6.540</td>
<td>11.92</td>
<td>154</td>
</tr>
<tr>
<td>5</td>
<td>Verbenone</td>
<td>7.200</td>
<td>8.52</td>
<td>150</td>
</tr>
<tr>
<td>6</td>
<td>Camphene</td>
<td>8.690</td>
<td>0.31</td>
<td>136</td>
</tr>
<tr>
<td>7</td>
<td>Geranyl acetate</td>
<td>9.361</td>
<td>1.50</td>
<td>196</td>
</tr>
<tr>
<td>8</td>
<td>Caryophyllene</td>
<td>10.135</td>
<td>2.75</td>
<td>204</td>
</tr>
<tr>
<td>9</td>
<td>Humulene</td>
<td>10.708</td>
<td>0.66</td>
<td>204</td>
</tr>
<tr>
<td>10</td>
<td>Patchouline</td>
<td>14.418</td>
<td>0.03</td>
<td>206</td>
</tr>
<tr>
<td>11</td>
<td>D-limonene</td>
<td>14.445</td>
<td>0.21</td>
<td>152</td>
</tr>
<tr>
<td>12</td>
<td>Farnesol</td>
<td>19.584</td>
<td>0.11</td>
<td>222</td>
</tr>
</tbody>
</table>

Abdullah *et al.* (2010) \(^{(20)}\) mention the GC analysis revealed that the major components determined in *R. officinalis* essential oil were 1,8-cineol, camphor, α-pinene, limonene, camphene and linalool, while Christiane *et al.* (2016) \(^{(21)}\) revealed the major components of the essential oil indicated three compounds: cineole, camphor and alpha-pinene. The variation in the chemical compositions of *R. officinalis* essential oil across countries might be due to different ecological conditions. Our results are in agreement with the findings of Jan *et al.* (2017) \(^{(22)}\) that also identified eucalyptol, α-Linalool, Camphor, L- Borneol, Verbenone, Caryophyllene, etc. as major components of *R. officinalis* essential oil.

*R. officinalis* is an aromatic plant popularly known as rosemary which has important biological properties, especially due the phenolic and the essential constituents, such as carnosol, carnosic acid and rosmarinic acid present in the extract of rosemary and α-pinene, bornylacetate, camphor and eucalyptol present in the essential oil of this species \(^{(23)}\).

**High-Performance Liquid Chromatography (HPLC)**

Individual phenolic compositions of *R. officinalis* essential oil were analyzed by HPLC method. Figure (3) shows that the essential oil of *R. officinalis* contains 16 polyphenolic compounds, and 6 compounds were identified depending on retention time according Hossain (2010) \(^{(24)}\), Romo-Vaquero (2012) \(^{(25)}\) and Meziane-Assami *et al.*, 2013 \(^{(26)}\), the result shows four phenolic acids were identified, Rosmarinic acid (compound 1), Caffeic acid (compound 2), p-Coumaric acid(compound 4), 4-hydroxybenzoic acid(compound 6), and lignans (medioresinol), while Isorhamnetin was the only flavonol detected.
Total phenolic content of *Rosmarinus officinalis* essential oil extract

Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals \(^{(27)}\).

The results of total phenolic content in the *Rosmarinus officinalis* essential oil were (30.52, 41.80 and 54.47 mg/g) in (50, 100 and 200 µg/ml) respectively as shown in (Table 3).

**Table (3): Total Phenolic content of *Rosmarinus officinalis* essential oil**

<table>
<thead>
<tr>
<th>Concentration µg/ml</th>
<th>Total phenol (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>30.52 ± 0.21</td>
</tr>
<tr>
<td>100</td>
<td>41.80 ± 0.09</td>
</tr>
<tr>
<td>200</td>
<td>54.47 ± 0.18</td>
</tr>
<tr>
<td>LSD value</td>
<td>0.601 **</td>
</tr>
</tbody>
</table>

\(^{**} (P\leq0.01).\)

Genena *et al.* (2008) \(^{(28)}\) mention the amount of phenolic compounds content ranged from 7.45 to 13.51 g of TAE/100 g of extract, with an overall mean of 10.06 g of TAE/100 g of extract. Phenolics are a class of compounds, which act as free radical scavengers and are responsible for the antioxidant activity in many medicinal plants \(^{(29)}\).
Determination of Antioxidant Activity Using (DPPH) Radical Scavenging Method

The antioxidant activity of essential oil was assessed by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method. The free radicals scavenging activity increased gradually with the increase in the concentration of essential oil as shown in Table (4). Furthermore, the antioxidant activity is expressed as an effective concentration (IC50). The half-maximal effective concentration (IC₅₀) often refers to the concentration of a drug, toxicant, or antibody which induces a response halfway between the baseline and maximum after a specified exposure time is commonly used as a measure of the potency of a drug. (30) In this study, the radical scavenging capacity (IC50) of vitamin C and BHT were (0.2 and 0.4 µg/ml) respectively, while the essential oil was (1.8 µg/ml) as shown in Figure (4). Lee et al. (2007) (31) confirmed if the IC₅₀ value of the extract was less than 10 µg/ml it indicates the extract is an effective antioxidant. In this study, the IC₅₀ value of Rosmarinus officinalis essential oil is less than 10 µg/ml this indicates the extract was effective antioxidant.

Elansary et al. (2012) (32) revealed monoterpenes hydrocarbons are known to have noticeable antioxidant activities. Our study was higher than study presented by Benyoucef et al. (2018) (33) which concluded that R. officinalis essential oil showed the greatest antioxidant activity with an IC50 of 2.6 mg/L.

<table>
<thead>
<tr>
<th>Concentration µg/ml</th>
<th>essential oil</th>
<th>BHT</th>
<th>Ascorbic acid</th>
<th>LSD value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.312</td>
<td>14.06 ±0.04</td>
<td>47.16 ±0.02</td>
<td>89.70 ±0.03</td>
<td>0.113 **</td>
</tr>
<tr>
<td>0.625</td>
<td>28.34 ±0.14</td>
<td>70.33 ±0.01</td>
<td>91.80 ±0.01</td>
<td>0.295 **</td>
</tr>
<tr>
<td>1.25</td>
<td>39.16 ±0.19</td>
<td>86.39 ±0.13</td>
<td>94.82 ±0.05</td>
<td>0.484 **</td>
</tr>
<tr>
<td>2.5</td>
<td>60.76 ±0.08</td>
<td>90.51 ±0.09</td>
<td>96.44 ±0.04</td>
<td>0.258 **</td>
</tr>
<tr>
<td>5</td>
<td>81.59 ±0.14</td>
<td>92.34 ±0.05</td>
<td>97.42 ±0.14</td>
<td>0.431 **</td>
</tr>
<tr>
<td>LSD value</td>
<td>0.420 **</td>
<td>0.248 **</td>
<td>0.231 **</td>
<td>---</td>
</tr>
</tbody>
</table>

** (P≤0.01).

Figure 4: IC₅₀ of Rosmarinus officinalis essential oil
Determination the antifungal activity

In this study, the antifungal activity of rosemary essential oil was evaluated. The minimal inhibitory concentrations (MICs) of rosemary oil against four species of Candida were detected by Resazurin Microtiter plate Assay as showed in Figure 5.

![Figure 5: Minimal inhibitory concentration of serial dilutions of rosemary oil against four species of Candida by Resazurin Microtiter plate Assay](image)

The results revealed that MIC of C. albicans and C. krusei was 3.125%, while the MIC of C. tropicalis was 12.5% in contrast with the highest MIC which recorded for C. tropicalis at 25% from rosemary oil. The findings of the current study indicated to the significant effect of rosemary oil toward the important pathogenic yeast C. albicans at low concentrations. Fu et al. (2007) (34) investigated the antimicrobial activity of the essential oils from rosemary (Rosmarinus officinalis L.) and studied the Minimum inhibitory concentrations (MICs) against three Gram-positive bacteria, three Gram-negative bacteria and two fungi were determined for the essential oil The essential oil possessed significant antimicrobial effects against all microorganisms tested, where the MICs of rosemary oil ranged from 0.125% to 1 % (v/v).

In a previous study by Abdulaziz et al. (2015) (35), the diameter of inhibition zone with rosemary essential oil was (11.8±2.8) and the serial two-fold dilutions of the tested essential oil showed exhibited antifungal activities even at very low concentrations. In a study by Tavassoli and Emamdjomeh (2011) (36) the antimicrobial activity of rosemary leaf extract against some of bacteria, Saccharomyces cerevisiae, and Candida krusei was determined by MIC measurements and the results indicated that the rosemary extract had a stronger inhibitory effect against the bacteria.

The mechanism of action of essential oils is related to changes in cell membrane permeability. The liposoluble nature of essential oils and their constituents facilitates their interaction with cellular structures that
have lipid components, resulting in increased membrane permeability, which in turn can cause electrolyte imbalance and cell death \(^{(37)}\). It was found that the antifungal action of the \textit{M. alternifolia} essential oil against \textit{C. albicans}, \textit{C. glabrata} and \textit{Saccharomyces cerevisiae}, is related to the changing in the permeability and membrane fluidity of these fungi \(^{(38)}\).

The effect of rosemary essential oil on the biofilm formation of the local isolates of \textit{Candida} spp. was investigated as showed in table 5.

**Table 5: The percentages of biofilm reduction by rosemary oil against four \textit{Candida} spp at different sub-inhibitory concentrations**

<table>
<thead>
<tr>
<th>Yeast Species</th>
<th>Rosemary oil concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.78</td>
</tr>
<tr>
<td>\textit{Candida albicans}</td>
<td>50.75 – 55.25</td>
</tr>
<tr>
<td>\textit{Candida glabrata}</td>
<td>19.75 – 24.25</td>
</tr>
<tr>
<td>\textit{Candida tropicalis}</td>
<td>9.75 – 14.25</td>
</tr>
<tr>
<td>\textit{Candida krusei}</td>
<td>41.90 – 53.51</td>
</tr>
</tbody>
</table>

*The reduction of biofilm included 3 isolates of each species.

The current results revealed that the reduction of biofilm formation among \textit{C. albicans} and \textit{C. krusei} was obvious at the lower concentrations (1.56%), where the percentage of biofilm formation in \textit{C. albicans} was (91.25%) and \textit{C. krusei} was (84.25%), while \textit{C. tropicalis} exhibit (86.32%) for biofilm reduction at the concentration (12.5%) of rosemary essential oil, also it was found that the effect of essential oil on \textit{C. glabrata} biofilm formation was at the concentrations 3.125% and 6.25%. The local study of Raheem and Ghaima (2021) \(^{(39)}\) revealed that the Nystatin had the inhibitory activity against \textit{C. albicans} at the concentrations 6.25 and 12.5 μg/ml, while the highest antibiofilm activity by Nystatin were demonstrated at the subinhibitory concentration 50 μg/ml with biofilm eradication percent (75.80%).

As the findings of the present study, Cavalcanti \textit{et al.} (2011) \(^{(40)}\) found that the essential oil of \textit{R. officinalis} had an anti-adherent effect on \textit{C. albicans}, where the oil at the concentration 2.25 mg/mL caused significant cell disruption and inhibition of adhesion and the intermediate effect was observed at 1.12 mg/mL. The rosemary essential oil was found to be more active against the gram-positive pathogenic bacteria and drug-resistant mutants of \textit{E. coli}, similarly, it was found to be more active toward nonfilamentous, filamentous, dermatophytic pathogenic fungi and drug-resistant
mutants of Candida albicans. The results of the previous study revealed that R. officinalis extract provided a significant biofilms reduction after 5 min treatment, with rates of 99.96 ± 0.07% for C. albicans; 67.84 ± 12.05% for S. aureus; 77.64 ± 15.67% for E. faecalis; 79.32 ± 7.34% for S. mutans; and 98.23 ± 2.17% for P. aeruginosa. In the biofilm of C. albicans with S. mutans was also observed reductions of 92.04 ± 5.24% and 64.55 ± 15.12%, respectively.

Rosmarinus officinalis essential oil when coated to nanoparticles strongly inhibited the adherence ability and biofilm development of C. albicans and C. tropicalis to the catheter surface, as shown by viable cell counts and scanning microscopy examination and the sub-inhibitory concentrations of these substances resulted in a reduction of the amount of sterol extracted as well as the capsule size, suggesting that they play an important role, in particular by causing the cell wall destruction and membrane irregularities, the presence of vesicles and cell wall thickening in C. albicans.

Conclusion

The phytochemical constituents present in the essential oil extract of Rosmarinus officinalis showed twenty-four constituents such as Eucalyptol, Linalool, Camphor, Borneol, Verbenone, and Caryophyllene and contains 16 polyphenolic compounds with 6 compounds were identified. The free radicals scavenging activity increased gradually with the increase in the concentration of essential oil and it was found that the essential oil was effective antioxidant. The findings of this study exhibited that the essential oil has antifungal activity against pathogenic C. albicans isolates at low concentrations. Also, the current results revealed that the reduction of biofilm formation among C. albicans and C. krusei was obvious at the lower concentrations.

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Ethical Clearance: Yes

Conflict of Interest: Nil

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The Technical Value of Magnetic Resonance Imaging and Ultrasonography in Identifying Characterization of Ovarian Cysts

Ali Hani Karim¹, Mahmood Radhi Jobayr², Lamyaa F.A. Al-Barram³

¹Research Scholar, Al-hakeem Hospital, Najaf, Iraq, ²Professor, Corresponding Author, Middle Technical University (MTU), College of Health and Medical Technology, Dept. Radiology Technology, Baghdad, Iraq, ³Research Scholar, Middle Technical University (MTU), College of Health and Medical Technology, Dept. Radiology Technology, Baghdad, Iraq

Abstract

An accurate diagnosis of ovarian cysts is of utmost importance to determine the timely treatment to preserve fertility. We sought to determine the effectiveness of ultrasonography and MRI modalities for diagnosing the most common ovarian lesions and differentiate between benign and malignant lesions in order to guide patients to appropriate treatment. This study was conducted on 94 women, the age group between 14-70 years, in the period of January 2020 to October 2021. Were all women suffering from abdominal pain, swelling, nausea or vomiting and bleeding and irregular menstrual cycle. Was suspected clinically in the presence of lesions in the ovary was confirmed by tests of ultrasound and magnetic resonance. The entire cases were subjected to transvaginal and transabdominal ultrasound and magnetic resonance imaging to assess ovarian lesions in terms of content (somatic, cystic solid), nodules, septal characteristics, wall thickness, vascular lesion and ascites. In USG, there were 16% cases of malignant ovarian lesion and 84% cases had benign lesions. MRI reports showed that 10.6% cases had malignant ovarian lesions and 89.4% cases had benign lesions. Findings of USG with HPE have 100.0% sensitivity, 89.1% specificity and 91.07% diagnostic accuracy. In MRI findings with HPE have 100.0% sensitivity, 100% specificity, and 100.0% diagnostic accuracy. MRI had high specificity and more accuracy value in terms of diagnostic performance than USG. The ultrasonography diagnostic value in case of characterization of adnexal mass lesion was significantly lower than in the MRI.

Keywords: USG, MRI, histopathology, adnexal lesions, ovarian cyst, adnexal masses.

Introduction

One of the common disorders in gynecology is ovary cysts, also known as adnexal masses or ovarian masses. Ovarian masses pose a dilemma for gynecologists since the diagnosis of variation is complex and difficult. Among the most serious types of gynecological cancer are ovarian malignancies, which are characterized by a late onset and poor response to therapy. Adequate surgical management of ovarian cancer remains as the cornerstone treatment for possible malignancy of the ovary and minimal invasive surgery (i.e. laparoscopy or laproectomy) if the tumor is benign. One of the routine gynecological investigation and primary diagnostic in cases of suspected a lesion and identification ovarian mass is an ovarian ultrasound. Where the mass in adnexal region is recognized as undefined on ultrasound when it couldn’t be surely located into either the malignant or benign tumor, even after thorough examination including evaluation by the Doppler, or for which the location of source, from the uterus, ovary
or other structures of the pelvis, stays to be decided (3). When findings of the ultrasound are equivocal or nondiagnostic, the magnetic resonance imaging can be solve the problem, an auxiliary method of assessing lesions in adnexal region, appropriate for even providing information on surgical readiness with avoiding the radiation exposure. Magnetic Resonance Imaging (MRI) can be used specifically to provide accurate details of fat, collagen, and hemorrhage (5-7). Also, it can detect several kinds of masses in the pelvis and distinguish between malignant and benign ovarian tumors with high accuracy ranging from 88-93% (1, 7). MRI due to its higher contrast to the soft tissue and high spatial resolution abilities can improve characterization and definition of normal anatomy pelvic organs with diffuse and focal conditions of the uterus. MRI has the advantage of a noninvasive test, without radiation risk, less operator-dependent and not need anesthesia (8). Characteristics of benign tumors are more indicative, a wall thickness smaller than 3mm, completely cystic consistent, a diameter smaller than 4cm, absence of inner structure and the lack abdominal fluid (as cites), adenopathy or disease of peritoneal (9). MR imaging has become an essential device in the valuation of patients with adnexal lesion, and its role continues to develop. Some benign structures can be diagnosed by magnetic resonance imaging with a high grade of sureness, such as endometriomas, simple and hemorrhagic cysts, fibromas, and hydrosalpinx (10). In cases of malignant tumors, MR imaging may be more exact than other modalities for staging, lesion characterization, and follow-up (11). Most significant morphological characteristics of ovarian masses at significant risk involve (a) the lesions is solid or solid / cystic with a high diameter greater from 4 cm; (b) The appearance of unequal, non-fatty, Solid avascular zones larger than 28 mm in diameter (c) In a cystic lesion, the existence of papillary bump and thick wall and septa larger from 3mm (12, 13).

In this study, we aimed to investigate the effectiveness of ultrasound and magnetic resonance imaging (MRI) for diagnosing the most common ovarian lesions. In addition, we studied the cases to determine the precision of ultrasonography (USG) and magnetic resonance imaging (MRI) in ovarian cyst characterization to differentiate between benign and malignant lesions in order to guide patients to appropriate treatment.

**Patients and Methods**

Ninety four consecutive patients, with age group (14–70) years; the mean age was 39.13 years diagnosed with adnexal lesions by ultrasound and MRI in the department of radiology in Al-Hakeem in Najaf teaching hospital, the women present with variation of symptoms include abdominal swelling, irregular cycle, abdominal pain, bleeding, incidental. MR imaging was achieved on 1.5T unit of MR to do T1-weight edimages(T1W), T2-weighted images, and fat-suppression image T1-weighted pre and post intravenous MRI contrast agent (gadolinium). Ovarian cysts have features that include shape, size, content (solid - cyst), nodal or vascular septum, and reinforcement. In addition, other features appeared includes peritoneal disease, enlarged lymph nodes and ascites. We differentiate surgical and pathologic findings with the image features. On all MR imaging features, multiple logistic regression analysis was performed without clinical details. They were classified as benign or malignant, according to the image features that were differentiated to the pathological findings and surgical.

**U/S and Doppler Protocol**

The systems used were the PhilipsHD11xe and GE Vivid E9, and GE volusion E6. All devices had a transabdominal probe of about 2–5 MHz and an endovaginal probe with 5-7 MHz. All devices had both pulsed Doppler and colorability. Where U/S and Doppler of the abnormality in ovarian may have been endovaginal performed, transabdominal Doppler U/S of the abnormality may have been used for non characterization outcomes. Before menstruation of females were arranged for the USG in eight days to the beginning their menses.
The protocol of MR Imaging

MR imaging was performed on 1.5-T units (Siemens Medical Systems). For imaging the pelvic, we used the multi-coil array, while for imaging the abdomen, we used body-coils. The sequences used to females pelvis was transverse T2-weighted sequence fast spin-echo (5,100–6,100/104–128 (TR msec/TE msec)) with a number of echoes acquired in given TR (ETL) of 15, a thickness of slice 4–10mm, and gap 1–2.5mm. 256 × 256 matrix size with twain signals obtained. These series recurred as seen in sagittal and coronal sequences. A transverse T1-weighted image sequence (510-810/12-20) with T2-weighted sequence similar to spatial resolution was then accomplished. After injections intravenous (10-15) mL of gadolinium, the T1W images recurrent. By an axial fat-suppressed T1W spin-echo sequence and an axial T2W fast spin-echo sequence, the residue of the pelvis and abdomen were imaged. There was 16 of an echo train length, an 5-9mm thickness of the slice, and a gap 1-2.5-mm in the T2-weighted sequence (4,100/101-125). The matrix was 256 or 256×192 by 2 to 4 signals obtained, in the planes of coronal and sagittal, this sequence was repeated. The sequence of T1-weighted (400–610/12–21) an 4–8-mm thickness of slice with a gap 2-mm. The two signals with a matrix o 256×128–192.

Results

Table 1: The Distribution of patients according to finding by ultrasound and MRI regarding ovarian cyst (N=94)

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Ultrasound</th>
<th>MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian cyst size (cm²)</td>
<td>(25.46 ± 22.61)</td>
<td>(27.97 ± 25.92)</td>
</tr>
<tr>
<td>Content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystic</td>
<td>80 (85.1)</td>
<td>82 (87.2)</td>
</tr>
<tr>
<td>Solid</td>
<td>14 (14.9)</td>
<td>12 (12.8)</td>
</tr>
<tr>
<td>Nodule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>1 (1.1)</td>
<td>4 (4.3)</td>
</tr>
<tr>
<td>Absent</td>
<td>93 (98.9)</td>
<td>90 (95.7)</td>
</tr>
<tr>
<td>Septum characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>9 (9.6)</td>
<td>10 (10.6)</td>
</tr>
<tr>
<td>Absent</td>
<td>85 (90.4)</td>
<td>84 (89.4)</td>
</tr>
<tr>
<td>Vascularity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>9 (9.6)</td>
<td>9 (9.6)</td>
</tr>
<tr>
<td>Absent</td>
<td>85 (90.4)</td>
<td>85 (90.4)</td>
</tr>
<tr>
<td>Laterality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>86 (91.5)</td>
<td>86 (91.5)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>8 (8.5)</td>
<td>8 (8.5)</td>
</tr>
</tbody>
</table>
Table 2: Sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of US results in diagnosis of ovarian cyst in comparison to histopathological results

<table>
<thead>
<tr>
<th>Ultrasound diagnosis</th>
<th>Histopathology</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malignant ovarian cyst</td>
<td>Benign ovarian cyst</td>
</tr>
<tr>
<td>Malignant ovarian cyst</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Benign ovarian cyst</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>46</td>
</tr>
</tbody>
</table>

Sensitivity of US = 10/10 * 100 = 100.0% ; Specificity of US = 41/46 * 100 = 89.1% ; PPV of US = 10/15 * 100 = 66.7% ; NPV of US = 41/41 * 100 = 100.0% ; Overall accuracy of US = (10+41)/ 56 * 100 = 91.07%

Table 3: Sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of MRI results in diagnosis of ovarian cyst in comparison to histopathological results

<table>
<thead>
<tr>
<th>MRI diagnosis</th>
<th>Histopathology</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malignant ovarian cyst</td>
<td>Benign ovarian cyst</td>
</tr>
<tr>
<td>Malignant ovarian cyst</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Benign ovarian cyst</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>46</td>
</tr>
</tbody>
</table>

Sensitivity of MRI = 10/10 * 100 = 100.0% ; Specificity of MRI = 46/46 * 100 = 100.0% 

PPV of MRI = 10/10 * 100 = 100.0% ; NPV of MRI = 46/46 * 100 = 100.0% ; Overall accuracy of MRI = (10+46)/ 56 * 100 = 100.0%

Figure 1: 27 years female patient presenting with dysmenorrhea and irregular cycle transvaginal ultrasound show cystic lesion with homogenous low level internal echo.
Figure 2: T2 weighted images sagittal MRI views high signal intensity.

Figure 3: Sagittal T1-weighted image.

Figure 4: Axial T1-weighted image with fat suppression high signal intensity showing right endometrioma.
Discussion

The study was achieved on 94 females who had clinically suspected ovarian lesions who were primarily investigated with U/S then by MRI. The last diagnosis was confirmed by histopathological (HPE). Age patients ranged from 14 to 70 years; the mean age was 39.13 years. Most cases (91.5%) had unilateral lesions while 8.5% had bilateral lesions. These bilateral lesions were confirmed as malignant. 16% cases were suffered with multiple presentation, 62.7% abdominal pain, 4.3% cases were with abdominal swelling, 8.5% cases were with abnormal bleeding and 58.5% cases were suffered with irregular cycles of menstruation (figure 2). Also, this study showed most of the benign lesions are simple cysts, thin wall of cyst with absent vegetations, nodules, or a solid component and without of significant contrast enhancement. Whereas, typical characteristics of ovarian lesion in MRI which pointed to a malignant pathology were, thick wall, present enhancing, septations, vascularity, solid areas, ascites, present nodules this agree with Arun Prasad et al. (14).

USG findings showed most lesions were cystic in nature 80 (85.1%) than solid cystic 14(14.9%), Nodules was absent in 93(98.9%) cases and nodules was seen in 1 (1.1%) case. Ascites was absent in 89(94.7%) cases and present in 5(5.3) cases, 85(90.4%) cases absent with Septum while 9(9.6%) cases had with septum, vascularity was absent in 85(90.4%) and present in 9 (9.6%) as shown in table 1.

MRI findings of this study stated that most lesions were cystic in nature 82 (87.2%) than solid cystic 12 (12.8%) Nodules was absent in 90(95.7%) cases and nodules was seen in 4(4.3%) cases. Ascites was absent in 89(94.7%) and seen in 5(5.3%) cases, septum was absent in 84(89.4%) and septum was seen in 10(10.6%), vascularity was absent in 85(90.4%) and present in 9 (9.6%) as shown in table 1.

Also, on USG there were 15(16%) cases of malignant ovarian lesion and 79(84%) cases had benign lesions. MRI results showed that 10(10.6%) cases had malignant ovarian lesions and 84(89.4%) cases had benign lesions. Those results are roughly in agreement with Chinta Vittal Prasad et al. (15).

The comparison of findings of USG with HPE: The sensitivity of US results in diagnosis of malignant ovarian cyst was (100.0%) that mean ultrasound was able to diagnosed all patients with malignant ovarian correctly. The specificity of US results in diagnosis of benign ovarian cyst was (89.1%) that mean ultrasound was able to diagnosed (89.1%) patients with benign ovarian correctly. Positive predictive value (PPV) was (66.7%) that mean those diagnosed as malignant ovarian cyst by US was (66.7%) being malignant ovarian cyst by histopathology and negative predictive value (NPV) was (100.0%) that mean all those diagnosed as benign ovarian cyst by US being diagnosed as benign ovarian cyst by histopathology and overall accuracy was (91.07%) as shown in table 2. The sensitivity of MRI results in diagnosis of malignant ovarian cyst was (100.0%) that mean MRI was able to diagnosed all patients with malignant ovarian correctly. The specificity of MRI results in diagnosis of benign ovarian cyst was (100.0%) that mean MRI was able to diagnosed all patients with benign ovarian correctly. Positive predictive value was (100.0%) that mean those diagnosed as malignant ovarian cyst by MRI was (100.0%) being malignant ovarian cyst by histopathology and negative predictive value was (100.0%) that mean all those diagnosed as benign ovarian cyst by MRI being diagnosed as benign ovarian cyst by histopathology and overall accuracy was (100.0%) as shown in table 3. In this study ultrasound sensitivity was same as that MRI, however specificity and accuracy was more on MRI with small percentage difference in detecting malignancy in adnexal lesions that agree with Sadowski, E. A. et al. (16) and disagree with Chinta Vittal Prasad et al. (15) and Yasmeen Usmaniet al. (17).

Conclusion

In USG, there were 16% cases of malignant ovarian lesion and 84% cases had benign lesions. MRI reports showed that 10.6% cases had malignant ovarian lesions and 89.4% cases had benign lesions. The comparison
of findings of USG with HPE has 100.0% sensitivity, 89.1% specificity. 66.7% PPV, 100.0% NPV and 91.07% diagnostic accuracy. In comparison of MRI findings with HPE have 100.0% sensitivity, 100% specificity, 100% PPV, 100.0% NPV and 100.0% diagnostic accuracy. The results conclude that MRI had high specificity, accuracy value than USG. MRI is dominant in diagnosis and characterization of ovarian cystic lesion than ultrasonography. MRI is superior to ultrasound and can be used in difficult or equivocal cases.

**Ethical Clearance**: Taken from Middle Technical University ethical committee

**Source of Funding**: Self

**Conflict of Interest**: Nil

**References**


10. Taj-Aldean, K. A. H. The validity of ultrasonography (US) and magnetic resonance imaging (MRI) in characterizing adnexal masses (prospective study; Al-Qadisiyah Medical Journal. 2012; 8(14): 205-220


Evaluation of Gingival Inflammation, Plasma IL-6, and TMJ Clicking after Masseters Muscle Botulinum Toxin Injection and Intra-Articular PRP Injection In Bruxism Patients

Ali Sahib Hussein¹, Muhassad H. Al-Mudhafar², Karar Abdulzahra Mahdi³, Ali A. Al-Fahham³
¹Research Scholar, ²Research Scholar, ³Research Scholar, University of Kufa, Faculty of Dentistry, Iraq, ⁴Research Scholar, University of Kufa, Faculty of Nursing, Basic Medical Science Department, Iraq

Abstract

In this study, thirty patients complaining of bruxism at night and hyperactivity masseters muscle causing TMJ disk changes, TMJ clicking sound and all selected patient complaining of gingival inflammation as included criteria, and 50-unit botulinum toxin type A were injected in both side of masseter muscle. Regarding to T.M.J clicking sound there were three intervals of PRP injection for 3 months. The levels of IL-6 in the blood samples measured by ELISA technique, this study proposed to investigate the plasma IL-6 level in patient with early changes of T.M.J disorder and role of repetitive intraarticular PRP injection on the T.M.J clicking sound and the effect of decreasing stress force microtruma of masseter muscle on gingival score in previously diagnosed bruxism patient with gingival inflammation whether of causative factor of gingivitis. The mean difference of plasma IL-6 in between patient and control (25.6 ± 4.2 and 23.8 ± 1.8) respectively. There were highly significant differences in gingival index score of all sided of oral cavity after botox intramuscular injection. Force stress muscle microtruma of bruxism patient considered a contributory factor that increased gingival inflammation these fact suggested by botulinum toxin injection to decrease this force, and highly statically difference on gingival score after injection and also, the intraarticular injection of PRP in T.M.J disorder patient statically not affected on the clicking sound of joint as well as suggesting in our research for further studies to appear the role of IL-6 in early joint changes locally as an experimental study.

Keywords : Gingival Inflammation, IL-6, TMJ, botulinum toxin, PRP , Bruxism

Introduction

Bruxism is defined as non-desirable upper and lower teeth contact that causing in clenching or grinding of teeth due to repetitive, unconscious tightening of the masster and temporalis muscles (¹). One of the biggest problem faced the dentist in the daily work is the fracture of dental restoration, many dentists forget the force from excessive teeth contact as in bruxism that causes teeth wearing, fracture, tooth cracking and gingival, periodontal damage and tooth mobility (²).

The etiology of temporomandibular joint disorder stilled unknown, but many factors included, one of these factors occlusal interference that contributed in masster muscle over contraction and spasm and these microtrauma that causing temporomandibular disorder and pain was considered the most obvious symptoms.

Proinflammatory cytokines play an important role in inflammation and dysfunction of the joint and associated structure as well as muscle of mastication especially lateral pterygoid muscles and masster. Interleukin-6 (IL-6), IL-1 and TNF-a, have been important roles implicated in pain and tenderness by developing and maintenance of muscular hyperalgesia and also

Corresponding Author:
Ali Sahib Hussein
Research Scholar, University of Kufa, Faculty of Dentistry, Iraq,
increased level of proinflammatory cytokines leading to express psychological stresses and depression in patient with TMJ disorder (3).

Parafunctional habits have been thought to cause TMJ microtrauma or masticatory muscle hyperactivity; however, these habits are also common in asymptomatic patients. Although parafunctional habits may play a role in initiating or perpetuating symptoms in some patients, the cause-and-effect relationship remains uncertain. There is some evidence to suggest that anxiety, stress, and other emotional disturbances may exacerbate TMJ disorders, especially in patients who experience chronic pain. It is not clear which symptoms are more common in which TMJ disorders; however, it is generally assumed that joint clicking or grating signifies intra-articular derangement whereas headache, neck pain (4). Clicking sounds (a single sound) have energy concentrated in a short time interval (10–100 ms), while crepitus (a series of sounds) is longer in duration (0.1–1 s). Various attempts to characterize TMJ sounds have been made. Watt described waveforms of clicks and crepitus based on the oscillographic display of the analog sound recordings. Reduction of the articular disc has been suggested as one of the major causes of TMJ clicks (5).

Methods

In this study, thirty patients complaining of bruxism at night and hyperactivity masseters muscle causing TMJ disk changes, TMJ clicking sound and all selected patient complaining of gingival inflammation as including criteria, all of those patient diagnosed in private clinic of oral medicine specialist in Najaf City private dental center in period from 25/4/2020 to 15/2/2021.

All of patients informed the purpose of study and taking her consent, intraoral examination has been done and all of the gingival index score were obtained by specialist of dental prevention in this center, after extra and intraoral examination and including criteria that all selected patient without any sign and systemic disease were approved 50-unit botulinum toxin type A diluted in 2 ml distilled water and injections of 5 unit of diluted botox in each one of 3 marked point one the masseter muscle in both side below the line from angle of mouth to tragus of ear, botulinum toxin prevented release of acetylcholine neurotransmitter in neuromuscular junction and resulting in muscle paralysis and decrease of muscle tone, after 15 days of botulinum toxin injection gingival index score were also measured.

Temporomandibular joint clicking sound were hearing by stethoscope for each patient, all of patient injected by platelet rich plasma (PRP) made from 5 ml Venus blood in PRP tube as described by Mazzocca et al. (6).

The buffy coat was resulted from centrifugation at the superior surface of fluid in PRP tube and red blood cell at the bottom, the procedure was finished and 2/3 of poor plasma from platelet removed by syringe and late about 15 minute to insure the buffy coat was linked and dissolved after gentle shaking the tube in the area of visible gel. Finally, 1ml of PRP aspirated by syringe. Aspirated PRP injected in the TMJ joint in point below 2mm under the 10 mm line drawing from tragus to canthus of the eye. This procedure of PRP injection was done after aspiration to avoid auriculotemporal artery injection (7,8).

Three interval of injection for 3 month and joint sound were evaluated at the end of two weeks from last month. ELISA assays were performed to determine expression levels IL-6 in the blood samples, according to manufacturer instructions (BT LTD company Research Institute). Limitation of present study difficulty in patient follow-up the research begun with thirty patients and dropped to 25 patients in 3 months.
Result

Table (1): Descriptive statistics and differences in age and BMI between patients and control

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control</th>
<th>df</th>
<th>T - test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>26.78 ± 6.99</td>
<td>25.78 ± 6.69</td>
<td>44</td>
<td>0.49</td>
<td>0.62</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>30.29 ± 2.85</td>
<td>29.74 ± 3.01</td>
<td>44</td>
<td>0.60</td>
<td>0.54</td>
</tr>
</tbody>
</table>

In table (1) the mean age and body mass index between patients and controls were non-significant difference identified by applying independent t-test.

Table (2): Differences in IL-6 level of Pre-injection patients, Post-injection patients and control groups

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>F-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>25.6 ± 4.2</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>20.3 ± 1.2</td>
<td>B</td>
<td>12.3</td>
</tr>
<tr>
<td>Control</td>
<td>23.8 ± 1.8</td>
<td>C</td>
<td></td>
</tr>
</tbody>
</table>

Different letters refer to high significant difference at P value < 0.01; SD: Standard Deviation

In table (2), the data of plasma level of IL-6 was calculated by applying F-test that comparing in between groups. There are non-significant differences p value 0.07.

Table (3): Differences in joint sound of patients before and after intra-articular PRP injection

<table>
<thead>
<tr>
<th></th>
<th>Post</th>
<th>Chi-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>12</td>
<td>12.3</td>
<td>0.06</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

In table (3), the data joint sound was measured by using Chi-test that. There are non-significant differences p value 0.06.

Table (4): Differences in gingival index score in the molar region of the left lower jaw of patients before and botox injection

<table>
<thead>
<tr>
<th></th>
<th>Pre Patients (MS ±SD)</th>
<th>Post Patients (MS ±SD)</th>
<th>n</th>
<th>Z test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>gingival index score</td>
<td>1.47 ± 0.51</td>
<td>0.34 ± 0.48</td>
<td>19</td>
<td>3.82</td>
<td>0.000 *</td>
</tr>
</tbody>
</table>
Table (5): Differences in gingival index score in the molar region of the right lower jaw of patients before and botox injection

<table>
<thead>
<tr>
<th></th>
<th>Pre Patients (MS ±SD)</th>
<th>Post Patients (MS ±SD)</th>
<th>n</th>
<th>Z test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>gingival index score</td>
<td>1.26 ± 0.54</td>
<td>0.13 ± 0.34</td>
<td>21</td>
<td>4.01</td>
<td>0.000 *</td>
</tr>
</tbody>
</table>

MS : Mean of Scores ; SD : Standard Deviation ; * p < 0.01 : High significant difference by Wilcoxon Signed Rank Test

In table (5), gingival index score in the molar right region of lower jaw was statistically highly difference after botox injection with p value 0.000

Table (6): Differences in gingival index score in the molar region of the left upper jaw of patients before and botox injection

<table>
<thead>
<tr>
<th></th>
<th>Pre Patients (MS ±SD)</th>
<th>Post Patients (MS ±SD)</th>
<th>n</th>
<th>Z test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>gingival index score</td>
<td>1.43± 0.50</td>
<td>0.30 ± 0.47</td>
<td>21</td>
<td>4.01</td>
<td>0.000 *</td>
</tr>
</tbody>
</table>

MS : Mean of Scores ; SD : Standard Deviation ; * p < 0.01 : High significant difference by Wilcoxon Signed Rank Test

In table (6), gingival index score in the molar left region of upper jaw was statistically highly difference after botox injection with p value 0.000

Table (7): Differences in gingival index score in the molar region of the right upper jaw of patients before and botox injection

<table>
<thead>
<tr>
<th></th>
<th>Pre Patients (MS ±SD)</th>
<th>Post Patients (MS ±SD)</th>
<th>n</th>
<th>Z test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>gingival index score</td>
<td>1.60± 0.65</td>
<td>0.21 ± 0.42</td>
<td>20</td>
<td>3.92</td>
<td>0.000 *</td>
</tr>
</tbody>
</table>

MS : Mean of Scores ; SD : Standard Deviation ; * p < 0.01 : High significant difference by Wilcoxon Signed Rank Test
In table (7), gingival index score in the molar right region of upper jaw was statistically highly difference after botox injection with p value 0.000

**Discussion**

It has been suggested that trauma from occlusion in the presence of periodontal inflammation may be an important contributory factor in the pathogenesis of periodontal disease (9,10). Thus, occlusal forces may alter the path of spread of gingival inflammation and thereby facilitate direct penetration into the periodontal ligament enhancing angular bone resorption and infra bony pocket formation (11,12).

Therefore, in our study highly significant differences in gingival inflammation after botulinum toxin injection in masseter muscle suggesting decrease level of force applied on teeth by muscles botox injection lead to decrease spreading and penetration of gingival inflammation as well as the considering bruxism as causative factors or contributory factor in gingivitis and needing further studies in futures.

The importance of IL-6 in OA is also supported by previous results. It was shown that IL-6 concentrations in synovial fluid were considerably higher in patients with cartilage defect or OA than in donors without joint pathology. Follow-up study showed that increased serum concentrations of IL-6 were associated with articular changes observed in radiographs (13).

In present study there is no significant in plasma IL-6 level in patient with T.M.J changes as a clicking sound and our suggestion that local changes of cytokines in early stage of T.M.J disorder and changes occur without developing pain response that result agree with previous research (14).

Pain is one of the common sensations which usually indicates tissue damage as a consequence of various external or internal factors. From the results of previous report, it seems that masticatory muscles are capable to adapt to minor occlusal changes and chronic stress without developing the changes in pain response, but when this two proposed etiological factors work together they cause a higher pain response (14).

In the present study, similarly to pain response results, there was a lack of significant changes in both tissue and plasma IL-6 levels between the chronically stressed animals and rats with occlusal interference in comparison to the control group, while masseter IL-6 significantly increased in animals submitted to combination of experimental procedures. These results suggest that only the combination of occlusal interference and chronic stress leads to a strong local inflammatory reaction characterized by the alteration in masseter IL-6 level. Results of other studies showed increased levels of IL-6, prostaglandins, serotonin, and growth factors in muscle tissue after the contraction and muscle damage (15). Also, cytokines (IL-6, IL-1, IL-8, TNF-a) and prostaglandins, when locally injected, influence nociception causing hyperalgesia (16).

Contrary to positive association between pain response and masseter muscle IL-6 level found in this study, in most plasma samples the IL-6 level was below the detectable limit of the assay and there were no differences in plasma IL-6 levels between groups as well as no correlation with pain. Although not significant, there was a negative correlation coefficient between the pain and blood IL-6 level. Low level of blood IL-6 found in this study, especially in the group with combination of occlusal interference and chronic stress and negative correlation coefficient regarding to pain response point to the difference between local and systemic cytokine response. It has been suggested that blood IL-6 level does not depend on its local level and in contrarily to local proinflammatory reaction, the systemic cytokine response might be directed towards suppression of inflammatory reaction (17).

After 3 intervals of PRP injection there were no significant differences statically in between group and that came in agreement with previous studies stated that the joint sound was evaluated using VAS scale (18) other studies it was calculated on joints affected by sound or crepitus, (19) and in another two number of patients reporting sound was scored (20). In all of the
works analysed joint sound was found to improve during follow up. But remained results were not statistically significant, nevertheless an improvement was noticed (20).

**Conclusion**

force stress muscle microtrauma of bruxism patient considered a contributory factor that increased gingival inflammation these fact suggested by botulinum toxin injection to decrease this force, and highly statically difference on gingival score after injection and also, the intraarticular injection of PRP in T.M.J disorder patient statically not affected on the clicking sound of joint as well as suggesting in our research for further studies to appear the role of IL-6 in early joint changes locally as an experimental study.

**Ethical Clearance** : Taken from University of Kufa ethical committee

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**Conflict of Interest** : Nil

**References**


Comparison between 3 Different Types of Mouthwash on the Healing Process of Periodontal Diseases

Ammar Alwan Ali¹, Zainab Kasim Mohammad¹, Abeer Isam Abdulhameed¹

¹Researcher, High Diploma in Periodontics / Dental Health Specialist Center in Sheikh Omar / Baghdad, Iraq

Abstract

Dental plaque is the principal etiological agent for the development and progression of gingival and periodontal diseases. Three different mouthwash types were compared in this study for the periodontal healing process based on the results. For all bacterial strains, the culture type collection is used. The serial dilution process is used for preparing every mouthwash and adding it to the tubes with a specific dilution. In certain microorganisms of the respective mouthwash, the last tube dilution that does not show any turbidity will be considered MIC. The three types of mouthwashes were compared in terms of the ability to inhibit microbial growth. Twenty-four hours after the agar was solidified and the colony forms (CFU) units counted, the plates were incubated. This study showed that bacterial growth inhibition might occur in the three types of mouthwash. Both, Behsa and Kimgingival mouthwashes had a significant difference in their antimicrobial effect. This study showed that they were unable to perform s after 24 hours of in vitro incubation while the bacterial count was lowered after two weeks of in vivo use in the boht washings. Continuous exposure to bacteria may be necessary to wash the mouth, especially for Behsa and Boht. It is difficult to remove all bacteria from the mechanical plaque controls; antibacterial mouthwash can cause additive. In the current study, three types of mouthwash have compared antimicrobial effects. The results showed only that bacterial counts are affected by mouthwash. Based on this study’s results, Boht mouthwash is more effective than Kin Gingival and Behsa mouthwash for oral microorganisms, but further clinical studies are necessary to confirm our findings.

Keywords: Dental plaque, Periodontal diseases, Mouthwash, Bacterial, Boht, Behsa, Kin-gingival

Introduction

A variety of microorganisms, mainly bacteria, can be colonised and developed through the mouth; one influence factor in teeth and periodontal tissue destruction is bacterial plaque (1). The primary etiological factor for tooth decay, gingivitis, and periodontal disease is dental plaque. Dental and periodontal conditions are diseases associated with the prevention of biofilm. Dental plaque is the primary etiological agent in gingival and periodontal disease development and progression (2). Dental and periodontal susceptibility varies according to risk factors, including genetics, systemic factors and oral hygiene (3).

The primarily mechanical removal of plastic plaque through regular tooth brushing is used to prevent different periodontal diseases’ development and progression (4). The use of mouthwash to control plaque bacteria is about 5000 years long when the Chinese suggested that urine for children be used to prevent gingivitis (5). Various bacterial and fungal communities integrated into a highly specialised extracellular matrix are present in oral biofilms. The decrease in oral biofilm accumulation (dental plates) and the control/reduction of dental disease risks are essential for good oral care practices (6).

The primary effect of Mouth rinses on the supragingival and marginal plaque is limited due to tight gingival contact with the tooth in healthy persons (7). It
must always be used in conjunction with mechanical plaque control measures but should never be used solely as a means of oral hygiene. Mouth washing can help prevent oral infections, reduce inflammation, decrease halitosis and local fluoride prevent caries. Mouth washing has several preventative and therapeutic purposes. Usually, mouthwash is based on anecdotal evidence instead of on-the-counter (OTC) scientific evidence.

The use of mouth washing and chemical cleansing of teeth is typically to reduce dental accumulation. It can even be used as oral care only for patients who cannot brush their teeth either after surgery or due to the motor or cognitive constraints. Chlorhexidine (CHX) is long considered a short-term gold standard of action for bacteria, spores and fungi, including many antiseptic components of oral mouth washing. Mouthwash is a safe and effective anti-plaque and antimicrobial agent, which prevents adhesion, colonisation, metabolic activity and bacterial proliferation. Due to the variety of antibacterial efficacy, cytotoxicity and kinetics of different solutions, it isn’t easy to decide whether to use a particular mouthwash. In decreasing oral microbial counts, CHX is regarded as the gold standard.

In preventing periodontal illnesses, mechanical plaque control measures (tooth brushing and flossing) are essential. Benzydamine chloride can also help avoid periodontal diseases by chemical plaque control measures, such as utilising only 0.12 or 0.2 per cent mouthwashes preparations with chlorhexidine. If there is unevenness between the host defence and the bacterial niche, periodontitis may occur. The primary aetiology of the gingival tissues and the paradental attachment is the bacterial plaque. Mechanic removals of the plaque or calculus and topical antimicrobial agents that can inhibit periodontal pathogens are commonly used in periodontal therapy. Moreover, in current periodontal and other applications, extracts from plants have been offered as anti-plaque agents.

Body rinse can produce a therapeutic effect on the tooth’s entire surface, including interproximal areas where toothpaste is very effective. Although it is effective in plaque control, chlorhexidine cannot be used for a long time because of some of its unpleasant side effects after a long time. The use of mouthwash in oral hygiene is ancient, in contrast to the popular notion. The use of mouthwash originates from religious codes of behaviour such as the outdated Manu laws of India that must rinse their mouths after a meal. This study aimed to compare three different mouthwash types for the periodontal healing process based on the findings.

**Methodology**

This study aimed to determine the effect of three types of mouthwash on the treatment of periodontal disease. The impact on Kin Gingival, Behsa and Boht are assessed on the selected bacterial. Thus, minimum inhibiting concentrations (MIC) were used. The collection of culture type is used for all bacterial strains. The serial dilution method has been used to prepare every mouthwash with specified dilution and added to the tubes. The last tube or the last mouthwash dilution not showing turbidity is considered the MIC in certain microorganisms of the respective mouthwash. In terms of the ability to inhibit microbial growth, the three mouthwash types’ MIC was compared. The tubes without turbidity (transparent) were then transferred to a solid media after 24 hours of incubation, which showed bacterial growth inhibition with the respective mouthwash and evaluated for the microbial growth to determine the MBC of mouthwash. In terms of the solid medium culture, the last tube, which was negative, indicated the minimum concentration (MBC) of bactericidal fluid.

For all bacterial strains, this procedure was done. 0.5 ml diluted samples have been transferred in empty plates for counting bacterial colonies. The agar was cooled to 50°C and poured into each plate. The pouring of the agar was done into each plate. The plates were incubated 24 hours after the agar had solidified and the colony forms (CFU) units counted. On dilute samples collected before the patients’ used mouthwashes or water, the zone of growth inhibition test was done in vitro. On the agar surface with the swab, bacteria were streaked. The filter
paper impregnated disks were then placed at the centre of each section and slightly pressed to the agar with the water and mouthwash. The plates were then incubated for 24 hours at 37°C inverted positions. The inhibition zone was measured after 24 hours.\(^{(10)}\)

**Results**

![Figure 1: The MIC and MBC effect of the three mouthwash on the selected bacteria](image1)

![Figure 2: The effect of the three mouthwash on the zone of inhibition in selected bacteria](image2)
**Discussion**

This study showed that the three types of mouthwash could cause bacterial growth inhibition. The antimicrobial effect of Boht, Behsa and Kin, gingival washes of the gum, were significantly different (Figure 1). The SM growth inhibition areas in the three study groups are shown in Figure 2. Two-week use of Boht, Behsa and Kin gingival mouthwash, before and after periodontal patients, is summarised in Figure 3. In patients with periodontal disease, the summary of bacterial counts is also presented in Figure 3 before and after two weeks.

Mueller Hinton Agar was the artistic medium of this study, used as a growth speciality for those bacteria in procedures commonly conducted for aerobic and optional anaerobic bacteria (neogen.com). Therefore, it would appear logical to assume that both aerobic and optional bacteria cultivated on agar plates were actual.

The results of this study demonstrated a difference in the antibacterial effects shown by Behsa and Boht, as they were unable to make s after 24 hours of in-vitro incubation, while boht washings showed a reduced bacterial count after two weeks of in-vivo use. The culture medium was treated only once and then after 24 hours in inhibition tests with mouth washing. At the same time, bacteria were repeatedly exposed to the effect of mouth washing for the in vivo testing for two weeks. The results were obtained after 24 hours only. It may require continuous exposure to mouth washing, especially for the Behsa and Boht, to reduce bacterial counts. Mechanical plaque controls are difficult to remove all bacteria; antibacterial mouthwashes may help additives to this effect. Antimicrobial effects were compared in three types of mouthwash in the current study.

The role of bacterial plaques was demonstrated by dental caries aetiology and by periodontal diseases. The mechanical methods of plaque inhibition are limited; this problem is proposed to chemical methods for plaque inhibition (6). Therefore, the utilisation of mouth washing as disinfectants can help mechanically...
reduce plaques. Mouth washing takes place in few ways, including apoptosis, bacterial growth inhibition, metabolic inhibition of cells, and bactericidal inhibitions based on their concentration \(^{11}\).

A great deal of study has shown that washing chlorhexidine’s mouth is the best way to wash the mouth. The supremacy of most studies comparing mouthwashes, with only a few studied products competing in antibacterial characteristics with chlorhexidine, has been demonstrated. Streptococci are the primary etiologic agent for tooth decay \(^{18}\).

Removal of streptococci prevents plaque formation and disease spreading. In the study of S. mutans susceptibility to mouthwashes, Jarvinen et al. showed that S. mutans is resistant to antimicrobial agents. S. mutans have the highest mouthwash strength and even greater varnish resistances \(^{19}\).

We also have confirmed that S. mutans are somewhat resistant to chlorhexidine. The most significant anti-caries effect of fluoride chlorhexidine was achieved using a study comparing Behsa polyphenol extracts with fluoride washes, showing its synergistic effect on microorganisms \(^{20}\).

A study comparing oral-B with other mouthwashes has demonstrated greater efficacy in reducing the S. mutans by washing Boht in plaques around orthodontic brackets, indicating high antimicrobial activity mouth \(^{14}\). Kin Chlorhexidine Gingspace effectively eliminates Streptococci causing decay and beneficial antimicrobial and anti-gingival effects, as they support initial plaques in these microorganisms. Studies have shown that a higher concentration of the antimicrobial effect. The main impact on the microorganism concentration is Kin gingival mouthwash \(^{20}\).

The first microorganisms to develop dental caries are lactobacilli and chemical or mechanical removal. The differences between the chlorhexidine compound may lead to previous studies since different combinations have other products \(^{21}\).

Streptococcus mutans are the primary etiologic agent for dental caries. It may adhere to the acquired film as the first step in plaque formation. By eliminating this bacterial species, I prevent plaque formation and caries development \(^{7}\). Mechanical methods of plaque inhibition have several limitations; dental plaque inhibition techniques have therefore been suggested. The use of disinfectants to wash the mouth can help to reduce plaque. The effect of Behsa mouthwash on SM inhibition has been evaluated and compared with Boht and Kin Gingival mouthwash’s effectiveness \(^{22}\).

The results showed that all three washers could inhibit SM’s spread with the highest Boht-inhibiting effect. Many oral studies in microorganisms have shown that Boht is the most appropriate gold standard for chemical treatment with SM and dental cavities. The study showed the inhibition by Boht containing mouthwash and Total Care Kin gingival of the formation of the plaques by various Streptococci species \(^{23}\). They also say Boht’s mouthwash is more efficient than Total Care Kin’s gingiva mouthwash. Dental caries and pathology decreased, damaging the innocent bacterial species that competed with SM effectively inhibited by washed-in \(^{1}\).

Boht’s positive effect on reducing the SM and Lactobacillus colonies has been described in the literature. Boht is a large anode and adsorbs the tooth, plaque and mucus surface and increases adsorption to the above characters by its cationic nature. The extracellular polysaccharides cause the absorption of this antibacterial mouth washing \(^{24}\).

However, previous authors, contrary to this study, have shown a better effect on the Kin gingival mouthwash plankton and biofilm bacteria than Boht (diluted). Boht also decreased the number of plaques and gingivitis, but no antibacterial activity existed when Boht was diluted. In its antimicrobial activity, Boht concentration appears to play a vital role \(^{2}\).

Another discovery of this study was Behsa mouthwash’s best, higher, antibacterial effect than Kin Gingival mouthwash. The essential oils available are kin gingival. The antimicrobial activity of Kin gingival against oral microorganisms has already been evaluated and has been confirmed \(^{2}\).
The main flavonoids in tea are epigallocatechin-3-Gallate and epicatechin. The catechin’s anti-cancer activity may be attributed to a direct anti-bacterial effect on SM inhibition adherence to tooth surfaces. Behsa purifies the oral cavity, and reduced dental caries affects people who drink large quantities of Behsa (8). The combination of several antibacterial agents in a single product is one way of increasing anti-plaque efficiency. In Behsa mouthwashes, the higher antibacterial effect is higher than that of Kin gingival, given confirmations from various studies on the impact of Behsa extract and Kin Gingival mouthwashes in SM (25).

The positive effect of Behsa extract on the decrease in SM and Lactobacillus colony figures has previously been demonstrated in line with this study. Contrary to current research, the use of Behsa extract mouthwash has been shown to reduce the number of oral Kin Gingival-like micronutrients and have similar implications for both mouthwash types (17). The differences in the concentration of active agents in the formulation of mouth washing can be due to the study results. Unfortunately, the manufacturer’s Behsa concentrations in the Kin gingiva mouthwash have not been reported, and studies’ attention is not similar (26).

Therefore, Boht may be used every day as the natural component is present in Behsa and the tooth’s lack of dental colour potentials (5).

**Conclusion**

The results showed just that mouthwash affects bacterial counts. Since the oral cavity for commensal species has nevertheless played a positive part, there is still discussion of the need to maintain a constantly low number of bacteria within the mouth. This study demonstrated that three types of mouthwash could reduce the number of bacteria in the oral cavity. The S. mutans proliferation was more efficient than Kin gingival due to the Behsa containing a mouthwash. But Boht was less potent than both types of mouthwash. This study helps doctors to select the best antimicrobial agent on the market. Based on this study’s findings, boht mouthwash is more effective for oral microorganisms than Kin Gingival and Behsa mouthwashes, but further clinical studies are required to confirm our findings.

**Conflict of Interest:** No

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Histological Evaluation of Effect of Combined Local Application of Vitamin D3 and βeta-Tricalcium Phosphate on Bone Formation in Rabbits

Noor Abdulkareem Razouki1, Ban A.Ghani Jamil2
1Assistant Lecturer, AL-Rafidain University College, Baghdad- Iraq, 2Professor, Department of Oral Diagnosis, College of Dentistry, University of Baghdad, Baghdad- Iraq

Abstract

Bone, a hard and rigid constituent of the skeleton of vertebrates is composed primarily of calcium salts and connective tissue. Osteogenesis, or bone formation, is the process by which new bone is formed. Beta-Tricalcium phosphate (β-TCP) is alloplastic biomaterials, multiple studies conducted on the β-TCP and bone interaction, that histological examination shows an initial bone neoformation in inter granular areas and in the porous surfaces that helps guided for new bone formation. Calcitriol is active form of Vit.D3, can affect human osteoblast growth and differentiation stimulating bone formation and mineralization. The aim of study is evaluation of effect of β-TCP alone and combined with vitamin D3 on bone deposition in induced bone defect. Twenty-four adult male New Zealand rabbits weighting an average of (1.5 – 2 kg) used in this study. three intra bony holes created in both tibias (right tibiae for experimental groups and left for control group) of each animal which will be divided as follows: Group I(C): Bone defect left to heal spontaneously as control. Group II(TCP): Bone defect filled with β-TCP. Group III(TCPD): Bone defect filled with combination of β-TCP and Vit.D3. Animal scarification was done for the healing durations (7 and 21days). Routine processing and sectioning technique was performed for histological evaluation. Histological and Histomorphometrical findings at 7 days revealed new bone entrapping osteocytes and enclosed bone marrow in TCP and TCPD groups. TCPD group after 21 days showed defect area filled with dense bone trabeculae entrapping osteocytes and rimmed by osteoblasts. Haversian canals started to develop and reversal lines between new and old bones were also noticed. The highest mean value of osteoblasts, osteocytes and trabecular area were recorded in (TCPD) group. Trabecular number and osteoclasts recorded highest mean value in (TCP) group at 21days healing period. Conclusion: Within the limitations of this study the observed histological results indicated that local Vit.D3 with β-TCP application may accelerate new bone formation.

Keywords: Beta-Tricalcium phosphate, Vitamin D3, bone formation.

Introduction

βeta-Tricalcium Phosphate β-TCP( has been utilized in bone reconstruction as a scaffold that may successfully regenerate bone over critical sized bone defects in an in vivo model (1). β-TCP is reabsorbed in progressive manner, leaving area for bone neoformation (2) Multiple studies conducted on the β-TCP and bone interaction, that histological examination showed an initial bone neoformation in inter granular areas and in the porous surfaces that guided for new bone formation. Unlike hydroxyapatite, β-TCP is continuously and totally reabsorbed, thereby discharged calcium and phosphate ions that participate actively in the new osseous tissue formation (2, 3). It’s a challenge to Repairing large bone defects using osteoconductive biomaterials alone, because osteogenic cells are not probable to be enrolled in the center of large defects without osteogenic property, and lack of absorbability, there remain substantial impediments. Therefore, biomaterials used to repair burdened bone defects should...
have osteoconductive and osteoinductive properties. \(^4\). Calcitriol is active form of Vit.D3, can affect human osteoblast growth and differentiation stimulating bone formation and mineralization \(^5\). Calcitriol significantly accelerated the production of mature matrix vesicles in the pre-mineralization. It directly accelerates osteoblast-mediated mineralization via increased production of mature matrix vesicles in the period prior to mineralization. The accelerated deposition of mature matrix vesicles leads to an earlier onset and higher rate of mineralization. These effects are independent of changes in extracellular matrix protein composition \(^6\).

**Materials and Methods**

Twenty-four adult male New Zealand rabbits weighting (1.5 – 2 kg) were used in this study. The general anesthesia given by intramuscular injection of xylazine 20mg(Arendonk, Belgium) (0.2 ml/kg-B.W.) plus ketamine HCL 50 mg (Holland) (20 mg/kg B.W.) \(^7,\ 8\). The operation site was the proximal tibia metaphysis of the right limb \(^9\). Exposure of tibia and initial intermittent drilling was done by small round bur (no.010) and fissure bur (no.010) with speed 1500 rpm and vigorous irrigation by normal saline ,the drilling accomplished by round and fissure bur (no.012) to diameter of about 3mm and depth 4 mm. Intra bony holes were created in both tibias of each animal divided as follows:

1- Group C: Bone defect left to heal spontaneously as control.

2- Group TCP: Bone defect filled with \(\beta\)-TCP (Portugal) only.

3- Group TCPD: Bone defect filled with combination of \(\beta\)-TCP and Vit.D3 (Calcitriol) (Turkey) in a ratio of 1:1 \(^10\).

Animal scarification done by over dose of general anesthesia and specimens immediately stored in 10% freshly prepared formalin(Tedia, U.S.A) for fixation

Bone decalcification done by 10% formic acid then processed to paraffin blocks as a bone defect faced outside of block toward microtome knife. Tissue sections of 5 \(\mu\)m thickness were prepared followed by Hematoxylin and Eosin staining procedure, histological and histomorphometrical evaluation using light microscope (OpticaB-350, Italy). The following microarchitectures were measured

1- Osteoblast cell number (OB/ \(\text{mm}^2\)).

2- Osteocyte cell number (OC/ \(\text{mm}^2\)).

3- Osteoclast cell number (OCL/ \(\text{mm}^2\)).

Trabecular and bone marrow area:

1- Trabecular area(\(\text{mm}^2\)).

2- Bone marrow area (\(\text{mm}^2\)).

3- Trabecular number(\(\text{mm}^2\)).

Measurement of trabecular and bone marrow area was performed by software program (ImageJ. exe). Statistical analysis of recorded data was done by (ANOVA) test, least significant difference (LSD) test.

**Results**

**Histological and Histomorphometrical findings:**

Histological findings at (7 days) duration of defect area in control group shows blood islets and osteoblasts rimming newly deposited bone spicules also osteocytes entrapped in bone are noticed Fig.(1 A). \(\beta\)-TCP group shows osteoblasts at the periphery of new bone that entrap osteocytes and encloses bone marrow and also areas of remaining \(\beta\)-TCP material are illustrated Fig. (1 B). While bone defect area filled with \(\beta\)-TCP and Vit. D3 (TCPD group) shows new bone matrix deposition, rimmed by osteoblasts and entrapped osteocytes Fig. (1 C).
Histological View at (21 days) duration in control group shows bone marrow with progenitor cells, osteoblasts rimming bone and osteocytes embedded Fig.(2 A). Defect area of TCP group shows bone trabeculae rimmed by osteoblasts, numerous osteocytes trapped inside trabeculae and reversal line demarcating new bone from the old one, fat cells and progenitor cells also seen Fig.(2 B). TCPD group after 21 days shows defect area filled with dense bone trabeculae entrapping osteocytes and rimmed by osteoblasts. Haversian canals start to develop and reversal lines between new and old bones are also noticed Fig.(2 C).
Regarding histomorphometrical analysis, the mean values of trabecular number, bone marrow area, trabecular area, osteoblasts, osteocytes and osteoclasts showed highly significant difference in all groups in both durations (7days and 21days). All measured parameters in both durations had increased mean value with time except for bone marrow area and osteoblasts decreased in all groups, the highest mean value of osteoblasts, osteocytes and trabecular area were recorded in (TCPD) group. Trabecular number and osteoclasts recorded highest mean value in (TCP) group at 21days healing period. Bone marrow area showed the highest values in (C) group. In 7 days healing period revealed highly significant difference between groups for studied parameters. Regarding 21days duration high significant differences were estimated in all bone parameters in different groups, except trabecular number there was no significant difference between C and TCPD experimental group Fig. (3 and 4).

Figure (3): Histomorphometrical analysis of bone architecture parameters at 7 days

Figure (4): Histomorphometrical analysis of bone architecture parameters at 21 days.
Discussion

This study was designed to evaluate bone healing after application of osteoconductive graft material (β-TCP) and active form Vit.D3 in experimentally induced bone defects in rabbit’s tibiae. Rabbits are potentially, manageable, not overlooked species for bone healing experiments (11). The beneficial effects of Vit.D3 in bone metabolism and regeneration is experimentally and clinically evident, but no consensus exists regarding the applicable benefit of local application in bone defect healing which still controversy. The Vit.D3 receptors (VDR) are principally involved in mineral metabolism, is expressed in osteoblasts and studies didn’t found the corresponding clinical or microscopic side effects after local calcitriol application to concure the previous evidence (12, 13). The choice of β-TCP in present study as bone substitutes and carrier for Vit.D3 was for the following reasons:

1- The microporous geometric topography of β-TCP gives allocation for osteoblasts differentiation.

2- β-TCP is useful as a growth factor carrier and scaffold for bone augmentation. That habitat standard and control releases of Vit.D3 over time (14).

In the current study, histological examination at 7 days duration of some bone sections showed residues of β-TCP material surrounded by new bone deposition , mean values of trabecular area and trabecular number were higher in TCP and TCPD groups with high significant difference for all bone microarchitectures in agreement with Luvizuto,2011 (15) who found that β-tricalcium phosphate presents very promising osteoconductive properties, including the defect closure with new formed bone.

At 21days duration , bone trabeculae surrounding β-TCP were seen with signs of bone maturation, in accordance with the findings reported by Hirota et al., 2009 observed that β-TCP could be a good scaffold material for bone regeneration when used in combination with osteogenic materials (16). The aforementioned results of histomorphometric analysis of induced bone defect at both 7 and 21 days healing periods which showed higher mean values of different studied microarchitectures of TCPD and TCP groups in line with Sivolella et al.,2013 who explained main purpose of the use delivery system at the defective bone site is to ensure adequate concentrations for as long as it takes to enable the regenerative cells to migrate, proliferate and differentiate. In histological examination of TCP and TCPD groups, remnants of β-TCP material were noticed in some sections which may be attributed to:

1- It biodegrades relatively slowly occurs through osteoclastic activity, which is generally recognized to be in association with bone formation (17, 18).

2-It’s restorability allows a gradual biologic degradation over a period of time and a progressively replaced by a natural host tissue, pure β-TCP may be completely resorbed at skeletal sites over 24 weeks’ duration (19).

Vitamin D3 suppresses bone resorption through VDR in osteoblast-lineage cells and that the role of Vit.D3 in stimulating osteoblast development through exerting anti-apoptotic effects (20) which may support results of this study regarding TCPD groups as compared to control group in 7 and 21 days healing intervals.

Conclusion: Within the limitations of this study the observed histological results indicated that combined local Vit.D3 and β-TCP application may accelerate new bone formation.

Conflict of Interest: No

Source of Funding: Self funded

Ethical Clearance: Not Required

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Assessment of Serum Ferritin, Folate, Vitamin B12, C-reactive Protein, D-dimer and Homocysteine in Patients with Severe and Critical Covid-19 Infection

Nawar Mazin AL-Alwan¹, Zainab A. Razak Al-Sharifi², Abbas Hashim Abdulsalam³

¹Postgraduate, ²Assistant Prof. dr. University of Baghdad, College of Medicine / Department of Biochemistry/Iraq, ³Assistant Prof. dr. University of AL-Mamoon/Iraq

Abstract

Background: Corona virus disease 2019 (COVID-19) pandemic need urgent measurement and correlation of laboratory parameters in severe and non-severe cases. Serum ferritin, folic acid, vitamin B12, C-reactive protein, D-dimer and homocysteine was measured in patients who were infected with COVID-19.

Aim of the Study: To evaluate the usefulness of serum ferritin, folate, B12, C-reactive protein, D-dimer and homocysteine level as biomarkers for prediction of patients with severe and critical COVID-19 infection.

Subject, Material, Method: A cross sectional study was conducted at AL-Kindy hospital, Baghdad, Iraq from October 2020 to December 2020. Serum ferritin, folate, Vitamin B12, C-reactive protein, homocysteine and blood D-dimer were measured in 88 patients who suffered from COVID-19 infection, 50 cases with severe infection and 38 with non-severe infection. The age range of patients between 20-59 years old. Samples were collected from AL-Kindy hospital, Baghdad.

Result: Serum folic acid was significantly low in severe group than in non-severe group, while serum ferritin, CRP and blood D-dimer were high in severe group than in non-severe group.

Conclusion: In this study, measurement of serum ferritin, CRP and blood D-dimer were important in follow up of patients who infected with the novel virus as the level of them were higher in severe than in non-severe cases. Folic acid has role in determining the severity of COVID-19 infection because level was low in patients with severe than non-severe infection.

Footnote: COVID-19, folic acid, vitamin B12, C-reactive protein, D-dimer, ferritin and homocysteine.

Introduction

Corona viruses infect humans and animals, in general human corona viruses (HCoVs) cause 15-30% of common colds. Any animal reservoir corona viruses can however be transferred to humans, causing outbreaks in the human population, these zoonotic viruses infect both humans and animals, unlike HCoVs, and cause serious respiratory diseases such as acute respiratory distress syndrome (ARDS) and pneumonia, resulting in death¹.

90 % of patients with COVID-19 have irregular chest radiographs, In serious and critically ill patients, lymphopenia is common, and in patients with mild symptoms, it is uncommon, COVID-19’s chest computed tomography features include bilateral opacity of ground glass, consolidation, and local or bilateral patchy shadowing, In COVID-19 patients, GI symptoms are common, and a meta-analysis has shown that these symptoms occurred in 17.6% of infected patients and are more common in serious patients².

Respiratory tracts are the primary focus of SARS-CoV-2, contributing to clinical symptoms such as fever, dry cough, fatigue and dyspnea, in 10-20% of patients,
the disease usually progress into a serious type, requiring hospital admission or even intensive care unit (3).

Ferritin levels were high in patients who died from COVID-19 upon admission to hospital and during the hospital stay (4). In COVID-19 infection, pregnant women who classified as severe and critical were less than non-pregnant women, The factor protecting these patients against SARS-CoV-2 infection may be folic acid supplementation during pregnancy(5).

A significant modifiable risk factor for COVID-19 morbidity and mortality in the elderly and those with diabetes mellitus could be vitamin B12-folate-homocysteine dependent biochemical reactions that are crucial for wide-ranging aspects of DNA synthesis, cellular regulation and body repair(6).

In most patients with COVID-19, elevated serum C-reactive protein (CRP) levels were observed(7).

Elevated D-dimer and the increased rate of poor prognosis-related conditions, and the initiation of anticoagulant administration identified with D-dimer as a potentially useful method to avoid complications and have a beneficial effect on the course of corona virus disease 2019 (COVID-19)(8).

Aim of the Study

To evaluate the usefulness of serum ferritin, folate, B12, CRP, D-dimer and homocysteine level as biomarkers for prediction of patients with severe and critical COVID-19 infection.

Subject, material, method:

Across sectional study was conducted at Al-kindly hospital in Baghdad/Iraq, from October 2020 to December 2020, where 88 patients with COVID-19 infection documented by polymerase chain reaction (50 patients with severe symptoms who need hospitalization and 38 patients with non-severe criteria), the age of the patients range from (18-59) years old.

The permission was obtained from Al-kindly hospital, the blood samples were drawn from the patients after their agreement.

Whole blood samples were collected in a clot activator tube, centrifuge the sample after clotting for 5 minutes at 3000 rpm, clear serum was obtained which is divided into five eppendorf tube (1ml each eppendorf tube). Then the serum was frozen at -50°C. Repeated freeze-thaw cycle were avoided.

For measurement of D-dimer, human whole blood was needed and the test must be done within 24 hours.

Serum level of ferritin was measured by Vidas ferritin is an automated quantitative test for measurement of human ferritin in human serum or plasma using Enzyme Linked Fluorescent Assay (ELFA).

Serum level of folic acid, vitamin B12 and homocysteine were measured by Enzyme Linked Immuno-sorbent Assay (ELISA) for quantitative measurement of human serum.

Serum C-reactive protein was measured by NycoCard which was a diagnostic test for quantitative determination of C-reactive protein in human.

D-dimer was quantitatively measured by Fluorescence immune-assay (FIA).

Statistical Analysis

Data translation was done in computerized database, SPSS version program 25 was used to do analysis, p value less than 0.05 was statistically significant results.

Result

Study conducted on 88 patients who suffer from COVID-19 infection, split into two groups (50 patient with severe infection and 38 patients with non-severe infection).

The most frequently observed category Male ($n = 26, 46.43\%$) and ($n = 30, 53.57\%$) for non-severe and severe groups.
Table 1: Gender distribution of patients according to severity of COVID-19 infection:

<table>
<thead>
<tr>
<th>Gender</th>
<th>Non-severe n (%)</th>
<th>Severe n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>12 (37.5)</td>
<td>20 (62.5)</td>
<td>0.415</td>
</tr>
<tr>
<td>Male</td>
<td>26 (46.43)</td>
<td>30 (53.57)</td>
<td></td>
</tr>
</tbody>
</table>

In non-severe COVID-19 patients, the most frequently observed category of age was 40 - <50 years (n = 13, 34%). As compared with 50 - <60 years (n = 40, 80%) for Severe cases. Age had an average of 53.24 ± 6.60, and Median was 55.5 in severe group. For non-severe, the average of age was 42.66 ±10.14 years, and median was 44 years.

Figure(1): Age distribution in patients with COVID-19 infection.

The most frequently observed category of biochemical parameters in severe and non-severe patients with COVID-19 infection show that the folic acid was significantly lower in severe group than in non-severe group (p<0.05).

There was significant difference in vitamin B12 between the Covid-19 patients’ groups, p < 0.05. Severe group was significantly lower than non-severe group.

There was significant difference in homocysteine between Covid-19 patients groups p < 0.05. Patients with
Severe infection was significantly lower homocysteine level than non-severe group.

There was significant difference in D-dimer level in blood between Covid-19 patients groups $p < 0.05$, Patients with Severe infection was significantly higher level than non-severe group.

### Table 2 Laboratory findings in patients with COVID-19 according to severity of infection

<table>
<thead>
<tr>
<th>Laboratory results</th>
<th>Severity of COVID-19 infection</th>
<th><em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Severe (n= 50)</td>
<td>Non-severe (n= 38)</td>
</tr>
<tr>
<td></td>
<td>Median + IQR</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Folic acid (nmol/L)</td>
<td>4.28 (3.22-4.86)</td>
<td>4.22±1</td>
</tr>
<tr>
<td>B12 (pmol/L)</td>
<td>463.56 (445.26-480.97)</td>
<td>474.83±65.74</td>
</tr>
<tr>
<td>Homocysteine (nmol/ml)</td>
<td>2.61 (2.19-3.22)</td>
<td>3.45±3.83</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>622.5 (436-702.75)</td>
<td>580.54±212.41</td>
</tr>
<tr>
<td>D-dimer (µg/ml)</td>
<td>1.38 (0.81-3.20)</td>
<td>2.49±2.51</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>51 (17.75-90.75)</td>
<td>55.68±41</td>
</tr>
</tbody>
</table>

### Discussion

Serum folic acid level was lower in severe group than serum folic acid in non-severe group, the results agree with (Elias J, *etal*, 2020) which was found that Pregnant women seem to have a lower risk of contracting COVID-19 infection, and those who infected were more likely to be asymptomatic. Pregnant women from ethnic groups with low folate RBC concentrations due to genetic factors and/or who do not receive folic acid supplementation, have a higher hospitalization rate$^{(9)}$.

Serum vitamin B12 levels were normal in patients with severe and non-severe COVID-19 infection. In non-severe COVID-19 infection, mean of serum vitamin B12 level in severe group was higher than non-severe group. These results were disagreeing with (Dalbeni A, *etal*, 2021) which was found that Excess serum B12 level has been linked to a rise in mortality rates$^{(10)}$.

Serum homocysteine levels were normal in non-severe COVID-19 infection patients, while serum homocysteine levels were low in severe COVID-19 infection. The results were disagree with (Ponti G., *etal*, 2020) which show that plasma homocysteine must be measured on a routine basis as a potential marker for severe disease in SARS-CoV-2 patients$^{(11)}$.

Serum ferritin levels were significantly increased in patients with severe COVID-19 infection in comparison with non-severe COVID-19 infection. These results were agree with (Henry B, *etal*, 2020) and (Cheng L., *etal*, 2020). Who show that severe group had substantially higher serum ferritin levels than non-severe also they
recommend that serum ferritin must be used to monitor COVID-19 patients’ prognosis\(^{(12)}\)\(^{(13)}\).

Serum C-reactive protein showed significantly high levels in patients with severe COVID-19 infection. These results were agree with (Liu F., etal, 2020). Their research looked into the connection between CRP and COVID-19 and discovered that patients with CRP levels greater than (41.8 mg/L) were more likely to develop serious disease\(^{(14)}\).

D-dimer levels showed significantly high levels in patients with severe COVID-19 infection while the in patients with non-severe COVID-19 infection was within normal range. The results were agree with (Li Y., etal, 2020) which show that patients with severe COVID-19 infection had a higher D-dimer than non-severe infection in the patients without cardiovascular disease\(^{(15)}\).

**Conclusion**

1- High Serum ferritin, C reactive protein and whole blood D-dimer levels were important to confirm the severity of the patients with COVID-19 infection.

2- Low serum folic acid level was associated with severe COVID-19 infection.

3- Serum vitamin B12 level was not affected by the severity of the COVID-19 infection.

4- Serum homocysteine level had no role in detection of the severity of COVID-19 infection because it was affected by drugs which were received as a protocol for COVID-19 treatment.

**Recommendation:**

1- A large sample size of patients are needed, follow up of patients until they discharged from the hospital in addition to calculate the mortality rate.

2- Two groups of patients with severe COVID-19 infection, first group receive folic acid tablet and the other group receive placebo then follow up to the patients.

3- Measurement of other parameters in COVID-19 infection such as lactate dehydrogenase, troponin and procalcitonin to detect the severity of infection.

4- Measurement of serum ferritin, C-reactive protein and whole blood D-dimer levels before intake of vaccine.

**Conflict of Interest:** No

**Source of Funding:** Self funded

**Ethical Clearance:** Not Required

**References**


2179.
The Effect of Acute Bronchitis on Blood Pressure

Haider Noori Dawood
Dr. FIBM, FABM, FIBM (Resp.)/ Consultant of Internal Medicine and Respiratory Diseases/Iraq

Abstract

Background: Acute bronchitis is one of the top 10 conditions for which patients seek medical care. Acute bronchitis is a clinical term implying a self-limited inflammation of the large airways of the lung. A possible link between inflammation and elevated blood pressure has been suggested by several cross-sectional and longitudinal studies.

Objective: To assess the effect of acute bronchitis on the blood pressure.

Patients and Methods: The cross sectional study was carried out in Baghdad. 100 patients with acute bronchitis, their age group of 17-76 years were included in the study. Blood pressures (systolic and diastolic) for the patients were taken in sitting position on the right arm during the attack and after improvement (five to seven days later).

Results: 100 patients with acute bronchitis were enrolled in the study. Mean age was 45.51±15.46. Female were 54 (54%), and 46 (46%) were male. There was 42 (42%) have history of hypertension. Only 15 patients (15%) have history of smoking. Mean arterial pressure (MAP) during attack was normal in 58 (58%), and high in 42 (42%). MAP after improvement was normal in 84 (84%), and 16 (16%) remain high. About clinical features, 100% have cough, 71% sputum, 36% dyspnea, and 6% hemoptysis. Systolic, diastolic blood pressure, and mean arterial pressure during the attack were higher than after improvement with highly statistically significance (P value < 0.001). There was no statistically significant effect for the history of hypertension and smoking on blood pressure during the attack and after improvement (p value 0.441, 0.309 respectively).

Conclusion: Systolic, diastolic blood pressure, and mean arterial pressure during the attack of acute bronchitis were higher than after improvement.

Key words: Acute bronchitis, systolic blood pressure, diastolic blood pressure, mean arterial pressure.

Introduction

Acute bronchitis is one of the most common diseases. (1) It affect approximately 5% of adults per year, and although they occur throughout the year, the incidence is higher in the autumn and winter (2). The usual causes of acute bronchitis are viral infections of the upper airways including influenza A and B, parainfluenza, coronavirus (types 1-3), rhinovirus, respiratory syncytial virus, and human metapneumovirus (3). However, reports indicate that more than 60 to 90 percent of patients with acute bronchitis who seek care are given antibiotics (9, 10).

Cough constitutes the most prominent manifestation of acute bronchitis(6). It is a self-limiting upper respiratory infection that takes about two to three weeks to resolve, however, a cough can last to a maximum of 6 weeks (7). Initially, the cough is non-productive, but after about a week there is an increase in mucus production, and in the second week, the colour of the sputum often changes from grey-white to purulent. Despite being a self-limiting condition, most patients with acute bronchitis seek medical advice, mainly because of bothersome cough (8).

Acute bronchitis is thought to reflect an inflammatory response to infections of the epithelium of the bronchi.
Epithelial-cell desquamation and denuding of the airway to the level of the basement membrane in association with the presence of a lymphocytic cellular infiltrate have been demonstrated after influenza a tracheobronchitis. (9)

In patients with acute cough and sputum production suggestive of acute bronchitis, the absence of the following findings reduces the likelihood of pneumonia sufficiently to eliminate the need for a chest radiograph: 1- heart rate > 100 beats/min; 2- respiratory rate > 24 breaths/min; 3- oral body temperature of > 38°C; and 4- chest examination findings of focal consolidation, egophony, or fremitus (10).

High blood pressure is a major public health epidemic in the United States. There are 76.4 million adult Americans with hypertension as defined by a blood pressure “140/90 mm Hg or greater (11). The WHO’s 2016 Global Health Observatory (GHO) data estimated that high blood pressure would cause 7.5 million deaths, about 12.8% of the total of all deaths (12).

Within Europe, high blood pressure is particularly an issue as it has been shown to have an increased prevalence of 60% when compared with the U.S. and Canada – two prominent non-European developed countries (13). Angiotensin converting enzyme (ACE) is an endogenous regulator of blood pressure that is highly expressed in the lung and has been linked to risk for acute respiratory distress syndrome (ARDS) (14).

The mean arterial pressure (MAP) is a term used to describe an average blood pressure in an individual. It is defined as the average arterial pressure during a single cardiac cycle (15). MAP may be used similarly to Systolic blood pressure in monitoring and treating for target blood pressure. Both have been shown advantageous targets for sepsis, trauma, stroke, intracranial bleed, and hypertensive emergencies (16).

Patients and Methods

Setting and study design:

The cross sectional study was carried out in in Baghdad. 100 patients with acute bronchitis (history of cough for two weeks or less with or without sputum, dyspnea, hemoptysis), their age group of 17-76 years were included in the study. From May 2018 to May 2019.

**Inclusion criteria:** 1. Adult patients 2. With clinical features of acute bronchitis

**Exclusion criteria:** 1. Patients complaint of fever as it can cause tachycardia which may increase blood pressure. 2. Heart rate > 100 beats/min; 3. Respiratory rate > 24 breaths/min; 4. Chest examination findings of focal consolidation, because not all patients did chest x ray (10), and 5. Patients who did not come for fellow up.

**Definitions of the outcomes:**

Self-administered questionnaires on age, respiratory symptoms (cough, sputum, dyspnea, and hemoptysis) were performed. History of hypertension, smoking were assessed by self-report for all patients.

Blood pressures (systolic and diastolic) for the patients were taken in sitting position on the right arm, by Mercury Sphygmomanometer.

The patients had two visits, during the attack and after improvement (five to seven days later). With each visit asked about respiratory symptoms, and blood pressure measured.

MAP can be estimated from measurements of the systolic pressure P sys and the diastolic pressure P dias. MAP = P dias + 0.33(P sys – P dias) (18)

**Statistical Analysis**

The statistical analysis of this prospective study performed with the statistical package for social sciences (SPSS) 21.0 and Microsoft Excel 2013. Categorical data formulated as count and percentage. Chi-square test describe the association of these data. Numerical data with normal distribution were described as mean and standard deviation, independent sample t-test used in comparison between two groups. The lower level of accepted statistical significant difference is bellow or equal to 0.05.
**Results**

100 patients with acute bronchitis were enrolled in the study. Mean age was 45.51±15.46. Female patients were 54 (54%), and 46 (46%) were male. There was 42 patients (42%) have history of hypertension, while 58 (58%) have not. 15 patients (15%) have history of smoking, while 85 (85%) have not. Mean arterial pressure (MAP) during attack was normal in 58 (58%), and high in 42 (42%). MAP after improvement was normal in 84 (84%), and high in 16 (16%). As in table 1.

**Table 1: Characteristics of patients**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Bronchitis patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=100</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.51±15.46</td>
</tr>
<tr>
<td>Gender type</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>54 (54%)</td>
</tr>
<tr>
<td>Male</td>
<td>46 (46%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>42 (42%)</td>
</tr>
<tr>
<td>No</td>
<td>58 (58%)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15 (15%)</td>
</tr>
<tr>
<td>No</td>
<td>85 (85%)</td>
</tr>
<tr>
<td>MAP during attack</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>58 (58%)</td>
</tr>
<tr>
<td>High</td>
<td>42 (42%)</td>
</tr>
<tr>
<td>MAP after improvement</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>84 (84%)</td>
</tr>
<tr>
<td>High</td>
<td>16 (16%)</td>
</tr>
</tbody>
</table>

About clinical features of patients, all patients were have history of cough, 71% have sputum, 36% have dyspnea, and 6% have hemoptysis. As in table 2.

**Table 2: Clinical features of patients.**

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Bronchitis patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=100</td>
</tr>
<tr>
<td>Cough</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>100 (100%)</td>
</tr>
<tr>
<td>No</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Sputum</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>71 (71%)</td>
</tr>
<tr>
<td>No</td>
<td>29 (29%)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36 (36%)</td>
</tr>
<tr>
<td>No</td>
<td>64 (64%)</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>No</td>
<td>94 (94%)</td>
</tr>
</tbody>
</table>
For the relation between disease activity and blood pressure, the systolic blood pressure, diastolic blood pressure, and mean arterial pressure during the attack were higher than after improvement with highly statistically significance (P value < 0.001). As in table 3.

<table>
<thead>
<tr>
<th>Table 3: Relation between disease activity and blood pressure.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>during attack</strong></td>
</tr>
<tr>
<td>SBP</td>
</tr>
<tr>
<td>DBP</td>
</tr>
<tr>
<td>MAP</td>
</tr>
</tbody>
</table>

*P value < 0.001 is highly statistically significant

The mean arterial pressure outcome after improvement showed that 55 patient were normal mean arterial pressure during the attack and only 3 patients (5.2%) were higher than after improvement. 29 patient were high mean arterial pressure during the attack and only 13 patients (31%) were normal after improvement, with highly statistically significance (P value < 0.001). As in table 4.

<table>
<thead>
<tr>
<th>Table 4: Mean arterial pressure (MAP) outcome after improvement.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAP</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>After improvement</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

*p value 0.001**

*P value < 0.001 is highly statistically significant

The effect of history of hypertension and smoking on blood pressure outcome, showed no statistically significant effect for the history of hypertension and smoking on blood pressure during the attack and after improvement (p value 0.441, 0.309 respectively). As in table 5

<table>
<thead>
<tr>
<th>Table 5: Relation of history of hypertension and smoking on blood pressure outcome.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outcome</strong></td>
</tr>
<tr>
<td><strong>Improved</strong></td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

In this study patients with history of hypertension was 42%, this was higher than that of Cathleen study (19) in which the overall age-adjusted prevalence of hypertension among persons aged ≥18 years was 29.6%, however in the later study there is race /ethnicity difference which showed that Hispanic blacks had a higher rate of hypertension (41.3%) than non-Hispanic whites (28.6%), so prevalence of hypertension may be different from country to other depend on factors like race, and our study took small number if compared that study.

The history of smoking in this study was 15%, while according to the WHO 2015 (20) data, there was 21.5 % in Iran, 19.9% in United Kingdom, and in United States of America 19.5%, but no data available from Iraq. However again the number in our study is small to detect prevalence.

In this study all patients were have history of cough, 71% have sputum, 36% have dyspnea, and only 6% have hemoptysis. In Boldy study (21) the cardinal symptom was the acute onset of cough (100%), usually productive (90%), wheezing was noted by 62% of patients. Transient bronchial hyper responsiveness appears to be the predominant mechanism of the bothersome cough of acute bronchitis (22). Acute purulent bronchitis is characterized by infection of the bronchial tree with resultant bronchial edema and mucus formation. Because of these changes, patients develop a productive cough and signs of bronchial obstruction, such as wheezing or dyspnea (23).

In this study the systolic blood pressure, diastolic blood pressure, and mean arterial pressure during the attack of acute bronchitis were higher than after improvement with highly statistically significance (P value < 0.001). This may be explained by fact that inflammation of the bronchial epithelium in acute bronchitis, this inflammation causes the bronchial and tracheal mucosa to thicken as well as epithelial-cell desquamation and denuding of the basement membrane airway (9, 24). Inflammatory processes are important participants in the pathophysiology of hypertension and cardiovascular disease (25). Tissue expression and plasma concentrations of inflammatory markers and mediators are increased in patients with cardiovascular disease, those molecules include CRP (26). Furthermore, the CRP level is positively associated with systolic blood pressure, pulse pressure, and hypertension (27).

Other inflammatory mediators are increased in patients with essential hypertension and in experimental models of hypertension include, interleukin (IL)-6,IL-1 (28), tumor necrosis factor-a(TNF-a) (29), monocyte chemotactantprotein-1(MCP-1), intercellular adhesion molecule-1(ICAM-1) and vascular cell adhesion molecule-1(VCAM-1) (26), and have been linked to the activation of the nuclear factor kappa B (NF-kB) system (30). So in both acute bronchitis and hypertension inflammation play important role in the pathogenesis.

There is a strong association between smoking and cardiovascular disease, acute tobacco consumption is only associated with a temporary rise in blood pressure per cigarette consumed – a rise which subsides after 30 minutes. (31). However, chronic tobacco consumption causes arterial stiffness that can persist for years after
smoking cessation \(^{(32)}\). Further, heavy consumers have increased incidence of hypertension \(^{(33)}\). In this study there no statistically significant effect for the history of hypertension and smoking on blood pressure during the attack and after improvement, this indicate that the effect of acute bronchitis on blood pressure is independent from history of hypertension and smoking.

**Conclusion**

During the attack of acute bronchitis blood pressure (systolic, diastolic blood pressure, and mean arterial pressure) may be elevated, and may decreased after improvement of the bronchitis without need for antihypertensive drugs. So just need fellow up the blood pressure during the attack and after improvement.

**Conflict of Interest:** No

**Source of Funding:** Self funded

**Ethical Clearance:** Not Required

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Common complications of Behcet’s Disease in Baghdad Teaching Hospital

Barq Wameedh Azeez¹, Atta AH Al-Sarray², Ali H. Al-Hafidh³

¹Post Graduate, ²Professor Dr. Middle Technical University/Iraq, ³Lecturer Dr. Middle Technical University/Iraq

Abstract

Background: Behcet’s disease is multi-systemic vasculitis, which usually is frequent oral & genital ulcerations as well as eye and skin lesions.

Objectives: To determine the proportion of complications of BD & associated epidemiological variables.

Patients and Methods: A cross-sectional study was conducted and performed in the Baghdad teaching hospital’s Rheumatology unit and Dermatology and Venereology Center, involving (116) patients over the course of six months, beginning November 2nd and ending April 2nd, 2020.

Results: The patients ranged in age from 13 to 64 years old at the time of the study. The mean age and SD was 35.08±10.46 and 70.7% from male while 29.3% from female. Recurrent oral ulceration, Recurrent genital ulceration, Eye lesion, Skin lesion, and Joint symptoms were the most common clinical manifestations of Behcet’s disease, with 96.6 percent, 37.9%, 67.2 percent, 62.1 percent, and 75.9%, respectively. The most common Behcet’s disease complication was ocular complication (34.5 percent), and the first major symptom of BD (94 percent) was oral ulcer. The age at onset of disease at (20-29) years with higher frequency (38.8%).

Conclusion: Higher percentages of study sample had ocular complications (83%) while the lower percentage (2%) had gastrointestinal complication.

Recommendations: More research is required to determine the etiology, enhance diagnosis precision, and develop new therapeutic strategies. To prevent visceral and ocular involvements, early diagnosis and treatment with the help of a dermatologist with extensive experience is essential.

Keywords: Behcet’s Disease, common complications, Iraq, Baghdad

Introduction

The disease is defined as a chronic, relapsing, multisystemic idiopathic inflammatory problem characterized by an episodic occlusive retinal vasculitis with no specific treatment¹. This ubiquitous disorder exhibits a distinct geographic variation and is endemically higher particularly in Turkey, Iraq, Iran, Korea and Japan, the population derived historically from the ancient Silk Road that was used for centuries as a trade-making passage from the East to the West (²). Behcet’s disease is an inflammatory multisystem disease of unknown etiology with unpredictable exacerbations and remissions. The disease was first described in 1937 by the Turkish dermatologist Hulusi Behcet as a trisymptom complex, characterized by recurrent oral ulcers, genital ulcers and uveitis (³).

Behçet’s disease usually starts around the third or fourth decade of life (⁴). Epidemiological surveys suggest that sex distribution is roughly equal. However, there are some exceptions. BD shows male predominance in some Middle Eastern and the Mediterranean countries, and female predominance in Japan and Korea (⁵).

There is no standard test for diagnosing of Behçet’s disease, there are multiple criteria sets in use up until 1990. The first evidence-based criteria for Behçet’s
disease were introduced by the International Study Group (ISG) in 1990. In most cases, a combination of local and systemic therapy is used. Immunosuppressants, corticosteroids, and colchicine are some of the medications used.

**Objectives of the Study**

To determine the proportion of complications of BD & associated epidemiological variables.

**Patients & Methods**

**Study Design**: A cross sectional study.

**duration of the study**: The data collection continued for the period of 6 months starting on 2nd of November 2019 ending to 2nd of April 2020.

**Place of Study**: The place of this study was performed in the Rheumatology unit & Dermatology and Venereology Center in Baghdad teaching hospital.

**Inclusion and Exclusion criteria of study:**

**Inclusion criteria**: All patients with BD attending to Baghdad teaching hospital in during the period of study

**Exclusion Criteria**: Patients with Behcet’s disease who visit the Rheumatology unit or Dermatology and Venereology Center in other hospitals in Baghdad and other governorates, as well as patients who visit other hospitals in Baghdad or other governorates.

**3.11 Statistical data analysis**

Analysis of data was carried out using the available statistical package of SPSS-25 (Statistical Packages for Social Sciences- version 25). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of difference for different percentages (qualitative data) were tested using Pearson Chi-square test (c²-test). Statistical significance was considered whenever the P value was equal or less than 0.05.

**Results**

**Table 1**: The higher percentages of patients (96.6%) in both genders have recurrent oral ulceration while the lower percentages (3.4%) had gastrointestinal lesions and the P value of gender with clinical manifestations show not significant (P value >0.05) except gender with recurrent genital ulceration which find significant (p value = 0.04).

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Recurrent oral ulceration</td>
<td>Yes</td>
<td>78</td>
<td>95.1</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>4</td>
<td>4.9</td>
<td>-</td>
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<tr>
<td>Recurrent genital ulceration</td>
<td>Yes</td>
<td>36</td>
<td>43.9</td>
<td>8</td>
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<tr>
<td></td>
<td>No</td>
<td>46</td>
<td>56.1</td>
<td>26</td>
</tr>
<tr>
<td>Eye lesion</td>
<td>Yes</td>
<td>56</td>
<td>68.3</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>26</td>
<td>31.7</td>
<td>12</td>
</tr>
<tr>
<td>Skin lesion</td>
<td>Yes</td>
<td>55</td>
<td>67.1</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>27</td>
<td>32.9</td>
<td>17</td>
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</tbody>
</table>
### Cont... Table 1: Clinical manifestations of BD with gender

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Joint symptoms</td>
<td>26</td>
<td>31.7</td>
<td>14</td>
<td>41.2</td>
</tr>
<tr>
<td>Yes</td>
<td>56</td>
<td>68.3</td>
<td>20</td>
<td>58.8</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epididemyitis</td>
<td>4</td>
<td>4.9</td>
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<td>2.9</td>
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<td>Yes</td>
<td>78</td>
<td>95.1</td>
<td>33</td>
<td>97.1</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal lesions</td>
<td>2</td>
<td>2.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>80</td>
<td>97.6</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central nervous symptoms</td>
<td>1</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>81</td>
<td>98.8</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular lesions</td>
<td>116</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Distribution of the complications of BD patients according to gender

<table>
<thead>
<tr>
<th>Complications:</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Ocular</td>
<td>26</td>
<td>31.7</td>
<td>14</td>
<td>41.2</td>
</tr>
<tr>
<td>Yes</td>
<td>56</td>
<td>68.3</td>
<td>20</td>
<td>58.8</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurological</td>
<td>4</td>
<td>4.9</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Yes</td>
<td>78</td>
<td>95.1</td>
<td>33</td>
<td>97.1</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular</td>
<td>2</td>
<td>2.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>80</td>
<td>97.6</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIT</td>
<td>1</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>81</td>
<td>98.8</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Regarding the complications with gender, the higher percentages of patients (34.5%) with ocular complications and the P value not significance (P value >0.05).
**Figure 1**: Complications of BD

*Figure 1*: show The higher percentages of patients (83%) with ocular complications while the lower percentages (2%) had gastrointestinal complications.

*Table 3*: shows the family history of BD and complications, most cases of all complications (ocular, neurological, vascular, GIT) don’t have family history of BD and the higher percentage (35.0%, 4.9%, 1.9%, 1%) respectively of the patients and the association statistically was found to be non-significant (P>0.05).

*Table 3*: Relationships between the complications of BD and family history of BD.

<table>
<thead>
<tr>
<th>Complications</th>
<th>Family history of Behcet’s disease</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Ocular</td>
<td>No</td>
<td>30.8</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>69.2</td>
<td>65.0</td>
</tr>
<tr>
<td>Neurological</td>
<td>Yes</td>
<td>6</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>100.0</td>
<td>95.1</td>
</tr>
<tr>
<td>Vascular</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>100.0</td>
<td>98.1</td>
</tr>
<tr>
<td>GIT</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>100.0</td>
<td>99.0</td>
</tr>
</tbody>
</table>
Table 4: show the family history of immune mediated disease and complications, most cases complications (ocular, neurological) have family history of immune mediated disease and the higher percentage (35.3%, 5.9%) respectively of the study sample and the association statistically was found to be non-significant (P>0.05).

Table 4: Distribution between the complications of BD and family history of immune mediated disease.

<table>
<thead>
<tr>
<th>Complications</th>
<th>Family history of immune mediated disease</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Ocular</td>
<td>Yes</td>
<td>6</td>
<td>35.3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>11</td>
<td>64.7</td>
</tr>
<tr>
<td>Neurological</td>
<td>Yes</td>
<td>1</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>16</td>
<td>94.1</td>
</tr>
<tr>
<td>Vascular</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>17</td>
<td>100.0</td>
</tr>
<tr>
<td>GIT</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>17</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Discussion

Regarding the clinical features of patients, this result show a high percentage of patients with oral ulcers (96.6%). This result agree with the results of those published studies (82.1% by (8) in oman; 96.3% by (9) in china; 91.2% by (10) in japan; 100% by (11) in magnolia; 99% by (12) in korea). Regarding the relationship between gender and oral ulcer. this study which finds that females had oral ulcers more than males. this result similar with results of those published studies (13) in Russia; (9) in china & (10) in japan. This study show (37.9%) of patients with recurrent genital ulceration. this result similar with the result Omani study (8) which shows (40%) of patients with genital ulceration and also similar with another study conducted in Iran (14) that find (47.4%) of patients with genital ulcer. Another study in china (9) that results show differ from this study which finds that (71.2%) of patients had a genital ulcer. The explanation of This difference were probably due to differences in geographic regions, genetics or diagnostic criteria. Regarding the relationship between gender and genital ulcer. This study finds that males had genital ulcers more than females. This result agree with the results of those published studies (13) in Russia; (15) in Finland). Another study in china (9) differ from this study which found that female more than genital ulcer than male. The explanation of This difference were probably due to that the difference in hormonal balance influences the development of clinical manifestation of BD with gender like genital ulcer. Some previous studies conducted in turkey (16; 17) have reported the possible influence of sex hormones such as testosterone and androgen on BD. Regarding the ocular lesions in this study demonstrated
(62.1%) of patients with ocular manifestations. This result similar with results of those published studies (57.1% by \(^8\) in Oman ; 58.1% by \(^{18}\) in Iran ; 54% by \(^{13}\) in Russia ; 63.1% by \(^{11}\) in magnolia). This study show relationships between ocular lesion with gender. This result find that male had more than female , this result similar to the results of those reported studies (\(^{13}\) in Russia ; \(^9\) in china ; \(^{10}\) in japan ; \(^{19}\) in Greek ; \(^{11}\) in magnolia). Regarding the cutaneous manifestation, this study shows (62.1%) of patients with skin lesions. This result agree with the results of those published studies (63.1% by \(^{20}\) in china ; 64.4% by \(^{18}\) in Iran ; 71.7 % by \(^{21}\) in brazil). Regarding the cutaneous lesion with gender, this study show the male more than female with a skin lesion. this result similar to the results of those reported studies (\(^{13}\) in Russia ; \(^{21}\) in brazil & \(^9\) in china). This study found (75.9%) of patients with joint symptoms. These result agree with a results of another reported studies ( 63.2% by \(^{12}\) in korea ; 87.3% by \(^{22}\) in southeast America ; 72.4 % by \(^{23}\) in Serbia ; 79.5 % by \(^{24}\) in turkey ). This result disagree with Another results of those reported studies (4.1% by \(^{25}\) in Iran ; 28.4% by \(^{20}\) in china ). The probably explanation of this difference that a high frequency of joint involvement might be speculated, because they used the questionnaire to obtain the information regarding joint symptoms, and they also employed a broad concept, “arthropathy”, rather than inflammatory arthritis. In addition, the reason why there is hesitation to use arthritis among the primary criteria, to us, may be because complaints about joints in society are very common, and therefore, investigators hesitate to consider these complaints and findings among the symptoms of the illness.

Regarding the joint manifestation with gender, this study shows the female more than male had joint symptoms. this result agree with the results of those published studies (\(^{26}\) in Korea ; \(^{21}\) in brazil ; \(^{24}\) in turkey ). This study show (3.4%) of patients with a gastrointestinal lesion. This result similar with the results of those published studies ( 3.8% by \(^{26}\) in Korea ; 7.1% by \(^{27}\) in turkey ; 3.3 % by \(^{20}\) in china ; 7% by \(^{18}\) in Iran. Regarding the neurological involvement, this study shows (6.9%) of patients with neurological symptoms. This result agree with the results of those reported studies (6.1 % by \(^{28}\) in turkey ; 9.6% by \(^{20}\) in china ; 10.6 % by \(^{18}\) in Iran ; 3% by \(^{14}\) in Iran ; 10.2% by \(^{29}\) in turkey ; 7.8% by \(^{30}\) in turkey). This study show (6.9%) of patients with a vascular lesion. This result agree with the results of those published studies ( 9.4% by \(^{10}\) in japan ; 6% by \(^{31}\) in japan ; 9.1 % by \(^{18}\) in Iran ; 8% by \(^{32}\) in japan ). The complications of Behcet’s disease in this study in general according to the clinical manifestations of patients that find the higher percentages of is an ocular complication (34.5%) of the complicated cases. There was no previous study on the general complications of Behcet’s disease in Iraq. This study show the relationships of complications with ( family history of BD and family history of immune mediated disease ) and the associations between them show non significance (P value >0.05). There was no previous study on the complications of BD with family history of BD & immune mediated disease in Iraq.

**Conclusion**

Higher percentages of study sample had ocular complications (83%) while the lower percentage (2%) had gastrointestinal complication.

**Recommendations:** More research is required to determine the etiology, enhance diagnosis precision, and develop new therapeutic strategies. To prevent visceral and ocular involvements, early diagnosis and treatment with the help of a dermatologist with extensive experience is essential.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**


Clinical Manifestations with Different Treatment Protocols for Iraqi Patients with Wilson’s Disease

Fadwa Ghassan Hameed¹, Inam Sameh², Mohamed Mahmood³, Hala Sameh⁴

¹Post graduate, Department of Pharmacy, Al-Kitab University, Kirkuk, Iraq, ²Ass.prof., Department of Pharmacology and Toxicology, College of Pharmacy, University of Al-Mustanseriah, Baghdad, Iraq, ³Ass.Prof. Department of Clinical Pharmacy, College of Pharmacy, University of Al-Mustanseriah, Baghdad, Iraq, ⁴Prof. Department of Pediatrics, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Wilson disease (WD) is an autosomal-recessive disorder of copper metabolism caused by mutations in the ATP7B gene. ATP7B is also essential for biliary excretion of copper when cytoplasmic levels are high. Dysfunction of ATP7B therefore leads to accumulation of copper in the liver giving rise to cellular damage and disease, and the release of non-ceruloplasmin bound copper into the systemic circulation. Clinical presentation of Wilson disease can vary widely; therefore diagnosis is not always straightforward. Wilson disease is not just a disease of children and young adults, but may present at any age. The key features of Wilson disease are liver disease and cirrhosis, neuropsychiatric disturbances, Kayser–Fleischer rings, and acute episodes of hemolysis, often in association with acute liver failure. Diagnosis is particularly difficult in children and in adults presenting with active liver disease. None of the available laboratory tests is perfect and may not be specific for Wilson disease. To overcome the diagnostic challenge, several clinical signs (Kayser–Fleischer rings(KF.), neurologic symptoms) and laboratory features (copper in serum, urine, liver; serum ceruloplasmin; genetic testing) are scored 0 (absent) to 2 (present) and the Leipzig score is calculated. If the score is >4, the diagnosis of Wilson disease is very likely. For asymptomatic siblings of index patients, mutation analysis is the most reliable approach.

Keywords: WD.(Wilson disease), ATP7B gene, Kayser–Fleischer rings(KF); toxicity; Iraqi patients

Introduction

Wilson disease (WD) is an autosomal-recessive disorder of copper metabolism caused by mutations in the ATP7B gene. It presents in childhood, adolescence or adulthood with a wide range of clinical manifestations. The disease prevalence has previously been estimated as 1 in 30,000 (3,4), but some recent analyses have suggested a genetic prevalence of 1 in 7,000 (5,6).

Copper is absorbed from the stomach and duodenum, taken up by the liver, and secreted by the liver into the systemic circulation bound to ceruloplasmin. ATP7B transports copper through the trans-Golgi network in hepatocytes before it is incorporated into apoceruloplasmin which is secreted as holoceruloplasmin. ATP7B is also essential for biliary excretion of copper when cytoplasmic levels are high. Dysfunction of ATP7B therefore leads to accumulation of copper in the liver giving rise to cellular damage and disease, and the release of non-ceruloplasmin bound copper into the systemic circulation.

Copper also accumulates and is associated with cellular damage and disease in other organs, most notably the brain. The extent to which disease in the brain relates to high levels of free circulating copper and/or underlying dysfunction in neurons, which also express ATP7B, is not clear.

Originally referring to this condition as progressive lenticular degeneration, Samuel Alexander Kinnier Wilson first described the combination of neurologic disease with cirrhosis in 1912. He recognised that psychiatric manifestations were common but stated that the cirrhosis was rarely symptomatic during life.
Barnes and Hurst subsequently reported in 1925 that WD can present with symptomatic liver disease in the absence of neurologic features \(^{(11)}\). It is now accepted that symptomatic involvement of the liver or brain can occur in isolation or in combination at presentation. Asymptomatic, or pre-symptomatic, liver and brain disease in siblings can be identified and studied through family screening \(^{(12)}\).

A working party at the 8th International Meeting on WD in Leipzig in 2001 revised the phenotypic classification and differentiated cases into neurologic (N), hepatic (H) or other (O) presentations \(^{(14)}\). Any patients in whom neurologic and/or psychiatric symptoms are present at the time of diagnosis are classified as a neurologic presentation. They are then subdivided into those with (N1) or without (N2) symptomatic liver disease, or not investigated for liver disease (Nx). This classification requires a detailed neurologic examination to exclude neurologic symptoms at diagnosis and a liver biopsy to confirm the absence of marked liver disease. Hepatic presentations are subdivided into acute (H1) or chronic (H2) depending on the presence of acute jaundice due to hepatitis and/or hemolysis in a previously healthy subject (H1), or any type of chronic liver disease, with or without symptoms (H2). The presence of any biochemical evidence of liver disease indicates a hepatic (H), as opposed to other (O), presentation.

The relative frequency of neurologic and hepatic presentations has been examined in several large cohorts over the last three decades. The proportion of patients that would, under the 2001 classification, be referred to as neurologic, either N1 or N2, ranges from 37% to 80% \(^{(13)}\). While there is likely to be some variation in phenotype between individual populations, comparing these cohorts may be problematic for other reasons.

Firstly, selection bias may affect the relative frequency of different presentations in individual cohorts; centres of excellence for neurology are likely to report a higher number of cases with neurologic involvement. Secondly, neurologic and hepatic features may be subtle or identifiable only through specific investigations. The classification of presentations may therefore have been inconsistent in some cohorts, especially before the introduction of rating scales for WD such as the Unified Wilson’s Disease Rating Scale in 2008 and the Global Assessment Scale in 2009 \(^{(15,16)}\).

The majority of neurologic presentations consist of a movement disorder associated with bulbar symptoms. The movement disorder is usually characterized by tremor, dystonia or parkinsonism. These ‘core’ movement disorders often occur in combination and may initially be subtle. Bulbar symptoms consist of dysarthria, drooling and/or dysphagia. There are a range of additional neurologic features, including cerebellar dysfunction, chorea, hyperreflexia, seizures and cognitive impairment, which can also co-exist.

**Material and Methods**

A total of 42 Iraqi patients diagnosed with Wilson disease (20 males and 22 female) with age range between 10 – 20 years, who attended the Rare Disease clinic of the Al-Imaamin AL-Kadhmiyan medical city Hospital – Baghdad/Iraq. The diagnosis of WD was established by clinical features, low serum ceruloplasmin and copper and increased 24 h urinary copper excretion, Liver function tests.

**Inclusion Criteria**

- Patients age from 10-20 years old.
- Asymptomatic patients with WD with hepatic involvement diagnosed by screening method.
- Patients referred for unexplained elevation of liver enzymes and have Leipzig scale score.
- Patients with positive family history of Wilson disease.

**Exclusion Criteria**

- Patient >20 years old.
- Patients with other co-moribids.
- Pregnancy and breast feeding.
- Patients allergic to any of the study medications.
The eligible 42 patients were allocated into three groups:

**Group (1)**; include 23 asymptomatic Iraqi patients with WD and hepatic involvement diagnosed by screening method treated with Dietary Supplement: **Zinc acetate** (100-150 mg/d) in 2-3 divided dose for 90 days.

Blood tests will be performed (before and after the treatment): ALT, AST, ALP, Ca., s.Albumine, Total Albumin, PT, IRN, s.Copper, s.Ceruplasmine, 24-hr. urine copper.

**Group (2)**; included 10 Iraqi Patients referred for unexplained elevation of liver enzymes and (+ve) Leipzig scale score treated with **d-PCA** (20mg/kg/d for Ped., 750-1500 mg for adult) for 90 days.

Blood tests will be performed (before and after the treatment): ALT, AST, ALP, s.Albumine, Total Albumin, PT, IRN, s.Copper, s.Ceruplasmine, 24-hr. urine copper.

**Group (3)**; included 9 Iraqi patients referred for unexplained elevation of liver enzymes and (+ve) Leipzig scale score treated with **Trientine** (20mg/kg/d for Ped., 1500-2500 mg for adult) for 90 days.

Blood tests will be performed (before and after the treatment): ALT, AST, ALP, s.Albumine, Total Albumin, PT, IRN, s.Copper, s.Ceruplasmine, 24-hr. urine copper.

**Results**

Patients in this study were classified according to clinical manifestations into the following groups: pre-clinical (pre symptomatic, identified by family screening), hepatic manifestations (H1: acute hepatic WD; H2: chronic hepatic WD) and neurological manifestations (N1: associated with liver disease; N2: not associated with WD) as mentioned in table (1). No significant p.value were identified between these three groups.

<table>
<thead>
<tr>
<th>Table (1) Clinical manifestations between the study groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D-Penicillamine)</td>
</tr>
<tr>
<td>PRE-CLINICAL</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Hepatic Manifestation</td>
</tr>
<tr>
<td>- H1: Acute Hepatic</td>
</tr>
<tr>
<td>- H2: Chronic Hepatic</td>
</tr>
<tr>
<td>Neurological Manifestation:</td>
</tr>
<tr>
<td>- N1: Associated with liver disease</td>
</tr>
<tr>
<td>- N2: Not associated with liver disease</td>
</tr>
</tbody>
</table>

NS=Non-significant, * significant at p value ≤ 0.05, ** significant at p value ≤ 0.01
Discussion

Patients in this study were classified according to clinical manifestations into the following groups: pre-clinical (pre symptomatic, identified by family screening), hepatic manifestations (H1: acute hepatic WD; H2: chronic hepatic WD) and neurological manifestations (N1: associated with liver disease; N2: not associated with WD). The clinical presentations of patients with WD can involve multiple organ systems and vary from subtle to life-threatening. Diagnostic testing for WD is essential for patients of any age with unexplained hepatic, neurologic, or psychiatric abnormalities. Diagnostic testing algorithms for WD, the Leipzig scoring system because of its quantitative, systematic nature (17). Pre symptomatic patients identified through genetic screening: Chelators and zinc have been proven effective in preventing development of symptoms or laboratory test abnormalities of WD in pre symptomatic patients identified by testing for WD in a pro band’s first-degree relatives. Zinc is preferred treatment, based on its low cost and minimal adverse event profile (18). Patients with hepatic manifestations: WD patients with acute liver failure, notably those with classic Wilsonian acute liver failure, do not spontaneously recover and require urgent liver transplantation for survival (despite occasional single patient reports to the contrary) (19,20-22). These patients must be transferred to a transplant center for management. In contrast, WD patients with or without evidence of advanced hepatic WD should be treated with a chelating agent (23). Such patients typically respond to therapy in the first 2-6 months, and progressive improvement can continue for up to 12 months. Responses often include improvement of complications of portal hypertension in cirrhotic patients. Thus, whenever possible, patients with decompensated cirrhosis should be observed for evidence of a therapeutic response to chelation prior to performing liver transplantation (22). Patients with neurologic signs and symptoms: The initial goal of chelation or zinc therapies is elimination of excessive total body copper causing end-organ dysfunction. The subsequent goal is to prevent re accumulation of toxic concentrations of total body copper using maintenance therapy. Rapid removal of excessive copper from the central nervous system must be avoided, as it can worsen symptoms and result in irreversible neurologic deficits (18). In contrast, gradual removal of copper from the central nervous system, achieved by using lower doses of D-penicillamine or Trientine, has a higher probability of resolving signs and symptoms long term. Patience in using lower doses and de coppering slowly is key. Improvement in neurologic symptoms is a slow process and may require up to 3 years after starting chelation therapy. Unexpectedly, zinc therapy has also been implicated in the worsening of neurologic WD. A recent series reported neurologic worsening in 9.1% of patients treated with D-penicillamine, 8.8% on Trientine, and 7.3% on zinc salts (19). Approximately 10% of WD patients treated with either D-penicillamine or zinc had neurologic worsening during the first 6 months of therapy (24). Since liver transplantation rapidly restores biliary copper excretion in WD, it also markedly accelerates depletion of total body copper. Thus, liver transplantation is relatively contraindicated for active neurologic WD prior to removal of substantial amounts of brain copper. In selected patients with neurologic WD, liver transplantation has reversed the neurologic deficits (25).

Conclusion

The WD clinical phenotype includes hepatic, neurologic, and psychiatric manifestations that can present with a wide range of severity and can be combined in unpredictable ways. Copper accumulation in WD is due to a genetic defect affecting the hepatic copper transporter ATP7B, the Wilson ATPase, but clinical and experimental evidence indicates that genetic mutations are just one component of the pathogenesis and clinical presentation of WD. Specifically, two types of evidence indicate there are likely other factors that can affect disease presentation: first, the lack of convincing genotype-phenotype correlation; second, several case reports indicating that homozygous twins with the same ATP7B mutation can present with different phenotypes. Diagnostic tests include serum ceruloplasmin, neurologic exam, and slit-lamp examination for Kayser Fleischer rings. However, additional testing for basal 24-hour urinary copper excretion, hepatic copper concentration...
or sequencing of the ATP7B gene may be required for diagnosis. Family screening is recommended for first-degree relatives of patients and may detect the disease prior to the appearance of signs or symptoms. With improved methods of diagnosis, WD has become an important disease in childhood. The hepatic manifestations are varied, and the neuropsychiatric manifestations may be subtle or nonspecific. Treatment needs to be individualized; comprehensive follow-up is important for all patients. Scoring systems may assist with diagnosis or clinical decisions relating to need for liver transplantation.

Clinical presentation may be chronic liver disease, acute liver failure with distinctive features (ALF-WD) or it may be “silent” liver disease. A broad spectrum of neurologic or psychiatric conditions can occur in childhood or adolescence. While hepatic presentation is more common than neurologic in the pediatric population, manifestations of WD are characteristically multi systemic. WD can resemble autoimmune liver disease clinically. Particularly when there is inadequate response to immunosuppression therapy in autoimmune hepatitis, every effort must be made to ensure that a diagnosis of WD is not being missed. Current management includes chelators like D-penicillamine or trientine; zinc has multiple effects exclusive of chelation. As an oral chelator, D-penicillamine is specified as a first-line drug for use in symptomatic WD patients according to recommendations published by the European Association for the Study of Liver (EASL) (Grade II-1, B, 1) and the American Association for the Study of Liver Diseases (Class I, Level B).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**

15. Leinweber B, Moller JC, Scherag A, et al. Evaluation of the Unified Wilson’s Disease Rating Scale (UWDRS) in German patients with treated


Prevalence of Drug-Drug Interaction in Hospitalized Patient in Basrah City; Southern of Iraq

Zainab Najim Abdul-nabi¹, Noor Kadhim Mohammed-Jawad², Jubran K. Hassan³
¹Assistant Lecturer in Pharmacology, College of Pharmacy, University of Basrah, Iraq, ²Assistant Lecturer; ³Assistant Professor in Clinical Pharmacy, College of Pharmacy, University of Basrah, Iraq

Abstract

Objectives: Although multiple drugs administration mostly increase therapeutic effect, some combinations lead to adverse drug-drug interactions and increased morbidity. This study was designed to identify the types, frequency, severity, and significance of drug–drug interactions (DDIs)

Methods: This retrospective cross-sectional study was conducted from September 2018 to February 2019 in Al-Fayha’a teaching hospital in Basrah, Iraq. The data of 186 patients were collected from hospital patients case sheets. The type and significance of DDIs were analyzed using “Medscape drug Interaction Checker.

Results: At least one to two DDI are noticed in about three quarters of the patients, about 30% cases have three to nine DDIs and 15% of them have ten or more DDIs. According to their severity, there are 85 (11.5%) of serious or potent DDIs. The largest percentage of reported interactions 544 (73.5%) were moderate were close monitoring required. Out of 740 documented DDIs, 65.1% were pharmacodynamics and 19.5% were Pharmacokinetic interactions, in addition, there were 15.4% of DDIs due to Unknown mechanisms. Most of the major potential DDIs occur with the antibiotic ceftriaxone and blood thinning medications (heparin and warfarin).

Conclusion: The findings of this study revealed a high prevalence of drug-drug interactions in hospitalized patients particularly in patients with cardiovascular disease. Potential DDIs in this study sufficiently high to alert health care providers to pay more attentions in order to prevent or decrease their adverse effects on patients.

Keywords: Drug interactions; pharmacokinetics drug interactions, pharmacodynamic drug interaction

Introduction

Drug-drug interactions (DDIs) widely occur in hospitalized patient especially in patients use long list of medications. A drug interaction occurs when the pharmacological effects of the one medication alters the intensity of the other concomitant drug. When two or more medications are taken together, there is a chance of an interaction among the drugs that could be manifested as an increase or decrease in the therapeutic effects or lead to serious unwanted effects which may change the clinical outcome of the patients [1]. Polypharmacy and increased age are significant risk factor for these interactions [2,3]. DDIs generally classified as pharmacodynamic and pharmacokinetic interactions that sub-classified according to mechanism of interactions into absorption, distribution, metabolism and elimination [4]. Potential DDIs are one of the preventable mechanisms of adverse drug events and health damage [5]. Frequency of potential DDIs markedly increase in prescriptions for hospitalized patients [6]. The clinical outcome of a possible DDIs is usually unknown [7]. Studies show that the patients may expose to one or more major or moderate DDIs during hospitalization especially in internal medicine wards and the probability these interactions increased when use more than 6 drug items [8-10]. Although DDIs are common in the hospitalized patients, but there are few data reporting these interactions clinically. It is difficult to remember all the known important DDIs.
However, knowledge of the vital types of medications that are more likely to be involved will be useful alert while prescribing [11]. Predicted theoretical drug-drug interactions may not lead to noticeable toxicity or therapeutic failure, therefore clinical intervention do not always needed. However, any clinically significant DDIs should be identified and discussed with health care team and kept under monitoring. Therefore clinicians should have a sufficient knowledge about the potential drug interactions. Product monographs, info graphics, health information technologies and drug interactions software programs would help in alerting healthcare providers about the possible DDIs [12]. In recent years, many programs have been developed to detect potential DDIs. Numerous online drug interactions databases are available which is either free such as “Drugs.com” and “medscape.com” or copyrighted databases (e.g., Micromedex) [13]. The use of these applications could enhance patient safety by minimizing the incidence of DDIs, and also help in educating trainers [14]. In this study DDIs were checked using Medscape free online application (https://reference.medscape.com/drug-interactionchecker).

Materials and Method

The present study held in Al-Fayha’a teaching hospital based in Basrah, southern of Iraq. This hospital provides medical services for large number of population in Basrah city and receive both in- and outpatients from all age groups. The study designs as retrospective cross-sectional study was conducted from September 2018 to February 2019. This study was approved by the ethical committee of the college of pharmacy/ university of Basrah. Patients admitted with different diagnosis were included in this study. Hospital permission was obtained to access the patient’s medical files during hospital stay for research purpose. Medications prescribed during the hospital admission were allocated from medical reports. Patient’s age, sex, length of hospitalization period, causes of admission, morbidities and associated comorbidities, and details of medication therapy collected from hospital patients case sheets. The type and significance of DDIs were evaluated using “Medscape Drug Interaction Checker”. It grades DDIs into three categories: minor (no change required), moderate (monitor closely), and potent DDIs (use alternative). Data analysis performed by Medcalc® software v12.

Results

Demographic Data

Demographic characteristics had shown in Table 1. A total of 186 patients’ case sheets were studied. The mean age of the patients was 56.4 ± 19 years, most patients were in age range of 60-80 years. With 51% being males. Most patients were received more than 3 medications. The length of stay was at least one week for most patients, few percentage stay more than seven days. Table 1 also showed the medical conditions and comorbidity of the patients included in the study.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Frequency: N (%)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>56.4 ± 19</td>
<td></td>
</tr>
<tr>
<td>Age range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>7(3.8%)</td>
<td></td>
</tr>
<tr>
<td>20-40</td>
<td>24(12.9%)</td>
<td></td>
</tr>
<tr>
<td>40-60</td>
<td>59(31.7%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>60-80</td>
<td>77(41.4%)</td>
<td></td>
</tr>
<tr>
<td>&gt;80</td>
<td>19(10.2%)</td>
<td></td>
</tr>
</tbody>
</table>
Cont... Table 1: Demographic data of patients included in the study

<table>
<thead>
<tr>
<th>Gender</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>95 (51.1%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Female</td>
<td>91 (48.9%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No of comorbidities</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No comorbidity</td>
<td>68 (36.6%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>57 (30.6%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>42 (22.6%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15 (8.1%)</td>
<td></td>
</tr>
<tr>
<td>&gt;3</td>
<td>4 (2.2%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Length of hospital Stay (days)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 3</td>
<td>62 (33.3%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3-7</td>
<td>111 (59.7%)</td>
<td></td>
</tr>
<tr>
<td>≥7</td>
<td>13 (7%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of prescribed Medications</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 3</td>
<td>9 (4.8%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3-6</td>
<td>82 (44.1%)</td>
<td></td>
</tr>
<tr>
<td>6-10</td>
<td>73 (39.2%)</td>
<td></td>
</tr>
<tr>
<td>≥ 10</td>
<td>22 (11.8%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hospital ward used</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>180 (96.8%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>4 (2.2%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 (1.1%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of morbidities</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular system</td>
<td>93 (50%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes &amp; endocrine (other than Diabetes)</td>
<td>11 (5.9%)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary system</td>
<td>29 (15.6%)</td>
<td></td>
</tr>
<tr>
<td>Gastro-intestinal tract</td>
<td>24 (12.9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hepatic &amp; biliary</td>
<td>8 (4.3%)</td>
<td></td>
</tr>
<tr>
<td>Renal system</td>
<td>7 (3.8%)</td>
<td></td>
</tr>
<tr>
<td>Hematology</td>
<td>5 (2.7%)</td>
<td></td>
</tr>
<tr>
<td>Obstetrics &amp; gynecology</td>
<td>3 (1.6%)</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>6 (3.2%)</td>
<td></td>
</tr>
</tbody>
</table>
Types of drug-drug interactions (DDIs)

Details of the DDIs were reported in Table 2. At least one to two DDI are noticed in about three quarters of the patients. About 30% cases have three to nine DDIs and 15% of them have ten or more DDIs. According to their severity, there are 85 (11.5%) of serious or potent DDIs. The largest percentage of reported interactions 544 (73.5%) were moderate were close monitoring required.

The documented DDIs were mostly pharmacodynamics 482 (65.1%) (increase or decrease effects of medications). Pharmacokinetic interaction about 144 (19.5%) with most of them were due to change in metabolism (enzyme induction or inhibition). In addition, there were 114 (15.4%) of DDIs due to Unknown mechanisms.

Table 2: Documentation, severity and type of drug interaction reported

<table>
<thead>
<tr>
<th>Possible Drug -drug interactions /patient n=186</th>
<th>Frequency</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>48(25.8%)</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>45(24.2%)</td>
<td></td>
</tr>
<tr>
<td>3-5</td>
<td>38(20.4%)</td>
<td>&lt;0.0428</td>
</tr>
<tr>
<td>6-9</td>
<td>27(14.5%)</td>
<td></td>
</tr>
<tr>
<td>≥10</td>
<td>28(15.1%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Types of DDIs according to their potencyn=740</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor</td>
<td>111 (15%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>544 (73.5%)</td>
</tr>
<tr>
<td>Serious</td>
<td>85 (11.5%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of interaction according to mechanism n=740</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown mechanism</td>
<td>114 (15.4%)</td>
</tr>
<tr>
<td>Pharmacodynamics</td>
<td>482 (65.1%)</td>
</tr>
<tr>
<td>Pharmacokinetic interaction</td>
<td>144(19.5%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Types of Pharmacokinetic interactions n=144</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Absorption</td>
<td>33 (4.5%)</td>
</tr>
<tr>
<td>2-Metabolism</td>
<td>86 (11.6%)</td>
</tr>
<tr>
<td>3-Elimination</td>
<td>25 (3.4%)</td>
</tr>
</tbody>
</table>
Table 3: Ratios of prescribed medication, DDI per system case and DDI per prescribed medication

<table>
<thead>
<tr>
<th>System</th>
<th>Cases</th>
<th>Ratio of Prescribed medications / system case</th>
<th>ratio of DDI / system case</th>
<th>DDI / Prescribed medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular system</td>
<td>93</td>
<td>7</td>
<td>5.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Diabetes &amp; endocrine (other than Diabetes)</td>
<td>11</td>
<td>5.8</td>
<td>3.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Pulmonary system</td>
<td>29</td>
<td>7.2</td>
<td>4</td>
<td>0.6</td>
</tr>
<tr>
<td>Gastro-intestinal tract</td>
<td>24</td>
<td>3.5</td>
<td>1.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Hepatic &amp; biliary</td>
<td>8</td>
<td>4.3</td>
<td>2.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Renal system</td>
<td>7</td>
<td>3.3</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Hematology</td>
<td>5</td>
<td>6</td>
<td>3.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Obstetrics &amp; gynecology</td>
<td>3</td>
<td>4</td>
<td>1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>6</td>
<td>5.2</td>
<td>4.2</td>
<td>0.8</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.0845</td>
<td>0.0464</td>
<td>0.5632</td>
</tr>
</tbody>
</table>

Data analyzed using t test

Table 4: Associated comorbidities that recorded in the study

<table>
<thead>
<tr>
<th>System</th>
<th>No. of comorbidities</th>
<th>medication / comorbidity</th>
<th>interaction / comorbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular system</td>
<td>133</td>
<td>2.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Diabetes &amp; endocrine (other than Diabetes)</td>
<td>13</td>
<td>2.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Pulmonary system</td>
<td>29</td>
<td>3.6</td>
<td>2</td>
</tr>
<tr>
<td>Gastro-intestinal tract</td>
<td>5</td>
<td>2.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Hepatic &amp; biliary</td>
<td>6</td>
<td>2.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Renal system</td>
<td>7</td>
<td>1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Hematology</td>
<td>5</td>
<td>3</td>
<td>1.7</td>
</tr>
<tr>
<td>Obstetrics &amp; gynecology</td>
<td>0</td>
<td>4</td>
<td>1.7</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>4</td>
<td>3.1</td>
<td>2.5</td>
</tr>
<tr>
<td>P values</td>
<td></td>
<td>0.0322</td>
<td>0.0146</td>
</tr>
</tbody>
</table>
Potential interactions (alternative medications should be tried)

The serious interactions are about 85 (11.5%) of total interactions (780). The frequencies and potential risk of these interactions are shown in table 5. The most common interaction has been seen with ceftriaxone-heparin and ceftriaxone-calcium (19, 9) respectively. The potential interactions with blood thinning drugs like heparin, warfarin, aspirin and clopidogrel also frequently seen in this study.

<table>
<thead>
<tr>
<th>Drug-Drug</th>
<th>Frequency</th>
<th>Risk of Potential Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone- Heparin</td>
<td>19</td>
<td>Increase risk of bleeding</td>
</tr>
<tr>
<td>Ceftriaxone-Calcium</td>
<td>9</td>
<td>Potentially risk of particulate precipitation in lungs and kidneys.</td>
</tr>
<tr>
<td>Omeprazole-Clopidogrel</td>
<td>7</td>
<td>Decrease anticoagulant effect of clopidogrel</td>
</tr>
<tr>
<td>Aspirin-Captopril</td>
<td>6</td>
<td>Risk of decrease in renal function.</td>
</tr>
<tr>
<td>Heparin-Azithromycin</td>
<td>4</td>
<td>Increase risk of bleeding</td>
</tr>
<tr>
<td>Hydrocortisone-simvastatin</td>
<td>3</td>
<td>Decrease effect of Simvastatin</td>
</tr>
<tr>
<td>Hydrocortisone-simvastatin</td>
<td>4</td>
<td>Decrease effect of Simvastatin</td>
</tr>
<tr>
<td>Amikacin-Lasix</td>
<td>2</td>
<td>Increased risk of ototoxicity and nephrotoxicity</td>
</tr>
<tr>
<td>Omeprazole-Digoxin</td>
<td>2</td>
<td>Increase effect of digoxin</td>
</tr>
<tr>
<td>Digoxin-Bisoprolol</td>
<td>1</td>
<td>Increase risk of bradycardia</td>
</tr>
<tr>
<td>Ceftriaxone-Warfarin</td>
<td>2</td>
<td>Increase bleeding risk</td>
</tr>
<tr>
<td>Heparin-Warfarin</td>
<td>1</td>
<td>Increase risk of bleeding</td>
</tr>
<tr>
<td>Azithromycin-Digoxin</td>
<td>1</td>
<td>Increase digoxin effect</td>
</tr>
<tr>
<td>Asprin-Lisnopril</td>
<td>1</td>
<td>Risk of decrease in renal function.</td>
</tr>
<tr>
<td>L-Thyroxin-Heparin</td>
<td>1</td>
<td>Increase risk of bleeding</td>
</tr>
<tr>
<td>Azithromycin-Heparin</td>
<td>1</td>
<td>Increase risk of bleeding</td>
</tr>
<tr>
<td>Clarthromycin-Ondasteron</td>
<td>1</td>
<td>Increase risk of arrhythmias</td>
</tr>
<tr>
<td>Metoprolol-Digoxin</td>
<td>1</td>
<td>Increase risk of bradycardia</td>
</tr>
<tr>
<td>Hydrocortisone-simvastatin</td>
<td>3</td>
<td>Decrease effect of Simvastatin</td>
</tr>
<tr>
<td>Aldactone-potassium</td>
<td>1</td>
<td>Increase risk of hyperkalemia</td>
</tr>
<tr>
<td>Simvastatin-amiodarone</td>
<td>1</td>
<td>Increases toxicity of simvastatin</td>
</tr>
<tr>
<td>Carbamazepine-Omeprazole</td>
<td>1</td>
<td>Decrease the level or effect of omeprazole</td>
</tr>
<tr>
<td>Azithromycin-Warfarin</td>
<td>1</td>
<td>Increase risk of bleeding</td>
</tr>
<tr>
<td>Carbamazepine-Atorvastatin</td>
<td>1</td>
<td>Decrease effect of Atorvastatin</td>
</tr>
<tr>
<td>Amiodarone-Simvastatin</td>
<td>1</td>
<td>Increases toxicity of simvastatin</td>
</tr>
<tr>
<td>Metronidazole-Simvastatin</td>
<td>1</td>
<td>Increase effect of simvastatin</td>
</tr>
</tbody>
</table>
Discussion

Drug-drug interactions are common preventable health risk. The present retrospective study analyzes the type and number of DDIs in hospitalized patients using data from 186 patients case sheets in Al-Fayhaa Teaching Hospital in Basra- Iraq. There are various methods for identification of DDIs. In this study Medscape drug interaction checker program is used.

The prevalence of DDIs is especially high in hospitalized patients, and the incidence of DDIs is usually associated with polypharmacy and patient age\cite{15,16}. The mean age of patients included in this study are [56.4 ± 19] and most of them in age range 60-80 year. This compatible with previous studies that correlate DDIs with age\cite{17}. This result explained by fact that most elderly patients have multiple comorbidities as shown in table [4] that illustrate the significant increase in DDIs with increasing comorbidities especially cardiovascular disease. This agree with other studies which conclude that patients with cardiovascular disease have additional risk for DDIs\cite{18,19}. Additionally, the present of polypharmacy medication in hospitalized patients are very important factor that explain the high rate of DDIs recorded in this study. Table 1 show that most patient take three medications or more and about 22(11.8%) take more than nine medications. Similar conclusion found by Assefa et al who show a higher incidence of potential DDIs in elderly people with polypharmacy and cardiovascular disease\cite{20}. The length of hospital stays also among the factor that lead to high rate of DDI\cite{21,22}. In the present study most of the studied patients were hospitalized about three to seven days [111(59.7% of cases)] and some of them stay more, as seen in table 1.

In the present study most patients (about 75%) experience at least one interaction during hospitalization. The total number of interactions are 740 DDIs identified from 186 case with average (3.9) interaction per case. Similar study found that 78.2% of the cases had at least one potential DDI\cite{23}. Lower rate of interactions, about 46% and 26% had been seen in other studies\cite{24,25} respectively. The heterogeneity in the results of various studies may reflect differences in the conditions of the included patients and level of care, as well as the various methodology used, especially the software used to identify these DDIs\cite{26}.

Most of DDIs in the present study occur due to pharmacodynamic mechanism482 (65.1%), followed by pharmacokinetic 144(19.5%) and about is occur by unknown mechanism 114 (15.4%). Moderate interactions are frequently found in the present study and 544 (73.5%) respectively. This agree with other studies which shows that moderate DDIs were highly incident\cite{27,28}.

The mild interaction is about 111 (15%). The mild and moderate DDIs may not associated with potentially harmful effects, however its need careful monitoring and adequate management. Serious DDIs or X- interactions is about 85 (11.5%) in our study and they are considered high compare with Shetty V. et al study that report 3.02% of X-interaction\cite{19}. Unfortunately, these interactions would cause clinically significant adverse effects on patients. Serious interactions reported in the present study include interactions blood thinning drugs with ceftriaxone and/or other medications and in most cases result in increasing the potential risk of bleeding. Another potential interaction seen in this study, is decrease the efficacy of clopidogrel by co-administration with omeprazole. This pharmacokinetic interaction occurs due to enzyme inhibition action of omeprazole on cytochrome p450. Instead of omeprazole, other proton pump inhibitor, pantoprazole not inhibit with the enzyme and not interfere with clopidogrel action.

Many potential interactions preventable adverse effect that need pharmacological knowledge. These DDIs not well documented and many of them unnoticed by health care organizations. Even in developed countries like united states, there is a study shows that DDIs did not recognize and/or adequately treated\cite{30}. Therefore, health care provider in our society, especially clinical pharmacists, should be encourage to use electronic based software for checking DDIs and discuss them with specialists for modification and or monitoring their clinical outcomes.

There are some limitations in the present results including that its retrospective study so the adverse
effects of these DDIs not followed clinically. DDIs are checked and recorded using Medscape drug interaction checker hence its needed identify real clinical interactions from theoretical one. Inaddition, the performance of drug-drug interaction software is different in their sensitivity and specificity.Finally, the data are collected from one hospital and we need further studies in multiple hospitals or health centers to shed light on true percentage of DDIs in our society.

Conclusion

There are many potential drug interactions in hospitalized patients especially who have cardiovascular comorbidities. The serious DDIs were unfortunately high in the studied patients and its sufficiently high to alert health care organization to pay more attention toward these avoidable adverse effects on patients. There is a need to provide health care team and clinical pharmacists with the useful software to check and monitoring the significant drug interactions in order to prevent or at least minimize their adverse effect on patients.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Evaluation of Implant Stability Following Sinus Augmentation Utilizing Bovine Bone Mixed with Platelet-Rich Fibrin

Ali H. Abbas Alhussaini¹, Thair A. Lateef Hassan², Heba Basim Mohammed³

¹Assistant Prof., Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Baghdad, Iraq, ²Prof., President of the Iraqi Scientific Council of Maxillofacial Surgery in Iraqi Board for Medical Specialization, Iraq, ³Assistant Lecturer, Department of Oral and Maxillofacial Surgery, College of Dentistry, Almustansiriya University/Iraq

Abstract

Background: Lateral sinus augmentation and simultaneous insertion of dental implants is a highly predictable procedure and associated with high rate of implants success.

Aims: To evaluate implant stability changes following maxillary sinus augmentation utilizing deproteinized bovine bone alone or mixed with platelet-rich fibrin.

Materials and Methods: A total of 34 lateral sinus augmentation procedures were performed and 50 dental implants simultaneously installed. The lateral sinus augmentation cases were allocated randomly into 3 groups: Group A comprised 13 procedures and 21 dental implants utilizing solely deproteinized bovine bone. Group B involved 10 cases and 16 dental implants using deproteinized bovine bone mixed with leukocyte and platelet-rich fibrin. Group C included 11 operations and 13 dental implants employing deproteinized bovine bone mixed with advanced platelet-rich fibrin. Resonance frequency analysis test was performed immediately after implant installation and 24 weeks postoperatively for the measurement of the implant stability.

Results: Implant stability quotient values increased significantly for all study groups 24 weeks after dental implants installation (P= 0.001). The implant stability quotient at T1 (day of implant installation) was 56.93 ±12.01 for group A, 58.34 ±12.82 for group B, and 61.35 ±8.47 for group C. The implant stability quotient at T2 (24 weeks after implant insertion) was 69.17 ±5.10, 69.43 ±5.32, and 68.50 ±7.44, respectively.

Conclusion: The addition of leukocyte and platelet-rich fibrin or advanced platelet-rich fibrin to the bovine bone for sinus floor augmentation did not increased the implant stability quotient value in comparison to the bovine bone alone.

Key Words: bovine bone, implant stability quotient, maxillary sinus augmentation, platelet-rich fibrin.

Introduction

Bone remodelling after tooth extraction and maxillary sinus pneumatization often create a clinical challenge for dental implant (DI) placement in the posterior maxilla (1,2). Several surgical approaches have been adopted to increase bone height in the posterior maxillary region for the insertion of DI. Two main techniques were reported for maxillary sinus augmentation; the crestal and lateral approaches (3).

Maxillary sinus augmentation through lateral approach (LSA) with concomitant insertion of DI is highly predictable procedure for gaining bone volume in atrophic posterior maxilla and associated with high rate of implants success (4,5).

Different types of biomaterials have been used for LSA including autograft, allograft, xenograft, alloplastic, and growth factors (6,7). The clinical suitability of deproteinized bovine bone (DBB) for maxillary sinus augmentation has already been proofed by many studies.
Biomaterials with osteoinductive properties, such as platelet-rich fibrin (PRF), which are rich in growth factors were introduced as additional or replacement materials in bone augmentation procedures, aiming to stimulate angiogenesis, enhance new bone formation, improve graft maturation and recovery period (10,11).

One of the most important criteria for implant success is osseointegration. Dental implant stability is an indirect indication of osseointegration, it is a measure of the anchorage quality of an implant in the alveolar bone. Implant stability can be defined as the combination of both primary (mechanical) and secondary (biological) stability (12,13).

Several methods are used for the measurement of implant stability. Resonance frequency analysis (RFA) devices are claimed to be more objective and superior to other methods in measurement of implant stability (13,14). It provides the clinician with valuable information about the present state of bone-implant interface at various times. Furthermore, it used as a guide for timing of implant loading (15,16).

The objective of the present study was to monitor the changes in DI stability after LSA with simultaneous placement of DI utilizing demineralized bovine bone (DBB) alone or mixed either with leukocyte and platelet-rich fibrin (L-PRF) or with advanced-PRF (A-PRF) as a grafting material during the first six months of healing.

Materials and Methods

Study sample

This randomized prospective clinical study was conducted at the Dental Implant Unit/Department of Oral and Maxillofacial Surgery/College of Dentistry/University of Baghdad, from January 2019 to August 2020. Twenty-five patients (15 females and 10 males), with a mean age of 51.5 years (ranged 25-72 years). A total of 34 cases with atrophic posterior maxilla who met the eligibility criteria and were suitable candidates for this research.

The LSA cases were randomly allocated into three study groups according to the type of the graft material which was inserted in the created subantral space: Group A comprised 13 procedures and 21 DI, utilizing solely deproteinized bovine bone (DBB) with particle sizes 0.5-1 mm (BEGO OSS, mebios GmbH, Germany), group B involved 10 operations and 16 DI, using DBB mixed with L-PRF, and group C included 11 LSA and 13 DI, employing DBB mixed with A-PRF.

Randomization was performed by drawing lots to distribute the grafting materials according to the study groups. The protocol of the study was approved by the Ethical Committee of the College of Dentistry/University of Baghdad (No. 035118). All patients were informed about the nature of the study and they signed a written consent form for their participation in this study.

Patients were selected according to the eligibility criteria: Healthy individuals without any systemic disease/local pathological lesion at the sinus zone, patients age ≥ 18 years, residual bone height (RBH) ≥ 3 ≤ 6 mm with residual bone width (RBW) ≥ 5mm, and healed implant insertion site at least 6 months after tooth extraction.

Radiological examination

Panoramic radiograph was obtained preoperatively for preliminary evaluation of the residual alveolar ridge. Preoperative CBCT scan was recommended when the candidates were selected for sinus augmentation to provides more informative preoperative assessment of the RBH, RBW, and maxillary sinus anatomy.

Surgical procedure:

One hour prior to the commencement of the surgical procedure, the patient received one capsule of Cefixime 400 mg orally and gargled with 0.2% chlorhexidine mouth rinse for 2 minutes. All surgical procedures were accomplished by an experienced surgeon with this sort of operations. Surgeries were performed under local anesthesia using lidocaine 2% with adrenaline 1:80,000 (Septodont, France). Three-sided flap was performed, followed by reflection of a full thickness mucoperiosteal flap to expose the lateral wall of the maxillary sinus. A lateral window approach was accomplished using conventional drilling technique with round diamond bur.
Gentle elevation of the Schneiderian membrane (SM) using Frios Sinus Set elevators (Dentsply Friadent, Germany). Preparation of implant insertion sites using NucleOss T6 surgical kit (Turkey). Undersized drilling protocol was done in an attempt to achieve optimal primary implant stability.

In all study groups, barrier membrane (GENOSS, South Korea) placed directly below the elevated SM and extended outside to cover the lateral window. Partial augmentation of the created subantral space with one of the optional graft materials according to the groups. Installation of the DI (NucleOss T6, Turkey) into the prepared osteotomy site. Finally complete the augmentation of the created space.

**PRF preparation:**

Preparation of PRF was performed by collecting 10 mL of autogenous venous blood which was poured into 10 mL plain glass tube and immediately centrifuged. Centrifugation was performed according to the following two protocols: either at 2700 rpm for 12 min for preparation of L-PRF, or at 1500 rpm for 14 min for preparation of A-PRF (17,18). Placing the PRF clot in a jar to be cut in small pieces with scissor and mixed with 1-2 cc DBB and being ready for sinus augmentation with one of these mixtures according to the study groups (B or C).

**Implant stability measurement**

Implant stability was measured by resonance frequency analysis test using Osstell ISQ device (Ostell; Gothenburg, Sweden) immediately after implant installation for baseline record (T1) and 24 weeks postoperatively (T2) before implant loading, as illustrated in figure 1. Two consecutive measurements, one from bucco-palatal and the other from mesiodistal direction for each implant were recorded. The mean of the two ISQ values was considered as the final primary ISQ (T1).

![Image of Osstell ISQ device](image.png)

**Figure 1: Osstell ISQ device for measurement of:**
(A) Primary implant stability (T1). (B) Secondary implant stability (T2).

Change in implant stability = Secondary implant stability (T2) - Implant stability (T1). Change in implant stability (%) = Change in implant stability (T2-T1) × 100% / Implant stability (T1).

**Statistical Analysis**

The new edition of IBM® SPSS® 24 was used for statistical analysis. The histogram revealed that the data was not distributed normally. The data is provided in the form of a mean and standard deviation. Kruskal-Wallis test was used to compare the mean stability of the groups. Wilcoxon Rank U test was used to assess changes within each category. The Mann-Whitney test is used to compare two different groups statistically. P value was considered not significant at \( P > 0.05 \), significant at \( P \leq 0.05 \) and highly significant at \( P < 0.01 \).

**Results**

Distribution of inserted dental implants according to the study groups
A total of 34 LSA procedures and simultaneous installation of DI (one-stage technique). The total number of DI installed concomitantly with the LSA for all study groups were 50 DI. Twenty-nine DI (58%) of which with a diameter of 4110 mm. Forty-six DI (92%) out of 50 DI were inserted in molar region (tables 2).

**Table 2: Distribution of dental implants according to the study groups.**

<table>
<thead>
<tr>
<th>Study groups</th>
<th>No. of DI</th>
<th>Implant dimension (mm)</th>
<th>Implant inserted region and site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35 10</td>
<td>41 10</td>
<td>41 12</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
<td>Frequency</td>
<td>Molar</td>
</tr>
<tr>
<td>A</td>
<td>21</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>B</td>
<td>16</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>C</td>
<td>13</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Total No.</td>
<td>50</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td>%</td>
<td>100</td>
<td>10</td>
<td>58</td>
</tr>
</tbody>
</table>

DI, dental implants.

Dental implant stability and study groups

Table 3 illustrates that the ISQ value increased significantly for all study groups from T1 to T2 (P= 0.001). For groups A and B, the statistical increase in stability was relevant with the increase in clinical ISQ scale (from low to medium stability, according to ISQ scale). In contrast, the significant increase in DI stability for group C was irrelevant clinically (within the medium ISQ scale).
Table 3: Implant stability measurement and mean change for each study group.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>No. of DI</th>
<th>Implant stability quotient (ISQ)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1 Mean ±SD (Min-Max)</td>
<td>T2 Mean ±SD (Min-Max)</td>
<td>T2-T1 Mean change &amp; (%)</td>
<td>P-value*</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>21</td>
<td>56.93 ±12.01 (31.5-68.5)</td>
<td>69.17 ±5.10 (52.5-75.0)</td>
<td>12.24 (21.5)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>16</td>
<td>58.34 ±12.82 (28.0-70.5)</td>
<td>69.43 ±5.32 (58.0-75.0)</td>
<td>11.09 (19.0)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>13</td>
<td>61.35 ±8.47 (40.5-70.0)</td>
<td>68.50 ±7.44 (49.0-75.0)</td>
<td>7.15 (11.6)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>58.87 ±11.1 (28-70.5)</td>
<td>69.03 ±5.95 (49-75)</td>
<td>10.16 (17.37)</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

*, Wilcoxon Signed Rank test.

Table 4 presents no statistically significant difference (P > 0.05) in ISQ values between the study groups at T1, T2 and in mean change (T2-T1).

Table 4: Implant stability measurements and mean changes between the study groups.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>No. of DI</th>
<th>Implant stability quotient (ISQ)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1 Mean ±SD (Min-Max)</td>
<td>T2 Mean ±SD (Min-Max)</td>
<td>T2-T1 Mean change</td>
<td>P-value*</td>
</tr>
<tr>
<td>A</td>
<td>21</td>
<td>56.93 ±12.01 (31.5-68.5)</td>
<td>69.17 ±5.10 (52.5-75.0)</td>
<td>12.24 (21.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>B</td>
<td>16</td>
<td>58.34 ±12.82 (28.0-70.5)</td>
<td>69.43 ±5.32 (58.0-75.0)</td>
<td>11.09 (19.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>C</td>
<td>13</td>
<td>61.35 ±8.47 (40.5-70.0)</td>
<td>68.50 ±7.44 (49.0-75.0)</td>
<td>7.15 (11.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>P** (A&amp;B) = 0.45</td>
<td>P** (A&amp;C) = 0.27</td>
<td>P** (B&amp;C) = 0.84</td>
<td>P** (A&amp;B) = 0.79</td>
</tr>
</tbody>
</table>

P** (A&B) = 0.20
P** (A&C) = 0.42
P** (B&C) = 0.91
P** (A&B) = 0.83
P** (A&C) = 0.83
P** (B&C) = 0.84
The ISQ value was highly significant increase in both gender and age groups from T1 to T2. Moreover, the mean change reveals that females had a statistically significant increase in ISQ value in comparison to males (table 5).

### Table 5: Relation of implant stability with gender and age variables.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of DI</th>
<th>T1 Mean ±SD (Min-Max)</th>
<th>P-Value*</th>
<th>T2 Mean ±SD (Min-Max)</th>
<th>P-Value*</th>
<th>T2-T1 Mean change</th>
<th>P-Value*</th>
<th>P- value **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>61.57 ±8.93 (37.0-69.5)</td>
<td>0.14</td>
<td>69.11 ±5.82 (49.0-75.0)</td>
<td>0.84</td>
<td>7.54</td>
<td>0.03</td>
<td>0.0001</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>56.14 ±12.7 (28.0-70.5)</td>
<td></td>
<td>69.05 ±5.79 (52.5-75.0)</td>
<td></td>
<td></td>
<td>12.91</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 40</td>
<td>9</td>
<td>53.78 ±14.33 (28.0-68.5)</td>
<td>0.26</td>
<td>65.78 ±7.31 (52.5-75.0)</td>
<td>0.10</td>
<td>12.0</td>
<td>0.48</td>
<td>0.008</td>
</tr>
<tr>
<td>≥ 40</td>
<td>41</td>
<td>59.57 ±10.58 (31.5-70.5)</td>
<td></td>
<td>69.80 ±5.17 (49.0-75.0)</td>
<td></td>
<td></td>
<td>10.23</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*, Mann Whitney U test; **, Wilcoxon Signed Rank test.

**Relation of implant stability to surgical-related variables**

Table 6 reveals that there was a statistically significant increase in ISQ value for each surgical-related variable from T1 to T2. Furthermore, a highly significant increase in ISQ values for RBH < 4 mm in comparison to RBH ≤ 4 mm at T2 (P= 0.005).
# Table 6: Implant stability and relation to surgical related variables.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of DI</th>
<th>T1 Mean ±SD (Min-Max)</th>
<th>P-value *</th>
<th>T2 Mean ±SD (Min-Max)</th>
<th>P-value *</th>
<th>T2-T1 Mean change</th>
<th>P-value *</th>
<th>P-value **</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBH (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 4</td>
<td>11</td>
<td>53.63 ±13.23 (28.0-67.0)</td>
<td>0.05</td>
<td>65.13 ±6.93 (49.0-71.0)</td>
<td>0.005</td>
<td>11.5</td>
<td>0.60</td>
<td>0.003</td>
</tr>
<tr>
<td>&gt; 4</td>
<td>39</td>
<td>59.91 ±10.61 (31.5-70.5)</td>
<td></td>
<td>70.19 ±4.91 (52.5-75.0)</td>
<td></td>
<td>10.28</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>DI insertion site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premolar</td>
<td>4</td>
<td>55.25 ±19.72 (28.0-70.5)</td>
<td>0.74</td>
<td>67.0 ±9.24 (58.5-75.0)</td>
<td>0.93</td>
<td>11.75</td>
<td>0.54</td>
<td>0.000</td>
</tr>
<tr>
<td>Molar</td>
<td>46</td>
<td>58.81 ±10.7 (31.5-70.0)</td>
<td></td>
<td>69.26 ±5.46 (49.0-75.0)</td>
<td></td>
<td>10.45</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>DI diameter (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>6</td>
<td>57.67 ±15.56 (28.0-70.5)</td>
<td>0.62</td>
<td>67.33 ±6.89 (58.5-75.0)</td>
<td>0.871</td>
<td>10.08</td>
<td>0.17</td>
<td>0.000</td>
</tr>
<tr>
<td>4.1</td>
<td>37</td>
<td>59.37 ±10.81 (31.5-70.0)</td>
<td></td>
<td>69.45 ±5.28 (49.0-75.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.8</td>
<td>7</td>
<td>54.78 ±11.7 (38.0-68.5)</td>
<td></td>
<td>68.57 ±7.6 (52.5-74.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RBH, Residual bone height; *, Mann Whitney U test; **, Wilcoxon Signed Rank test.
Discussion

The implant stability quotient (ISQ) value ranges from 1 to 100. In general, ISQ values for successful implants are reported from 57 to 82 ISQ \cite{13,14}. The ISQ > 70 represents high stability, ISQ between 60 and 69 exemplify medium stability, and ISQ < 60 ISQ considered low stability \cite{19}.

In the present research, the results were considered clinically significant or not dependent on the number of DI that remained in the same level of ISQ-scale or changed to another level. This clinical analysis of the data illustrated and confirmed that not all the statistically significant results essentially being clinically relevant. This idea is supported by Guller in 2008 \cite{20} who declared that statistically significant differences may be of no clinical relevance whatsoever”.

The implant survival was 100%. For all study groups, there was a significant increase in ISQ value from 58.78 ±11.1 ISQ (low stability) at T1 to 69.03 ±5.95 ISQ (medium stability) at T2 with a mean change of DI stability (10.16 ISQ), in which it was statistically significant and clinically relevant.

For study groups A and B, there was a statistically significant increase in stability from T1 to T2 which was harmonious with the clinical outcome (increase of ISQ values from low to medium stability level). In contrast, the statistically significant increase in ISQ value for group C was not associated with clinical relevance (ISQ values remained within medium stability level).

The statistically significant increase in stability from T1 to T2 for all study groups might be related to the increase in osseointegration. This outcome is supported by Sennerby et al. in 2005 \cite{21} who claimed that the increased ISQ values might be attributed to the successfully osseointegrated implants.

Undersized drilling protocol was accomplished in the maxillary posterior region to gain the requested primary implant stability. This method was enforced by several studies which proved that when DI were inserted in underprepared osteotomy sites using smaller diameter drills, maximum bone volume preservation and enhanced bone density were achieved as stated by Turkyilmaz et al. in 2008 \cite{22}, Tabassum et al. in 2010 \cite{23}.

No statistically significant difference (P > 0.05) noticed in ISQ values between the 3 study groups at T1, T2 and in mean change (T2-T1). Nevertheless, it was higher in groups A and B, 12.24 ISQ and 11.09 ISQ, respectively, when compared to group C (7.15 ISQ). However, it did not reach the level of statistical significance (P = 0.45).

In addition, it has been found that the addition of L-PRF and A-PRF to DBB for the study groups B and C, respectively; did not provide an enhancement to the ISQ value superior to the DBB alone in group A. This result comes in line with Pichotano and co-workers in 2019 \cite{24}, who found that the ISQ values at loading did not differ according to the grafting materials, following a split-mouth design, in which the right maxillary sinus was augmented using L-PRF mixed with DBB and the left side was filled with DBB alone. In contrast, Călin et al. in 2016 \cite{25} claimed that the use of the combination of A-PRF and bovine bone in sinus lift technique speeded healing time by approximately 50%. However, the authors in their study relied the assessment of implant osseointegration on clinical examination and panoramic radiograph and they did not measure the DI stability.

Relation of implant stability to patient-related variables

For all study groups, and from a statistical point of view, there was a highly significant increase in the ISQ value for gender and age variables from T1 to T2. The speculation might be related to the normal remodeling process during osseointegration in which the stability increased during time depending on primary stability and other factors as bone remodeling and implant surface conditions as proclaimed by Sachdeva et al. in 2016 \cite{14}.

Relation of implant stability to surgical-related variables

In this research, the effect of some factors on implant stability were standardized by operating on the same region (posterior maxilla), standard surgical technique (LSA), the same implant system (geometry
and surface characteristics) utilized for all cases, the same surgeon, undersized drilling technique that is to reduce the confounding factors that might influence implant stability.

There was a statistically significant increase in ISQ value for each surgical-related variable (RBH, DI insertion site, and DI diameter) from T1 to T2. This was ordinarily related to the normal sequelae of healing process and osseointegration since stability of DI increased in all surgical-related variables.

The RBH < 4 mm demonstrated a statistically significant increase in implant stability versus RBH ≤ 4 mm at T1 and T2. This result is supported by several studies which concluded that primary implant stability is influenced by quality and quantity of the residual bone, and the secondary implant stability in its turn affected by the primary stability (12,16,26).

**Conclusion**

There was a significantly increase in ISQ value for all of the DI 24 weeks after their installation irrespective to the type of graft material utilized to augment the sinus. Moreover, the addition of L-PRF or A-PRF to the bovine bone for sinus floor augmentation did not increased the ISQ value superior to the bovine bone alone.

**Ethical Clearance**

The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**


Evaluating the Effect of Air Abrasive Polishing on Friction and Surface Micromorphology of Ceramic Brackets Using Different Wires

Ahmed R. Mohammed Hassan¹, Shahbaa A. Mohammed²
¹Master Student, ²Assit. Prof. ; Department of Orthodontics, College of Dentistry, University of Baghdad, Baghdad/Iraq

Abstract

Objectives: The aim of this in vitro study was to evaluate the Resistance to Sliding (RS) provided by ceramic (Perfect clear) sapphire ceramic brackets using round 2 types of orthodontic wires (Fiber-Reinforced Polymer Composite (FRPC) and Epoxy fully coated Stainless-Steel), before and after the use of sodium bicarbonate airborne particle abrasion, in an experimental model with 3 non levelled brackets and to evaluate the surface micromorphology of these brackets by means of scanning electron microscopy.

Materials and Methods: One commercial brands of ceramic brackets were evaluated. The forty specimens were divided into two groups (n = 20) according to the two type of arch wires and each group was divided into four subgroup of five specimens according to the application or not of sodium bicarbonate airborne particle abrasion for (5, 10 and 20) seconds. A device adapted to a universal testing machine was used to simulate the movement of retraction in sliding mechanics, measuring the traction force needed to slide 10 mm of the wire over the test specimen brackets. The test speed was 5 mm/min. The data were analyzed by two-way analysis of variance (ANOVA) and Tukey test.

Conclusions: It may be concluded that it is not recommended to apply airborne particle abrasion on the slots of ceramic.

Key Words: One commercial brands of ceramic brackets were evaluated.

Introduction

With the advent of increasing adults’ number opting for the orthodontic treatment, the development of the orthodontic appliances with ample emphasis on the aesthetics coupled with the optimum performance has become an exceedingly essential object or rather necessity of the day [1]. It has been partially resolved by the introduction of aesthetic brackets manufactured of ceramic or composite [2].

The ceramic brackets available nowadays are made of alumina either in poly-crystalline or mono-crystalline forms. The manufacturing process of mono-crystalline brackets results in a purer structure, a smoother surface, and a considerably harder substance than the fabrication of polycrystalline brackets [3].

Ceramic brackets currently represent an aesthetic alternative, although their use is limited. They abrade the enamel, and fracture more easily, and they have a higher coefficient of friction, increasing resistance to sliding [4].

However, most arch wires are still made of efficient, albeit un aesthetic, metal alloys such as stainless steel (SS) and nickel-titanium (NiTi) so that an aesthetic arch wire is highly desired to complement aesthetic brackets in clinical orthodontics [5].

Coating metallic arch wires by plastic resin materials was the main solution to offer aesthetic characteristics to the wires of metallic or silver colour appearance [6]. As the patients favor that shiny metal arch wires were not as apparent as opaque, an alternative could be wires with transparent or translucent features [7]. Moreover,
aesthetic coating of alloy arch wires are not durable clinically and tends to dehisce over a period of time [8]. Through composite technology, an aesthetic arch wire has been developed from continuous fibers and polymer matrix, giving rise to the fiber reinforced polymer composite (FRPC) arch wire [9]. The translucent nature of the polymer matrix confers the aesthetic property to this wire, while the fiber content gives the flexibility, overcoming the inherent problem of brittleness [10].

Friction is defined as a force that retards or resists movement related to two objects in contact. In sliding mechanics, friction is determined by the type of arch, type of bracket, and type of ligature.[11] Although orthodontic treatment provides the functional and aesthetic correction of teeth, one of its inconvenient aspects is that the brackets (whether they are made of metal or ceramics) and other accessories allow a greater accumulation of residues and consequently, the formation of dental plaque, especially around the brackets. Professional prophylaxis must be performed to correct the patient’s deficient tooth brushing, particularly with the use of sodium bicarbonate abrasion, a system that releases controlled jets of air, water, and sodium bicarbonate particles. It was introduced at the end of the 1970s as a fast and easy mechanism to remove dental plaque,[12]

Consequently, professional tooth cleaning could be extremely important for the maintenance of oral health, especially when patient compliance is inadequate or when dexterity is poor.[13] Compared to professional tooth cleaning with rubber cup and pumice, air-polishing devices are more effective for removing dental plaque, and in addition they promote less operator fatigue due to reduced working time.[14]

With regard to alterations to the dental substrate, it has been shown that the use of sodium bicarbonate airborne particle abrasion does not cause surface alterations in healthy enamel, but it does affect and change the micromorphology of dentin and cementum. Therefore, the use of the sodium bicarbonate airborne particle abrasion is an efficient and safe method for removing dental plaque from healthy enamel, but its use on exposed dentin and cementum must be avoided. [12,14–17].

Many studies evaluated the factors that could influence the frictional resistance, such as proprieties related to bracket and wires materials,[18–23] type and force of ligation,[20,24–26] and biological variables.[27,28] However, little research has been conducted to evaluate the effect of sodium bicarbonate air abrasive polishing on frictional resistance at tooth alignment and levelling phase of orthodontic treatment. Therefore, the aim of this study was to evaluate in vitro the frictional resistance (static friction) provided by Perfect Clear Sapphire brackets, using 2 types of aesthetic orthodontic wires size 0.018 inch (Fiber-Reinforced Polymer Composite (FRPC) arch wire and Epoxy fully coated Stainless-Steel arch wire) before and after the use of the sodium bicarbonate airborne particle abrasion, in an experimental model with 3 non levelled brackets and surface micromorphology of the brackets before and after applying sodium bicarbonate airborne particle abrasion, by means of scanning electron microscopy.

**Materials and Methods**

The study samples included 0.018 inches upper preformed round arch wires from two commercial brands . The first one was Fiber-Reinforced Polymer Composite (FRPC) arch wire manufactured by (Dentaurum, Germany.) and the second was Epoxy fully coated Stainless-Steel arch wire manufactured by IOS® (Tooth Tone® arches, Ortho Technology, USA).

The distal end of wires were cut into (5 cm.) length and investigated with one type commercial brand Sapphire Ceramic brackets {Perfect Clear® sapphire brackets from Hubit Co. (South Korea)}. Frictional resistance of 40 samples in this study was obtained from grouping the samples into 2 groups (n = 20) according to the type of arch wire and each group was divided into four subgroup of five specimens according to the time of air powder abrasive application.

In this present study, we used an experimental model with 3 non levelled brackets to assess the frictional forces generated during the dental alignment process. To
prepare the samples, 120 upper first premolar Sapphire ceramic edgewise brackets.022”(MBT prescription with a 0.022x0.028 inch slot with torque of (-7) and angulation of 0) were employed. Sapphire ceramic brackets were used because they are more aesthetic than stainless steel brackets and made from mono crystal alumina because the manufacturing process of mono-crystalline brackets result in pure and clear structure, smoother and harder surface than other type of ceramic brackets (Swartz, 1988).29

The samples were prepared by bonding 3 brackets on a preformed plastic block (37 mm length, 12 mm width, 10 mm height) made by Computerized Numerical Control (C.N.C), which was designed to simulate a non-aligned dental segment. The brackets were bonded with cyanoacrylate adhesive (Gucex star 502, china) and the bonding procedure was standardized by using the (C.N.C) plastic design and positioner showed in [Figure 1]. The end of the arch wire with a dimension of 0.018 inch cut into 5 cm and ligate with ligature elastic to brackets specimen.

[Figure 1: A-from the left to the right (C.N.C) plastic design, block and positioner. B-precisely bonding of a non-aligned brackets .C-standardized application of air abrasive powder.]

The vertical discrepancy between the brackets was set at 1 mm to simulate a non-alignment situation in the segment of dental arch to be studied. The inter bracket distance was set at 11mm, according to a previous study.30

The brackets and wires were washed in 95% ethanol and air-dried, and then one wire segment of 5 cm was positioned on the brackets slots for each sample. Several studies have documented that a high force generated by a tight ligation will cause an increase in the measurement of frictional force.31 To reduce the potential for such bias, all ligations were done by the same operator using a needle holder in a standardized procedure. The ligatures used in this study were elastomeric modules (Super slick clear) IOS® (International Orthodontic Services, Stafford, USA)).

During the frictional force tests Static Friction (SF) and Kinetic Friction (KF) readings were performed. SF readings were obtained by determining the peak force (N) at the first 2 mm of wire displacement.

The test specimens were submitted to the tensile test in the mechanical testing machine computerized universal testing machine (Instron H50KT Tinius Olsen testing machine with 10 N load cell). Figure 2 shows the device and the bracket/wire positioned in the universal
testing machine. A maximum load of 5 kgf was used under dry conditions. Tensile force needed to slide 10 mm of the wire over the test specimen brackets for 2 minutes, at a speed of 5 mm per minute, was measured, and the maximum tensile force value obtained during the range of motion of each bracket was also measured. The data were obtained Newtons.

The sodium bicarbonate airborne abrasion was performed with a sodium bicarbonate appliance using AIR-N-GO Classic sodium bicarbonate polishing powder based in raspberry natural fresh flavour (Satelec A Company of Acteon Group, French). After each air abrasion session for every bracket the remaining powder was discarded and a new (15 gm) of powder were poured into the tank of Prophy-Mate neo polishing system airflow hand piece (NSK Company, Japan) to prevent the level of powder from reaching below 50% of the tank in accordance with Parmagnani and Basting (2012).

The airborne abrasion was applied perpendicularly to the brackets at a distance of 5 mm for 10 seconds with a 2.3 bar pressure.

Surface micromorphology of the brackets was examined by scanning electron microscopy before and after application of sodium bicarbonate air abrasive polishing visualized at (50X, 500X, 1000X, 2000X) magnification is shown in figure 3 to figure 5.

**Results**

According to Table 1, it was observed that mean resistance was higher in the group that received airborne particle abrasion, regardless of the type of wire.

Epoxy fully coated Stainless-Steel arch wire showed a higher mean resistance than the Fiber-Reinforced Polymer Composite (FRPC) arch wire, both in the control group and after airborne particle abrasion.

Surface micro-morphologies of the brackets before and after jet application are shown in Figures 3 to 5. For the ceramic brackets, there was no surface alteration both at (50X, 500X, 1000X, 2000X) magnification.

<table>
<thead>
<tr>
<th>Groups</th>
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<th>Mean</th>
<th>S.D</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
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<td>0.647</td>
<td>4</td>
<td>5.37</td>
</tr>
<tr>
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<td>5 sec.</td>
<td>4.914</td>
<td>0.903</td>
<td>4</td>
<td>5.95</td>
</tr>
<tr>
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<td>10 sec.</td>
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<td>0.817</td>
<td>4.065</td>
<td>6.32</td>
</tr>
<tr>
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<td>20 sec.</td>
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<td>0.523</td>
<td>4.9</td>
<td>6.32</td>
</tr>
<tr>
<td>Epoxy SS</td>
<td>Zero sec.</td>
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<td>1.294</td>
<td>12</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>5 sec.</td>
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<td>1.084</td>
<td>13.5</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>10 sec.</td>
<td>15.3</td>
<td>1.956</td>
<td>13.5</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>20 sec.</td>
<td>15.8</td>
<td>2.540</td>
<td>13.5</td>
<td>18.5</td>
</tr>
</tbody>
</table>
Figure 3: Micromorphology of slot of ceramic bracket before airborne particle abrasion at 2000X magnification and before insertion of any wires.


Figure 5: Micromorphology of slot of ceramic bracket after sliding 10 mm of the Epoxy fully coated Stainless-

Discussion

The sliding of the brackets along the arch wire results in frictional forces that prevent the supporting tissue from attaining the optimal force level required for tooth movement [33]. Therefore, to overcome the friction at the bracket-arch wire interface, it is important to understand the orthodontic force so that a proper magnitude of force can be used to produce optimal biologic tooth movement, as well as understanding the tooth movement affected by a combination of biological and mechanical factors [34].

There are several ways to reduce friction by improvement of the material surface of the arch wire [35]. With ion implantation, the measured frictional losses ranged from 23.4 to 45.9 per cent [36]. The influence of the bracket material with regard to friction caused by the wire during sliding mechanics has been assessed, and it was found that ceramic brackets show higher friction than those made of stainless steel, not only due to the type of material, but particularly due to the irregularities on ceramic bracket surfaces [31,38,39].

In the present study, mono-crystalline ceramic brackets were used. In general, the surface roughness of ceramic brackets is similar to that of a block of concrete in comparison with stainless steel brackets, which has a porous, irregular, and polyhedral surface, and this was observed in the scanning electron microscopy images in the present study, retaining a larger amount of sodium bicarbonate particles after the airborne particle abrasion and therefore, increasing friction. On the other hand, the sodium bicarbonate airborne particle abrasion did not cause surface alterations on ceramic brackets because the ceramic material hardness was greater than that of the metal material.

Therefore, it must be considered that jet application on the bracket slot should be avoided, and if it is done, abundant washing with water must be performed to remove the residues, which occurs mainly in the ceramic brackets due to their greater surface irregularity.

Conclusions

Sodium bicarbonate air abrasive polishing is not recommended because the airborne abrasion of the brackets promoted a significant RS increase when the 2 types of wires (FRPC and the Epoxy fully coated Stainless-Steel) were used. Concerning the Epoxy fully coated Stainless-Steel wire, the airborne abrasion effect on RS seems to be greater due to these material’s properties.

Regardless of the type of wire tested, mean resistance was higher in the group that received sodium bicarbonate airborne particle abrasion.

The micromorphologic analysis showed that the airborne particle abrasion caused no changes on the surface of the ceramic brackets.

The application of airborne particle abrasion on the slots of ceramic brackets is not recommended.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References


Angulation and Height of Canine in Different Type of Malocclusion

Iman I. Al-Sheakli¹, Muthanna I. Ali²

¹Professor, Department of Orthodontics, College of Dentistry, University of Baghdad, Iraq,
²M.Sc. Student, Ministry of Iraqi Health, Baghdad, Iraq

Abstract

Background: Canines are unique teeth because they have certain characteristics and responsibilities. They are located in the corner of the mouth, assist the incisors in cutting and tearing food, help the posterior teeth by guiding the mandible during the action of mastication. The present study aim to determine the most common angulation and height of buccally malposed canine and determine the gender difference of the angulation and height of buccally malposed canine measured in each side and in total samples and to study the relation between the angulation and height and the relationship of the angulation and height with the side of buccally malposed canine and with the sagittal malocclusion of dental arch.

Material and Method: The present study was a population-based, cross sectional study based on the surveying of panoramic radiographs of 11-17 years aged patient with buccally malposed canine seek orthodontic treatment in Baghdad city- Iraq. For each case, determine age and gender of the patient, as well as determine affected side and sagittal malocclusion of the dental arch. And measure angulation and height of buccally malposed canine on panoramic radiograph. T-test and F-test were used to determine gender difference (for each side and for total samples), side difference and sagittal malocclusion difference. Person correlation were used to reveal the relation between angulation and height of buccally malposed canine. 

Result: The total samples is 71 sample, consist of 41 female and 30 male. The gender difference was not significant in right and left sides and in total samples. No significant difference in angle and height between the sides, as tested by t-test. F-test reveal no significant difference among different sagittal malocclusion in angulation and in height. The Angulation and Height of buccally malposed canine in total samples show normal distribution of samples with median, mode and mean close to each other. The person correlation between the angle and height was (-0.552) with p-value (0.000) that reveal highly significant reverse relation

Conclusion: No gender difference found in the buccally malposed canine in the right and in the left side, so the gender difference can be measured in the total samples as one unit High frequency of buccally malposed canines was reported in female than in male. The side difference was not significant in total samples, High frequency of buccally malposed maxillary canines was found in class I & II sagittal occlusion more than class III. The sagittal malocclusion difference was not significant. The Angulation and Height of buccally malposed canine in total samples show normal distribution of samples so the mean was represent most common angulation and height of buccally malposed canine. There are highly significant reverse relation between height and angulation, so the buccally malposed canine be more vertical as it came close to the occlusal plane

Keywords: Canines are unique teeth because they have certain characteristics and responsibilities.

Introduction

Human beings have four pointed teeth one on each side of the dental arch called canine or cuspid. Each canine represents the third tooth from the median line after the central and lateral incisors forming the key stone or the corner stone of the dental arch (¹).
They are the longest teeth in the mouth; the crowns are usually as long as those of the maxillary central incisors, and the single roots are longer than those of any of the other teeth. The middle labial lobes have been highly developed incisally into strong, well-formed cusps (Nelson and Ash, 2015). The shape and position of the canines contribute to the guidance of the teeth into the intercuspal position by “canine guidance” (2).

Development: The calcification of crown of the permanent canine begins to take place early in life i.e. at the age of 4 to 5 month, and by the end of the first year it can be seen high in the maxilla above the bifurcation of first deciduous molar and below the floor of the orbit. From this position to the occlusal plane the path of eruption is more tortuous and difficult than any other tooth, as the root begins to form approximately at 7 years of age the canine moves towards the occlusal plane, the crown lying in close proximity to the root of the lateral incisor and emerging into the mouth at the age of 11 and 12 years. By 12 years the canine has usually reached occlusion, the lateral incisor has been.

Importance: The positions and forms of the permanent canine and their anchorage in the bone, along with the bone ridge over the labial portions of the roots, called the canine eminence, have a cosmetic value. They help form a foundation that ensures normal facial expression at the corners of the mouth. Loss of all of these teeth makes it extremely difficult, if not impossible, to make replacements that restore that natural appearance of the face for any length of time. It would therefore be difficult to place a value on the canines, and their importance is manifested by their efficiency in function, stability, and aid in maintaining natural facial expression. In function, the canines support the incisors and premolars, since they are located between these groups. The canine crowns have some characteristics of functional form, which bears a resemblance to incisor form and also to the premolar form (3).

Iteology: Sachan, Chaturvedi, (4) summarized the etiological factors of ectopic canines as followed: Early loss of deciduous teeth, Crowding of the permanent successor, Tooth size and overall arch length, High developmental position and long path of eruption and tortuous movement, Prolonged retention of the deciduous tooth, Failure of primary canine root resorption, Small or congenital missing permanent lateral incisor, Reduced in the length of the adjacent lateral incisor root, Ankylosis of permanent canine, Alveolar cleft, Malposed tooth germ, Hereditary factors, Endocrine deficiency, and Febrile diseases.

Many studies have been performed in Iraq to study the problems of the canines. Some of them took the maxillary canine as a part from the survey (5-9). Other Iraqi studies was study impacted canine (11, 12). Some of them concern with canine specifically. Kinaan (13) study the management of buccally malposed canine. Ghaib, (14) and Aziz, (15) study the prevalence of buccally malposed canine. Saloom, (16) and Al-Ani (17) concern with eruption course and eruptive anomalies of canine. Al-Fahdawi (18) and Aziz, (19) study the problems of the maxillary canine in Iraqi people. Al-Dabagh, (20) study buccally malposed mandibular canine in Iraqi Kurdish population.

This survey was conducted to study the angulation and height of the canine in deferent type of malocclusion.

Material and Method

The present study was a population-based, retrospective cross sectional study based on the surveying of panoramic radiographs.

The study sample The samples of present study are derived from any patients seeking fixed appliance orthodontic treatment in dental hospital of university of Baghdad / collage of dentistry and private practices in Baghdad city.

Age was considered according to the last birthday giving an age range from 11 years to 17 years.

Selection criteria: The sample specification for present study are: Patient indicated for fixed appliance orthodontic treatment who have unilateral or bilateral buccally malposed canine, have complete set of maxillary permanent dentition with/without the third molars, have no extracted or congenitally missed lateral incisor or premolar, no massive interproximal caries or
restoration, and no crown or bridge restoration.

Characteristic of radiographs: Digital panoramic radiography was carried out using PM 2002 CC Proline apparatus (Planmeca, Helsinki, Finland) using imaging values between 58–68 kV and 4–10 mA, depending on the subject’s size. And the magnification factor was 1:1. All re-reported measurements were adjusted according to this factor.

The study variables: The variables of present study incorporated age and gender of the patients, sagittal malocclusion class, as well as side, angulation and height of buccally malposed canine.

Inter- and intra-examiner: All panoramic radiographs were investigated by two observers in two time interval to assess intra- and inter-examiner reliability

The anatomic measures:

1. Angulation of malposed canine: The measurement of angulation of malposed canine on panoramic radiograph will be done by measure the angle between the long axis of malposed canine and the occlusal plane (21). The occlusal plane will be determined by drawing a horizontal line from the medial point of the incisal edge of the central incisal to the mesiobuccal cusp tip of the maxillary first molar. (21) The angulation of the malposed canine will be measured lateral to the midline and recorded in degrees (22, 23).

2. The height of the malposed: Canine will be measured as length of perpendicular line extend from the occlusal plan to the cusp tip of the canine and recorded in millimeters.

Statistical analysis: Data were entered into a spreadsheet during the study period and analyzed using a commercially available statistical software package SPSS (Statistical Package for Social Sciences, version 11.0; SPSS Inc, Chicago, IL).

For statistical descriptions, means with standard deviations, were calculated and presented by gender, side and sagittal class. T-tast and f-tast use to test the difference between two and three variables, respectively. For all analyses, a P value less than (0.05) was considered statistically significant.

Result

Gender difference of the angulation and height of buccaly malposed canine measured in each side.

The samples were divided to angulation and height of buccally malposed canine in the right side and in the left side, and test gender difference in each side.

The gender difference was not significant in both sides (p >0.05), table 1

Gender difference of the angulation and height of buccaly malposed canine measured in total samples.

The total samples is 71 sample, consist of 41 female and 30 male. The mean of angle in female was slightly more than in male. The mean of height was slightly more in male than female. The gender difference was not significant in total samples (p >0.05), table 2

Angulation and Height of buccaly malposed canine with side different.

The sample consist of 32 sample with right buccally malposed canine and 39 with left. The mean of angle and the mean of height nearly similar in both sides. No significant difference in angle and height between the sides, (p >0.05), table 3.

Angulation and Height of buccaly malposed canine with sagittal malocclusion difference.

The samples consist of 26 sample with class I sagittal relation. 26 sample with class II sagittal relation and 19 sample with class III sagittal relation. F-test reveal no significant difference among different sagittal malocclusion in angulation and in height. (p >0.05), table 4.
Table 1: Gender difference of the angulation and height of buccaly malposed canine measured in each side.

<table>
<thead>
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<td></td>
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<td>Height</td>
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Table 2: Gender difference of the angulation and height of buccaly malposed canine measured in total samples.

<table>
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<td></td>
<td></td>
<td>Females</td>
<td>21</td>
<td>4.571</td>
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<tr>
<td></td>
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<td>Males</td>
<td>19</td>
<td>5</td>
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<tr>
<td></td>
<td></td>
<td>Females</td>
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<td>5.25</td>
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Table 3: Angulation and Height of buccaly malposed canine with side different

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>Median</th>
<th>Mode</th>
<th>Mean</th>
<th>S.D.</th>
<th>Skewness</th>
<th>Kurtosis</th>
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<th>Max.</th>
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<tr>
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<td>71</td>
<td>4.00</td>
<td>3</td>
<td>5.01</td>
<td>4.406</td>
<td>1.031</td>
<td>.605</td>
<td>0</td>
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<tr>
<td>Angel</td>
<td>71</td>
<td>76.00</td>
<td>70</td>
<td>75.10</td>
<td>11.275</td>
<td>.337</td>
<td>.944</td>
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Table 4: Angulation and Height of buccaly malposed canine with sagittal malocclusion difference.

<table>
<thead>
<tr>
<th>Parameter</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td>Mean</td>
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<tr>
<td>Angle</td>
<td>Class I</td>
<td>26</td>
<td>76.615</td>
</tr>
<tr>
<td></td>
<td>Class II</td>
<td>26</td>
<td>75.538</td>
</tr>
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<td></td>
<td>Class III</td>
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<td>72.421</td>
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<tr>
<td>Height</td>
<td>Class I</td>
<td>26</td>
<td>4.423</td>
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<td></td>
<td>Class III</td>
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<td>5.684</td>
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</table>

Angulation and Height of buccaly Malposed canine in total samples

The Angulation and Height of buccaly malposed canine in total samples show normal distribution of samples with median, mode and mean close to each other, table 5.

Table 5: Angulation and Height of buccaly malposed canine in total samples.

Relation between the height and angle of buccally malposed canines

The person correlation between the angle and height was (-0.552) with p-value (0.000) that reveal highly significant reverse relation as in table 6.

Table 6: Relation between the height and angle of buccally malposed canines

<table>
<thead>
<tr>
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<th>Relation</th>
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</thead>
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<tr>
<td>Angle</td>
<td>r</td>
<td>-0.552</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.000  (HS)</td>
</tr>
</tbody>
</table>
Discussion

Angulation and Height of buccally malposed canine with gender difference measured in each side.

No gender difference found in the buccally malposed canine in the right and in the left side, so the gender difference can be measured in the total samples as one unit.

Angulation and Height of buccally malposed canine with gender difference in total samples.

High frequency of buccally malposed canines was reported in female than in male and this agree with (24). There is no significant difference between male and female in both angulation and height.

Angulation and Height of buccally malposed canine with side different.

High frequency of buccally malposed canines was reported in the left side more than the right, this agree with Al-Atabi, et al, (24), Al-Huwaizi (9) and Aziz (15), and disagree with Ghaib (14), Al-Fahdawi (18) and Al-Chalabi (10).

The side difference was not significant in total samples, and this agree with Al-Atabi, et al, (24).

Angulation and Height of buccaly malposed canine with sagittal malocclusion difference.

High frequency of buccally malposed maxillary canines was found in class I &II sagittal occlusion more than class III; because the dominance type of occlusion is class I & II, so it is reasonable to find a higher percentage of canine problems in class I & II, sagittal occlusion.

The sagittal malocclusion difference was not significant.

Angulation and Height of buccaly malposed canine in total samples.

The Angulation and Height of buccaly malposed canine in total samples show normal distribution of samples so the mean was represent most common angulation and height of buccaly malposed canine.

Relation between the height and angle of buccally malposed canines

There are highly significant reverse relation between height and angulation, so the buccaly malposed canine be more vertical as it came close to the occlusal plan.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References

9. Al-Chalabi ZMR. Occlusal features, perception of occlusion and orthodontic treatment need and...


Molecular detection of vicR, vicK and 16S rRNA genes of *Streptococcus mutans* Isolated from Dental Caries of Iraqi’s Patient

Zainab Sadeq Ali ¹, Raya Ezat Maroof ², Lateef Essa Alwan Aljorani ³, Abbas Sabah Mohammad ⁴, Sarmad Sameer Hameed ⁵, Ali Sadeq Ali ⁶

¹Researcher/Ministry of Health/Environmental- AL- Karkh Health Directorate / Al-Adel sector-primary health care/Iraq, ²Researcher/Ministry of Higher Education and Scientific Research/Middle Technical University/ Collage of Health and Medical Technology/ Baghdad/Iraq, ³Researcher/Ministry of Higher Education and Scientific Research/Middle Technical University /Institute Medical Technology- Baghdad/Iraq, ⁴Researcher/Ministry of Health/Environmental- AL- Karkh Health Directorate / AL-Amriyah Specialized Dental Center/Iraq, ⁵Researcher/Ministry of Health/Environmental- AL- Karkh Health Directorate / Al-Karkh sector-primary Health Care/Iraq, ⁶Researcher/Ministry of Health/Environmental-Medicine City Directorate / Oncology Teaching Hospital. /Iraq

Abstract

Background: This study was carried out to detect the distribution of *Streptococcus mutans* with dental caries in Iraqi’s patient samples. Out of 100 patient’s samples only (22) with age (3-17) years old were found to be effected with *Streptococcus mutans*. DNA was extracted amplified by Gradient PCR and then sequenced. vicR was showed highly conserved with no mutation while vicK showed that these were silent mutation and four isolates have missense mutation from eight isolate.

Material & Method: In this study, 22 from clinical *S. mutans* strains were isolated from caries- children to sequence the vicR, vicK and 16s rRNA genes. Genomic DNA was extracted from *S. mutans* strains and amplified using Gradient PCR. The PCR products were purified and then sequenced.

Result: The molecular study include vicR, vicK and 16s rRNA, genes by Gradient PCR. The result of sequencing appear that no mutation in vicR genes, although in 16s rRNA present transion and transverision mutation . Finally the sequencing of vicK gene appear silent mutation and four isolates have missense mutation from eight

Conclusion: the result of molecular study of vicR gene were appear no mutation in all isolates while 16s rRNA although have transition and transversion mutation, the Sequence of vicK gene that note more of mutation was silent mutation but some of them was missense mutation.

Keywords: Dental caries, molecular, *Streptococcus mutans*.

Introduction

Dental caries is a major common and common public health oral illness which hinders the attainment and safeguard of oral health in variation age groups (1). Dental caries is one of most important prevalent infectious disease of the human can be leads wastage tooth structure, there after changes in oral functions (2). Other define of dental caries is a bacterial disease of the dental hard tissue and happen in certain localized sites in the dentitions. In the iorder of frequency sites to attack “pits” and “fissures”, especially on the surfaces of the teeth, proximal contact of the surface, and “labial”, “buccal” and “lingual” surface of the dentition neighboring to the gingivae (3). So consider a complex disease caused by a physiological imbalance, between fluid and mineral dental biofilm (4). *Streptococcus mutans* is the plurality cariogenic pathogens in tooth decay. The highly acidogenic, industrialize short-chain acids which...
the soften hard tissues of dental. Three isoymes of glucosyltransf erases metabolize and catalyze; sucrose to industrialize insoluble extracellular polysaccharides, which increase attachment. The most isolated of significant from tooth caries samples are S. mutans (5) In the oral cavity Despite being ubiquitous, Streptococcus mutans spread often an indicates caries portability and no good oral hygiene. Streptococcus mutans is a gram positive cocci, food used not only for sticking to the dental, but also for synthesis, consequently detect acids. and allows low pH of the bacterium, preventing competitive bacteria from colonizing and eventually causing early decays (6). Usually, the appearance of S. mutans in the tooth cavities is followed by caries after 6-24 months (7).

In the bacteria, two component regulatory systems (TCRSs) are used as “molecular switches” in the response to ecology changes. Regulatory genes are essential for the bacterial adaptation, survival, and the virulence. Basically on the genome sequence, 13 TCRSs genes have been; identified in the S. mutans (8). One of these TCRSs, the vicRK signal transduction system, affects different virulence features of S. mutans (9). This system is consist of a histidine kinase sensor protein (vicK) located in the bacterial membrane and a cytoplasmic; response regulator protein (vicR). During phosphorylation reactions, extracellular signals is sensed via vicK, and then the vicK histidine kinase transmits the message to vicR, which is modulates gene expression (10). VIC genes regulate expression of several virulence associated genes that is affect produce and adhesion to polysaccharides, including gtfBCD, ffI, and gbpB. Furthermore it, compared with wild-type UA159 strain, strains without vicK form thumping biofilms, with a decrease rate of total format ion glucan (11). In addition, inactivation of vicK action a decrease level of lactic acid and best acid tolerance of S. mutans (10); a vicK knockout mutant have been present to be more sensitive to H2O2 than wild-type (12).

A vicR null mutation is present lethal to S. mutans. vicR acts directly on promoter regions of the gtfB, gtfC, and ffI genes. Over production of vicR transcript up regulates these genes (11). In addition, vicR binds specifically to; the comC gene, subsequently negatively affecting the transcription of comC, comDE, comX, and nlmC (13). The vicRK signal transduction system are basic for S. mutans by modulating gene expression. sequenced the vicR and vicK genes of the S. mutans strains isolated from; children with a distinct caries status to analyse effects of vicR and vicK polymorphisms on risk of Early childhood caries (ECC). Mutational analysis present that vicR in S. mutans plays an basic role in the viability vicR, vicK of this bacterium (11)."
Result & Discussion

A total of 22 dental caries in (3-17) age who attending to AL-Mamoon Specialized Dental Center and Yarmouk Health Center for Family Medicine in Baghdad city.

Genomic DNA Extraction The result of this study indicates that extracted DNA from bacterial cultuer sample dental caries was done

Detection of *vicR* gene

*vicR* gene was amplifid by Gradient PCR and then gel electrophoresis was verified by 1.5 % (w/v) agarose using a ladder (1500) as a molecular weight marker.

![Figure 1: Analysis of vicR gene product was electrophoresis on agarose gel (1.5 %) at 70 volt. 1x TBE buffer for 1 hours. vicR product band size (708 bp). Lane(N): DNA Marker sizer (1500) and lane 1-22 for sample dental caries.](image)

Sequencing of 708 bp amplicons of *vicR* gene

Eight samples was sent to Korea sequencing which had shown exactly 708 bp The sequencing reactions indicated the exact positions after performing NCBI for these PCR amplicons. Concerning the supposed 708 bp PCR amplicons of *vicR* gene, NCBI engine has shown extremely high sequences similarities between the sequenced samples and this target. NCBI (http:// www.ncbi.nlm.nih.gov) engine has indicated the presence of 100% of homology with the expected target that completely covered

appear analysis sequencing of *Streptococcus mutans* *vicR* gene no mutation because highly conserved among the clinical isolates

Detection of *vicK* gene

*vick* gene was ammpilfied by Gradient PCR and then gel electrophoresis was verified by 1.5 % (w/v)
agarose using a ladder (1500) as a molecular weight marker.

**Figure 2**: Analysis of *vicK* gene product was electrophoresis on agarose gel (1.5 %) at 70 volt. 1x TBE buffer for 1 hours. *vicK* product band size (1353 bp). Lane(N): DNA Marker sizer (1500) and lane 1-22 for sample dental caries.

**Sequencing of 1353 bp amplicons of *vicK* gene**

Eight samples was sent to Korea sequencing which had shown exactly 1353 bp The sequencing reactions indicated the exact positions after performing NCBI for these PCR amplicons. Concerning the supposed 1353 bp PCR amplicons of *vicK* gene, NCBI engine has shown extremely high sequences similarities between the sequenced samples and this target. NCBI (http://www.ncbi.nlm.nih.gov) engine has indicated the presence of 99% of homology with the expected target that completely covered

| Table ( 1 ) analysis sequencing of *Streptococcus mutans* *vicK* gene |
|---------------------------------|-----------------|--------------------------|--------------------------|
| Type mutation                   | Nucleotide change          | Amino acid change        | Isolation                |
| silent mutation                 | AAA->AAG               | Lysine> Lysine          | All isolation            |
|                                 | GAA->GAG,               | Glutamic acid> Glutamic acid |
|                                 | AAT->AAC                | Asparagine> Asparagine   |
|                                 | ACC->ACT                | Threonine> Threonine     |
|                                 | GTA->GTT                | Arginine> Arginine       |

Sequencing of 1353 bp amplicons of *vicK* gene

Eight samples was sent to Korea sequencing which had shown exactly 1353 bp The sequencing reactions indicated the exact positions after performing NCBI for these PCR amplicons. Concerning the supposed 1353 bp PCR amplicons of *vicK* gene, NCBI engine has shown extremely high sequences similarities between the sequenced samples and this target. NCBI (http://www.ncbi.nlm.nih.gov) engine has indicated the presence of 99% of homology with the expected target that completely covered

| Table ( 1 ) analysis sequencing of *Streptococcus mutans* *vicK* gene |
|---------------------------------|-----------------|--------------------------|--------------------------|
| Type mutation                   | Nucleotide change          | Amino acid change        | Isolation                |
| silent mutation                 | AAA->AAG               | Lysine> Lysine          | All isolation            |
|                                 | GAA->GAG,               | Glutamic acid> Glutamic acid |
|                                 | AAT->AAC                | Asparagine> Asparagine   |
|                                 | ACC->ACT                | Threonine> Threonine     |
|                                 | GTA->GTT                | Arginine> Arginine       |
This table 1 appear that most of mutation was silent mutation in all bacterial isolates that mean the mutation change to the same amino acid change may well have little effect on the protein since the substituted amino acids are similar to the original (they are all hydrophobic amino acid) such as Lysine > Lysine AAA>AAG, Glutamic acid > Glutamic acid GAA>GAG, Asparagine > Asparagine AAT>AAC, Threonine > Threonine ACC>ACT, Arginine > Arginine GTA>GTT But four isolates (2,3 and 7) was give a missense mutation, Glycine > Arginine GGT>CGT, Asparagine > Lysine AAT>AAA, Glutamine > Histidine CAG>CAC, Glutamine > Arginine CAG>CGG, this mutation may be cause high biofilm product, (benefit mutation or positive mutation), mutation lead to decrease production biofilm.

### Detection of 16S rRNA gene

16S rRNA gene was used to increase the confirmation of bacteria *S. mutans* deposite begin diagnosis by GP 24 kit, vicK gene detected by electrophoresis Amplification was verified by electrophoresis 1.5% (w/v) agarose using a ladder(1500) as a molecular weight marker. An amplification of 16S rRNA from 22 isolates was performed to confirm bacterial identification, Identification of S. mutans isolates by using 16S rRNA is more accurate than bacteriological and biochemical assays. (15) demonstrate that 16S rRNA gene PCR was sensitivit, specific,and used for diagnosis of culture-negative bacterial Infections also useful for identification of bacterial pathogens in patients pretreated with antibiotics.

Figure 3: Analysis of 16S rRNA gene product was electrophoresis on agarose gel (1.5 %) at 70 volt. 1x TBE buffer for 1 hours. 16S rRNA product band size (1250 bp). Lane(N): DNA Marker sizer (1500) and lane 1-22 for sample dental caries.
Sequencing of 1250 bp amplicons of 16S rRNA gene

Ten samples was sent to Korea sequencing which had shown exactly 1250 bp The sequencing reactions indicated the exact positions after performing NCBI for these PCR amplicons. Concerning the supposed 1250 bp PCR amplicons of 16S rRNA gene, NCBI engine has shown extremely high sequences similarities between the sequenced samples and this target. NCBI(http:// www.ncbi.nlm.nih.gov) engine has indicated the presence of 98- 99% of homology with the expected target that completely covered.

In this appear 16S rRNA gene despite the emergence of mutations in all isolates they gave a ratio 98-99% S.mutans The sequence was blasted in NCBI against standard strain of S. mutans complete genome. The identifying result showed 98-99%.

Conclusion

The result of molecular study of vicR gene were appear no mutation in all isolates while 16S rRNA although have transition and transversion mutation but the percentage of similarity with NCBI (http:// www.ncbi.nlm.nih.gov) appear 98-99% that Streptococcus mutation partial 16S rRNA gene. While the Sequence of vicK gene that note more of mutation was silent mutation but missense mutation present in isolated that cause change in biofilm production.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References


Periotest Evaluation of Stability after Insertion of Temporary Anchorage Device

Hussain Jabar Kadhem¹, Akram Fasial Alhuwaizi²

¹Post graduate, ²Prof.; Department of Orthodontics, College of Dentistry, University of Baghdad, Baghdad/ Iraq

Abstract

Introduction: Skeletal anchorage devices was developed for absolute anchorage during orthodontic treatment. Because of immediate loading on orthodontic miniscrews, early stability became a essential requirement for loading forces on miniscrews.

Objective: The aim of this study was to evaluate the early stability of the miniscrews after two weeks of insertion and the effect of the length and the side of insertion of the miniscrews on its stability using Periotest device.

Material and Method: Thirty- three orthodontic patients in a collage clinic were recruited who required bilateral miniscrews for orthodontic anchorage. Under local anesthesia, each patient received two miniscrews in the maxillary buccal area (one side 1.6x8mm and the other side 1.6x10mm). after two week early stability measured using Periotest device.

Results: There was no significant different in stability of miniscrew between right and lift sides and between short and long miniscrews.

Conclusion: length of miniscrew and side of insertion do not affect the early stability of miniscrew.

Keywords: Periotest ; stability ; Insertion ; Temporary Anchorage Device

Introduction

Preparation of the anchorage is a extremely important part in the orthodontic treatment. The success of orthodontic treatment generally relied on the anchorage protocol planned for particular cases. Planning for anchorage and preparation before tooth movement is necessary to avoid inappropriate tooth movements and not to interfere with the orthodontic result (19). Absolute anchorage is defined as no movements of the anchorage segment as a result of the reaction forces applied to move the teeth (7). The success rates are reported to be 80-90%, which is to some extent inferior than that of miniplate and palatal screw (15). Miniscrews can simply be placed and removed with a uncomplicated procedure, can be loaded instantly, are commercially accessible in a number of sizes (width and lengths), and are relatively cost-effective (4).

Because of immediate loading on orthodontic miniscrews, early stability became a essential requirement for loading forces on miniscrews (12). It is considered as clinical condition of immobility of miniscrew and ability to resist loads in different directions (10).

The early stability of miniscrews is mostly held by mechanical retention between bone and miniscrew surface (18).

Early stability is controlled by factors such as overloading, bone density, cortical bone thickness , design of screw and root proximity (6,1). The Periotest measurements after implant placement are helpful in the assessment of early stability. Periotest® M is an electronic instrument comprised of a hand piece containing a metal slug that is accelerated towards a tooth/implant by an electro-magnet, the tapping rod strikes 16 times in 4
seconds. The contact duration of the slug on the tooth/implant is measured by an accelerometer. The software in the instrument is designed to relate contact time as a function of tooth mobility. The result is displayed digitally as Periotest® values (PTVs). The Periotest® values (PTVs), ranging from -8 to +50. 

Materials and Methods

Patient selection:

A total of Thirty-three patients undergoing orthodontic treatment who required miniscrew placement between second premolar and first molar teeth in the buccal side of dental arches bilaterally as part of their treatment plan at the Department of Orthodontics, College of Dentistry, University of Baghdad were recruited as study participants. Age range of the participants was between 18 and 28 years and all the participants were healthy, with no significant medical findings, or special needs. The study was reviewed and approved by the ethical committee of the University of Baghdad College of Dentistry.

Miniscrew installation protocol:

The miniscrews used in this study were a self-drilling, 1.6 mm in diameter, made of Titanium alloy (Hubit Company, Gyeonggi-do, South Korea). In one side an 8mm miniscrew was inserted and in the other side a 10mm miniscrew was used. The surgical procedure involved the following:

1. From the pretreatment OPG, the site of insertion was determined in order to reduce radiation exposure.

It was located interdentally in the buccal side of the posterior segment of the maxilla between the upper second premolar and first molar.

2. Infiltration local anesthesia approximately 0.45 mL (a quarter of acarpool) of 2% lidocaine with 1:100,000 epinephrine, was administrated into the alveolar mucosa above the miniscrew insertion site, in order to provide an adequate degree of anesthesia, while still permitting the patient feedback in case the miniscrew make contact with the periodontal ligament.

3. Miniscrews (8mm and 10mm length) were randomly assigned to right and left sides.

4. Miniscrews were inserted manually by a screw driver through the attached gingiva at an angle between 30 and 60 degrees. No mucoperiosteal flap was raised and no pilot hole was made.

5. A periapical x-ray was taken to confirm miniscrew position.

6. Post-operative care instructions were explained to the patient and given in a written sheet. Post-operative care instruction included:

   1. Gently brush mini-screw and use of soft bristle tooth brush.

   2. Don’t touch mini-screw with the tongue or finger.

   3. Avoid eating hart food during first two day of insertion.

   4. Don’t tap mini-screw head with toothbrush.

Figure 1: Miniscrew insertion.
Stability measurement

After two week of miniscrew placement, early stability was measured using Periotest® M (Figure 3). Measuring procedure achieved by holding the Periotest® M at right angle to the center toward the miniscrew head to be examined, the maximum deviation angle from the ortho-radial direction of percussion is 45 degree. In addition, the rod of Periotest® M and the test surface of miniscrew superstructure must maintain 0.6-2.5 mm distance according the (Periotest® M operating instructions), measurements were taken by placing the Periotest parallel and gingivobaccally to occlusal plane to head of miniscrew. Two repeated measurements were obtained for each implant and the mean of these two readings was taken. An audible sound will be emitted and the damping capacity was measured as a Periotest® M value (PTV), this value can range from -8 to +50, the lower the values represented the better the stability (Figure 3).
Inter examiner calibration

The inter examiner calibration was done to assess the standardization of the Stability values (Periotest M values), this calibration was done by the researcher and another examiner (A.G Msc oral surgeon who work on Periotest during Msc research) at same time on 20 TAD (10 patients) and non-significant differences were found between two readings as in Table 1.

Table 1: Intra-class Correlation Coefficient

<table>
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<th>95% Confidence Interval</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
</tr>
<tr>
<td>Single Measures</td>
<td>.998a</td>
<td>.995</td>
</tr>
<tr>
<td>Average Measures</td>
<td>.999c</td>
<td>.997</td>
</tr>
</tbody>
</table>
Results

A total of thirty-three patients were included in the study. Their average age was 22.9 years (range 18–28 years). In total, seventeen of them were females and sixteen were males. Mean, median, and standard deviation values of miniscrew stability are shown in table 2.

Table 2: Side difference of miniscrew stability.

<table>
<thead>
<tr>
<th>Side</th>
<th>Descriptive statistics</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Median</td>
</tr>
<tr>
<td>Right</td>
<td>33</td>
<td>4.6</td>
</tr>
<tr>
<td>Left</td>
<td>33</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Discussion

One of the most techniques to assess the stability of implant objectively and noninvasively is the Periotest Mentor. PTVs evaluates the miniscrew mobility indirectly to predict the early stability of implantation, the amount of osseointegration, and the prognosis of the implant (8).

Insertion of miniscrews with the self-drilling technique used, as proposed by the manufacturers, to leave out a hole of pilot drilling since the size of pilot hole and its depth have been found to control the early stability of mini-implants (3).

The results of this study showed that miniscrew length had no significant effect on early stability of miniscrew that agrees with (6) who compared the success rate of each miniscrew anchor in 51 patients with 151 miniscrew anchors. He used three types of titanium screws with different lengths and diameters (length, 6 mm, diameter 1.0 mm; length, 11 mm, diameter, 1.5 mm; length, 14 mm, diameter, 2.3 mm) and miniplates with 2 screws (diameter, 2.0 mm; length, 5 mm) and one of result of his study was lack of relationship between the miniscrew length with its stability if the miniscrew was longer than 5 mm. did not find that the length of the miniscrew had a significant correlation with implant stability clinically. (2) study stability of miniscrew experimentally. He also found that the miniscrew length does not have significant impact on their stability when measuring insertion torque. (16)

However, (14) found that the length of the inserted screws was an important risk factor. They used 8, 10, 12, and 14 mm miniscrews in the upper and lower jaws and left unloaded for 2 weeks. The authors findings emphasized that the miniscrews length was associated with their success rate, with higher success rates in the longer miniscrews. (17) suggest that the smaller diameter and shorter miniscrew had lesser survival rates than the longer miniscrews.

For side factor, the result of our study shown no significant different between right and left side. (13) studied 70 orthodontic patients with 140 miniscrews in the maxillary buccal bone between the first molar and second premolar the on both sides and divided them randomly into 2 groups: the first group received self-tapping miniscrew, and the second group had self-drilling miniscrews. They found that the left side with either method of placement had a higher success rate.
than the right side, but the differences between both sides were not statistically significant.\(^{(11)}\) also showed that the left side had higher rate of success and was more stable than the right side. The former two studies attributed to that hygiene on the left side of the dental arch is better by right-handed patients, who are the majority of the population. Better hygiene could decrease inflammation around the miniscrew.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**


Evaluation of Chlorhexidine Application on Shear Bond Strength in Different Brackets and Different Etching Techniques in Vitro Study

Bayan Ghdhban¹, Nidhal H. Ghaib²

¹Post graduate; ²Professor/College of Dentistry, University of Baghdad, Iraq

Abstract

Purpose of this study was to assess the etch enamel surface of teeth, affect “shear bond strength” in application chlorhexidine and the “adhesive remnant index” scores.

Material and Method: Eighty premolar teeth half of the samples (Groups A,B/metal bracket.C,D/ceramic bracket) were conditioned with using 37% orthophosphoric acid gel (SDI, California,USA) for 30s washed/dried. The other half of the samples (Groups E,F/metal bracket.G,H/ceramic bracket) were conditioned with Er:CrYSGG laser system, Power: 2W, Wave length: 2,780, energy: 250 mJ , Pulse duration: 140 μs Frequency: 20 Hz. , Cooling system: 90%:%80air-water spray , Mode of application: swiping motion (Waterlase,1 plus from Biolase USA) and then washed and dried. The groups(B,D,F,H) sealant was applied with CHX varnish.

Results: statistical analyses presented that SBSs of laser radiation were significantly difference than that of acid etching.

Conclusions: laser etched resulted the bond Strength accepted clinically in the metal, ceramic and could be an substitute to the acid etching .CHX sealant not significant affect in orthophosphoric acid etching but more weaker in laser etching.

Keywords: chlorhexidine application; shear bond strength; etching techniques; vitro study

Introduction

key to therapeutic success is get highly bond strength and less damage to the tooth surface. The bond strength between the bracket base and tooth surface should be sufficient to withstand the thermal and mechanical effect of the oral environment and have to provide sufficient resistance against stresses from arch wires and force of mastication(1,2),it is consider acid etching has been standard procedure for bonding orthodontic brackets .But the decalcified enamel surface after acid etching has the prospective disadvantage of making the enamel more liable to a caries attack . There is an urgent to found simpler clinical techniques with decrease enamel loss and provide clinically better bond strength. Lately, It has attracted significant attention to using laser radiation are Er:YAG, and Er,Cr:YSGG because it results in an irregular surface pattern which is similar to that of an acid etching pattern(3) and can remove the smear layer and it does not involve heat or vibration, It can be modifies crystalline and chemical structure of the tooth surface and more resistant to caries. (4). Fixed appliances difficulties maintaining proper oral hygiene, and easily dental plaque can accumulate therefore increase prevalence of plaque-induced diseases lead to a higher risk of rising gingivitis and decalcification (5). Prevention of dental caries plays a vital role in orthodontic treatment, strategies chemotherapeutic treatment regimens have received much attention and have presented satisfactory result. Chlorhexidin (CHX) as chemical antimicrobial substances are proficient of inhibiting bacterial adhesion, metabolic activity, and colonization by reducing the bacterial growth. CHX collective with thymol in a varnish have excellent adsorption to the tooth surface for long period, prevent its immediate loss, thus acting
as slow-releasing reservoirs\(^6\), this study has been established to evaluate and comparable the laser etching with acid-etching and to decide the suitability of these with chlorhexidine varnish.

**Materials and Methods**

Sample organization and preparation: The selected (82) upper premolar teeth intact enamel, no caries and cracks. The teeth were pumiced/ washed-dried for (10) s. The teeth embedded in acrylic resin and align the buccal surface of the tooth with mounting jig was used to be parallel to the force applied through shear test\(^7\). The power of laser output can be various from 0-6W determined the tissue to be cut. The power settings for laser groups had been selected on the basis dentinal studies and pilot study, the using 2 W power, the rate repetition pulse 20 pulses / second (20 Hz), a duration of the pulse 140 microseconds\(^8\),\(^9\),\(^10\). The Er,Cr:YSGG laser (Waterlase, I plus from Biolase USA ), is produces accurate cuts for hard tissue via interaction with water at the enamel surface. The air levels were 90% and water 80%, respectively. To standardized the distance, The beam of laser was a vertical to the tooth surface at a distance of 1 m\(^11\), a sweeping manner was moved by hand over an about 4*4 an acrylic resin with a gap, was retained on the enamel surface during an exposure time of 15 seconds\(^1\) (Fig:1-1). All specimens were randomly divided into:

- **Group A (10teeth):** Enamel etched with 37% orthophosphoric acid for 30 second, then rinsed/dried for 15 second then bonding metal bracket as control group.

- **Group B (10teeth):** Enamel etched with 37% orthophosphoric acid for 30 second then rinsed/dried for 15 second then bonding metal bracket with chlorhexidine sealant.

- **Group C (10teeth):** Enamel etched with 37% orthophosphoric acid for 30 second then rinsed/dried for 15 second then bonding ceramic bracket without chlorhexidine sealant.

- **Group D (10teeth):** Enamel etched with 37% orthophosphoric acid for 30 second then rinsed/dried for 15 second then bonding ceramic bracket with chlorhexidine sealant.

- **Group E (10teeth):** Enamel irradiated with the Er,Cr:YSGG laser at 2 W for 15 seconds then washed/dried for 15 second then bonding metal bracket without chlorhexidine sealant

- **Group F (10teeth):** Enamel irradiated with the Er,Cr:YSGG laser at 2 W for 15 seconds then washed/dried for 15 second then bonding metal with chlorhexidine sealant

- **Group G (10teeth):** Enamel irradiated with the Er,Cr:YSGG laser at 2 W for 15 seconds then washed/dried for 15 second then bonding ceramic bracket without CHX sealant.

- **Group H (10teeth):** Enamel irradiated with the Er,Cr:YSGG laser at 2 W for 15 seconds and bonding ceramic bracket with chlorhexidine sealant.

The two teeth were utilized for observation by “scanning electron microscopy” (SEM) to decide the morphology/topography of the etching enamel.

Adhesive preparation: The group B,D, F,H were incorporated, with CHX varnish has equivalent, thymol+CHX (1 mg of each/gm) ,two drops of CHX were added to every one drop of primer 2:1 and then mixed\(^12\). Thin uniform coat was applied to etched enamel surface from mixture Transbond XT primer and curing for 15 second all groups\(^13\).

Bonding Procedure: each group of the brackets were bonded to premolars utilizing primer / resin (3M Unitek,Monrovia, U.S.A),in the center of the facial surface of the tooth. Sufficient pressure “ 200 gm. load on the upper part of the vertical arm of the surveyor” to promote excess adhesive, which was removed by probe, and then brackets light cured for 10s;5s for each side. the intensity of light was 1200 m W/cm .later exposed to thermocycling 500 times in distilled water between 5uC and 55uC, with a dwell time in each bath of 30s and a transfer time of 15s. All specimens accomplished by using “Tinius `Olsen universal testing machine with 5 KN load cell and a crosshead speed of 1 mm/minute” \(^13\),as a result that the readings in newtons
Then, values were altered into megapascals (MP) via dividing the force on the base of bracket area (metal 10.50mm$^2$/ceramic10.75 mm$^2$). The facial surface of every tooth were inspected under a stereomicroscope (10X magnification) later debonded bracket for conclude the ARI score to define quantity of resin residual on the enamel surfaces was predominant site of bond failure [Artun and Bergland, 1984](14). ARI ranges between 0 - 3 (0: no residual resin covering on tooth; 1: less than 50% resin covering on tooth; 2: more than 50% residual resin on tooth; 3: 100% residual resin covering on tooth). Data were collected and analyzed using SPSS program version 25. The descriptive statistics included means, standard deviations, frequency distribution and percentage, while the inferential statistics included Shapiro-Wilk test, independent sample t-test and Chi-square test.

Results

SBS in descriptive statistics was expressed in (MPa) for 8 groups tested are existing in (Table:1/Fig:1). Anova revealed the existence of significant differences (P<0.0001) between laser etching/acid etching and Tukey test informed that with conventional acid etching, the highest SBS values were recorded than laser etching. Adding CHX as the sealant lowers SBS in laser groups, but no more effect in acid etching special in metal bracket (Table:2/Fig:1). Chi-square comparison of the ARI scores between entirely groups (Table:3/Fig:2) shown that the groups were significantly different. The minimum quantity of residual adhesive was found in laser groups. SEM analysis revealed the different etching patterns gained for every group.

Table 1 : Descriptive statistics and comparison the effect of etching type with CHX on the shear bond strength of different brackets

<table>
<thead>
<tr>
<th>Etching types</th>
<th>Presence or absence of CHX</th>
<th>Descriptive statistics</th>
<th>Comparison (d.f.=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MB Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>Without CHX</td>
<td>13.963</td>
<td>2.576</td>
</tr>
<tr>
<td></td>
<td>With CHX</td>
<td>12.396</td>
<td>2.069</td>
</tr>
<tr>
<td>LASER</td>
<td>Without CHX</td>
<td>11.053</td>
<td>2.227</td>
</tr>
<tr>
<td></td>
<td>With CHX</td>
<td>5.923</td>
<td>1.152</td>
</tr>
</tbody>
</table>
Table 2: Descriptive statistics and comparison the effect of chlorhexidine on the shear bond strength of different brackets

<table>
<thead>
<tr>
<th>Etching types</th>
<th>Brackets' types</th>
<th>Descriptive statistics</th>
<th>Comparison (d.f.=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without CHX</td>
<td>With CHX</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>MB</td>
<td>13.963</td>
<td>2.576</td>
</tr>
<tr>
<td></td>
<td>CB</td>
<td>19.542</td>
<td>3.994</td>
</tr>
<tr>
<td>LASER</td>
<td>MB</td>
<td>11.053</td>
<td>2.227</td>
</tr>
<tr>
<td></td>
<td>CB</td>
<td>9.462</td>
<td>1.160</td>
</tr>
</tbody>
</table>

FIG. 1 Box plot of shear bond strengths (MPa) of the different groups tested.
Fig.2: Frequency distribution of adhesive remnant index

Table 3: Frequency distribution and percentage of ARI of different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Score 0</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0 (0%)</td>
<td>3 (30%)</td>
<td>3 (30%)</td>
<td>4 (40%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>B</td>
<td>2 (20%)</td>
<td>6 (60%)</td>
<td>2 (20%)</td>
<td>0 (0%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>C</td>
<td>0 (0%)</td>
<td>2 (20%)</td>
<td>5 (50%)</td>
<td>3 (30%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>D</td>
<td>8 (80%)</td>
<td>1 (10%)</td>
<td>1 (10%)</td>
<td>0 (0%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>E</td>
<td>2 (20%)</td>
<td>7 (70%)</td>
<td>1 (10%)</td>
<td>0 (0%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>F</td>
<td>9 (90%)</td>
<td>1 (10%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>G</td>
<td>7 (70%)</td>
<td>3 (30%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>H</td>
<td>10 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>10 (100%)</td>
</tr>
</tbody>
</table>

Discussion

Acid etching is considered the well technique of attachment resins to tooth surface\textsuperscript{(15)}. But it is more susceptible to acid attack as a result of plaque accumulation nearby to the brackets especially when saliva contamination and air bubbles disturb the resin penetrance. Recently introduced laser etching as an alternative to the acid etching, because it decreases the caries risk by altering the calcium to phosphorus ratio. But the lased surface is fissured and less homogenous\textsuperscript{(1)}. We aimed to find the most suitable enamel preparing techniques for bracket attachment in terms of SBS, ARI, and SEM observations, with minimum caries risk by CHX application.
In present study, the laser parameters determine based on a previous studies and pilot study \(^{(8)}(9)(10)\). The results of present study revealed that SBS values of brackets bonded with laser etching were significantly lesser than that of acid etching in both (LM, LC brackets). Reynolds \(^{29}\) recommended the SBS ranged from 6 to 8 MPa clinically acceptable bonding. So, the SBS values for LM and LC groups in present study are still acceptable clinically strengths for bracket bonding. These results agree with the findings to \(^{(8,10,16)}\). But is different from Findings \(^{(17,9)}\).

Probably this Contradictory the findings relating they utilized more various power outputs of laser technique and varies nature curvature enamel structures or formed micro pores, craters in enamel and melted bubbles formed during laser etching or may be as result of the using sweeping motion by hand controlled of the laser beam during the etching may be reason a inadequately standardized etching pattern during the irradiated area. Further might standrazition be aid to solve this problem, Our results also agree with the findings of Nakamura et al \(^{(18)}\). Chlorhexidine such as in Cervitec plus (1% Chlorhexidine +1%thymol) has proved to be better antimicrobial agent, therefor using as the sealant with primer agent, to reduce minimal time application and does not rely on patient compliance. Also retained on to the oral surfaces by attaches to the glycoproteins by reversal electrostatic binding thus gets CHX releases slowly into the oral environment permitting for a prolonged antimicrobial effect \(^{(19)}\). Laser in CHX sealant commonly weaker this is probably causing a existence of micro cracks under close enamel particles, this may formed a weakened substrate, this is more susceptible to the happening of fractures during measured bond strength testing \(^{(20)}\). But is more acceptable in acid etching this agreement with Karaman \(^{(21)}\).

In acid etching more ARI score was found on facial surface resultant in decreased enamel fracture risk and final more “bracket bond strength”. These results were in agreement with Samruajbenjakul et al \(^{(22)}\). reduce ARI score was found in laser etching and sealant with CHX. This means a smaller quantity of adhesive remaining on tooth surface resulting in relatively reduced SBS. These results were comparable with studies showed via Sibi et al, \(^{(23)}\).

Different etching patterns achieved in SEM analysis, according to Silverstone et al \(^{(24)}\), the acid etched enamel shows cracks in some regions, and produced enamel surface resembled type 1 pattern of etching (Fig: 4).

![Fig.4: Scanning electron microscope image of an enamel surface etched with acid](image)

**Conclusions**

The results of the study point to that etching of tooth surface with an” acid etching with 37% orthophosphoric acid for 30s” is more predictable bond strengths than did “Er,Cr:YSGG hydrokinetic laser system”. But laser can be consider acceptable bond strength more practical and quicker than acid etching. CHX sealant did not influence bond strength in acid etching but not significant in laser etching.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq  

**Conflict of Interest:** None  

**Funding:** Self-funding  

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19. DePaola LG, Spolarich AE. Safety and efficacy


Evaluation Hormonal State in Iraqi Infertility Females Under In-Vitro Fertilization Program

Zainab Sabah Hassab¹, Bushra J. Al-Musawi², Asmaa M. Salih Almohaidi³
¹Assist Lect., College of Science for Women, University of Baghdad, Iraq. ²Lect. Dr. Kumal Al-Samurai -IVF Hospital, Ministry of Health, Baghdad, Iraq. ³Prof. Dr. College of Science for Women, University of Baghdad, Iraq.

Abstract

Background: Uniqueness of the current study at first time in Iraq specially, across the comparison the clinical parameters among Iraqi infertility females according the IVF program outcome: failures and success implantation. While, aspect were neglected in IVF field. Also, to difficulty of some parameters comparing with control.

Methods: This study done during (December 2019 to November 2020) on Iraqi infertile females with age range (19 – 45)ys. It included the two groups: success implantation and failure implantation females.

Result: The current study revealed to that , secondary infertility is a good marker in implantation success prediction. Central circumference increasing has been associated with implantation failure (P<0.05). When hormonal profile comparison among two groups. Failure implantation females have non-significant increasing in basic prolactin values. Also, E2, progesterone and LH values were higher significantly(P>0.001), among success implantation. The hCG values were highly significant in failure implantation (P>0.001). MII oocytes and G2 embryos were highly significant in success implantation. Our result support that no. of embryo cells have positive impact on IVF outcome. The S. TC and TG were significant increasing among failure implantation.

Key words: hormonal state, Infertility female, IVF, toxicity

Introduction

Implantation is phase at which the embryo adheres to the wall of the uterus¹. The implantation required the synchronism among the endometrium maturation and embryo(s) development². The clinical window of implantation (WOI) is the time frame; in which IVF embryo(s) must be transferred. The optimal period for WOI is known as the period (20-24) of natural cycles, or after 2 or 3 days of egg-retrieval in an IVF cycles³. After IVF, 25-30 % of the embryo(s) transferred to the uterus are successful in implantation⁴.

After birth, 1-2 million cells remain in the ovary; no new oocytes are formed. Follicle stimulating hormone (FSH) promotes the growth of multiple follicles at different stages of development⁵. Luteinizing hormone (LH) triggers follicle bursts and the release of ova into the fallopian tube. The corpus luteum(CL) is created, when luteinized granulosa cells combine with luteinized theca and surrounding the stroma in the ovary⁶. CL was a temporary endocrine organ that secreted the hormones progesterone and estradiol (E2). E2 causes uterine swelling, which may aid in pressing the blastocyst into the endometrium⁷. After ovulation, progesterone is released during the luteal phase. The endometrium cannot be prepared for implantation, if progesterone levels are insufficient. While, Human chorionic gonadotropin (hCG) functions to maintain of gestational yellow body and controls the development of progesterone and estrogen⁸.

The purposes of present study: Evaluate the hormonal and demographic state of Iraqi infertility women during IVF.
Materials and Methods

Study done over a ten months (December 2019 to November 2020), on 70 Iraqi infertile females during IVF, age range was 19 – 45 years. Samples collected from: the Kamal Al-Samurai hospital. Iraqi infertility females were assessed clinically by physician. IVF program was beginning 2-3 day of cycle. There are forms to determine the number and quality each of ova and embryo during IVF. The embryos were transferred, at two or three days after cleavage began.

Subject undergo to questioning for obtain some demographic and clinical information. Also, Subject divided into two groups according IVF outcome: Implantation failure group comprised 50 infertile Women. While, other group 20 subjects of implantation success. Serum lipid profiles were measured. Also, Serum hormonal profile tests done by VIDAS- auto analyzer as the following:

i. Basal hormonal profile (LH; FSH; TSH and prolactin) in the second day of menstrual cycle for normal ovarian reserve.

ii. E2 Follow- up of IVF to assessment of ovulation occurrence

iii. At embryo transfer (ET) (LH; Progesterone). Furthermore, β-HCG after 14day of ET to IVF outcomes reported.

Results

Current study described the demographic and clinical data of Iraqi infertility females. 58.57% have primary, 41.43% secondary infertility type (P>0.05). Also, the study recorded the highly significant increasing (P<0.001) of abnormal ovulation through 36 cases (51.43%), 19 cases (27.14%) have unexplained infertility. While, Uterine factor were seen in 10 cases (14.29%), only 3 cases (4.29%) and 2(2.86%) cases with endometriosis and tubal factor, at respectively. While, patients distributed according outcome of implantation:

Success implantation forms 28.57%, failure implantation 71.43% (P<0.001). Infertility duration distributed into four categories: 1-5 ys (37.14%), 6-10 ys (41.43%), 11-15 ys (15.71%) and >15 ys (5.71%) (P<0.01). Ages distributed between 20-25 ys (30%), 26-30 ys (34.29%) and >30 ys (35.71%) (P<0.05). While, BMI distributed significantly(P>0.05) into three categories: 18-24 Kg (21.43%), 25-30 Kg (54.29%) and >30 Kg (24.29%). Waist/hip ratio (WHR) (Kg) recorded lower significant (P<0.05) for group <0.85 (25.71%) than for group >0.85(74.29%).

The success of implantation strongly (P<0.05) in secondary infertility (60%) than primary Iraqi infertility females (40%) as following in table (1):

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Success (No./%)</th>
<th>Failure (No./%)</th>
<th>Chi-square</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of infertility</td>
<td>Primary</td>
<td>8(40%)</td>
<td>33(66%)</td>
<td>3.980</td>
</tr>
<tr>
<td></td>
<td>Secondary</td>
<td>12(60%)</td>
<td>17(34%)</td>
<td></td>
</tr>
</tbody>
</table>

In obstetric history: Gravidity has non-significant increasing of failure implantation group than success. The later can be disturbuted to non- significant increasing to abortion among failure implantation group, as shown in Figure(1).
Figure (1) Comparison of Obstetric History between Success and Failure Implantation Groups

Non-significant differences of demographic parameters (Age, BMI, Waist/Hip Ratio, and Duration of Infertility) of Iraqi infertility females among two groups during IVF (P>0.05). While, This study recorded significant increasing (P<0.05) in central circumference among failure implantation group, as shown in table 2:

Table (2) Demographic Characteristics of Study Samples among Success and Failure Implantation Groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Success (Mean± S.E)</th>
<th>Failure (Mean± S.E)</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>29.1±1.27</td>
<td>29.65±0.85</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>BMI</td>
<td>28.73±0.752</td>
<td>29.24±0.908</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Waist/ Hip ratio</td>
<td>0.80±0.01</td>
<td>0.81±0.01</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Central circumference (Cm)</td>
<td>99±1.67</td>
<td>104.31±1.45</td>
<td>P&lt;0.05*</td>
</tr>
<tr>
<td>Duration of Infertility(ys)</td>
<td>7.4±1.075</td>
<td>8.16±0.569</td>
<td>P&gt;0.05</td>
</tr>
</tbody>
</table>

The current study recorded non-significant increasing (P>0.05) in LH and FSH basic in success implantation. While, non-significant decreasing of prolactin basic among the same group. E2 levels for success implantation had highest value (857.11±88.96) at (P>0.001).

At ET: There was non-significant increasing in LH among success implantation. Also, non-significant increasing in progesterone hormone among success implantation. But, the current study recorded highly significant increasing (P>0.001) in hCG values that were (41.60) in failure implantation than (12.05) in success, as shown table (3):
### Table 3: Hormonal Profile in Success and Failure Implantation Groups via IVF

<table>
<thead>
<tr>
<th>Hormonal profile</th>
<th>Outcome of implantation</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Success (Mean± S.E)</td>
<td>Failure (Mean± S.E)</td>
</tr>
<tr>
<td>Hormonal basic (CD2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>6.36±1.37</td>
<td>4.65±0.68</td>
</tr>
<tr>
<td>FSH</td>
<td>5.75±0.98</td>
<td>5.76±0.57</td>
</tr>
<tr>
<td>Prolactin</td>
<td>18.59±3.21</td>
<td>22.73±2.29</td>
</tr>
<tr>
<td>TSH</td>
<td>3.61±1.06</td>
<td>2.13±0.16</td>
</tr>
<tr>
<td>Follow up IVF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>857.11±88.96</td>
<td>563.44±43.40</td>
</tr>
<tr>
<td>At embryo transfer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>4.58±1.50</td>
<td>2.11±0.63</td>
</tr>
<tr>
<td>HCG</td>
<td>12.05±3.63</td>
<td>41.60±4.22</td>
</tr>
<tr>
<td>Progesterone</td>
<td>41.91±5.25</td>
<td>40.56±3.38</td>
</tr>
</tbody>
</table>

There are highly significant in total no. ova in success (12.40±0.97) than (7.58±0.62) failure implantation group of Iraqi infertility women during IVF (P>0.001). Also, highly significant (P>0.001) recorded in MII oocytes in success (7.95±0.88) than failure group (4.10±0.55). Total embryo(s) have highly significant increasing (P>0.001), among success implantation group. The mean were (4.65±0.48) in success, While it was (3.26±0.27) in failure implantation. Non-significant increasing of Grade 1 (G1) embryos (P>0.05). While, highly significant for Grade 2 (G2) embryos means, that were 1.25±0.39 in success and 0.26±0.08 of failure implantation. Mean embryo cells no. has significant increasing (P<0.01) in success implantation (6.80) than failure implantation group (5.44), as shown in table (4).

### Table 4: The Ova and Embryos Quality in Success and Failure Implantation Groups via IVF

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Outcome of implantation</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Success(Mean±S.E)</td>
<td>Failure (Mean± S.E)</td>
</tr>
<tr>
<td>Ova Quality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Ova</td>
<td>12.40±0.97</td>
<td>7.58±0.62</td>
</tr>
<tr>
<td>GV</td>
<td>0.90±0.35</td>
<td>0.76±0.16</td>
</tr>
<tr>
<td>MI</td>
<td>1.50±0.44</td>
<td>1.04±0.21</td>
</tr>
<tr>
<td>MII</td>
<td>7.95±0.88</td>
<td>4.10±0.55</td>
</tr>
<tr>
<td>Rapture</td>
<td>1.95±0.478</td>
<td>1.44±0.254</td>
</tr>
</tbody>
</table>
Embryo Quality

<table>
<thead>
<tr>
<th>Embryo Quality</th>
<th>Total Embryo</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>Embryo Cells No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.65±0.48</td>
<td>2.40±0.37</td>
<td>1.25±0.39</td>
<td>0±0</td>
<td>6.80±0.47</td>
</tr>
<tr>
<td></td>
<td>3.26±0.27</td>
<td>1.58±0.28</td>
<td>0.26±0.08</td>
<td>0.12±0.05</td>
<td>5.44±0.32</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.01*</td>
<td>P&gt;0.05</td>
<td>P&lt;0.01*</td>
<td>P&gt;0.05</td>
<td>P&lt;0.01*</td>
</tr>
</tbody>
</table>

MI Oocyte(pre-ovulatory); MII Oocyte(mature); GV Oocyte(immature)

According the Table (5): The total cholesterol (TC) levels have highly significant increasing in failure than success implantation (P>0.001). The Means were 133.9±5.64 in failure, 111.9±5.77 in success group. Also, there was increasing significant for S. triglyceride and S. HDL between failure and success groups (P>0.05, P>0.01). Means were (95.4±6.33), (113.57±5.62) for S. triglyceride(TG) and (51.12±1.95), (42.75±2.78) for S.HDL at receptively. While, non-significant deference was reported in S.LDL among failure and success implantation. VLDL had highly significant (P>0.01), in implantation failure than success group.

Table(5) Lipid Profile in Success and Failure Implantation Groups via IVF

<table>
<thead>
<tr>
<th>Lipid profile</th>
<th>Outcome of Implantation</th>
<th>P- Value</th>
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<tr>
<td></td>
<td>Success (Mean± S.E)</td>
<td>Failure (Mean± S.E)</td>
</tr>
<tr>
<td>S.TC</td>
<td>111.9±5.77</td>
<td>133.9±5.64</td>
</tr>
<tr>
<td>S.TG</td>
<td>95.4±6.33</td>
<td>113.57±5.62</td>
</tr>
<tr>
<td>S.HDL Chol.</td>
<td>42.75±2.78</td>
<td>51.12±1.95</td>
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<tr>
<td>S.LDL Chol.</td>
<td>54.19±5.5</td>
<td>60.9±4.17</td>
</tr>
<tr>
<td>VLDL</td>
<td>19.11±1.27</td>
<td>22.67±1.14</td>
</tr>
<tr>
<td>AIP</td>
<td>0.28±0.05</td>
<td>0.39±0.04</td>
</tr>
</tbody>
</table>

Discussion

Infertility type has significantly effect on outcome of implantation among Iraqi infertile females. Current result agree with Hambartsoumian,1998 study(9). Also, the present result, can be contributed according the Moragianni, 2012 suggest, to the counseling and psychological support are required to enhance the IVF success (10). In present study: Gravidity means seem non-significant higher among failure than success implantation. This is back to non-significant increasing of abortion in failure than success group . Although the current study does not accept with significant lowering for gravidity and parity among infertility females as
compared to the control (11).

The present study found no effect of age on the implantation success, which agree with Abdalla et al. study (12). Also, Other study reported no significant relation for BMI and age means between primary infertility and control (13). Furthermore, the age means for infertile females and the control group included in the study were identical (14). There was highly significant increasing of success implantation rate in BMI<30 than >30(15). Central obesity lead to decrease the chance of implantation success according the current study. Central circumference is a tool to assess lipid excess and ART prediction of infertility women (16).

Through comparable hormonal profile of current study, among success and failure implantation during IVF. Current basic hormonal were similar to Tawfeek et al. for each of: prolactin; FSH and LH (14). Thyroid dysfunction associated to fertility decreasing (17). Other research suggests that infertility women with an abnormal menstrual cycle should have checked TSH (18). The current study agree with important of E2 for induction of implantation to improved IVF efficiency (19).

At ET: non-significant decreasing of LH between failure implantation women of current study. This can be supported by the report that refer to that, Luteal phase deficiency (LPD) is widespread among failed implantation females (20). The change in progesterone levels in LPD, leads to a rise implantation failure rate (21). For high significance of hCG among failure implantation group of this study; disagree with non-significant differences of implantation rates in higher or lower the hCG cycles (22). The current study matched with Strom et al. 2012 decreased of hCG levels through ET at cleavage stage in success implantation(23). Also, Other report refer to: high doses of hCG administration is associated significantly with decreased of implantation, (24). The current study showed non-significant increasing progesterone level, that comparable with previous studies; through the progesterone kept the fetus from the mother’s immune, during secretory phase of cycle (25).

This study agree with Khalaf Allah et al., 2020 through the oocyte no. is essential to be evaluated the IVF outcome (26). Also, The no. of eggs obtained has no impact on the probability of implantation (22). With increasing MII oocyte and G2 embryos among success group in current study. Other reports refer to non-association with in MII no. (27).

Present results suggest the lipid may be used as indicator of fertility. Other designs revealed to the TG, TC, LDL levels were highest, but HDL were lowers in un-explain infertility females than fertiles (28;29).

**Conclusion**

Although, afraid the couples of IVF outcome. But, There are many factors that effect in the implantation success rate during IVF. Specially, central obesity and hormonal profile. So, the control of these factors across the BMI , LH, Progesterone, E2, hCG balance, Oocyte pick up number and quality will lead to significant increasing in success implantation during IVF.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**


Study the Synergistic Effect of AgNPs with Antibiotics on Klebsiella Pneumoniae Isolates Which Diagnosed by Molecular Methods

Sura Saadoun Khalaf1, Hanaa Abdellatif Yassin2

1Post Graduate; 2Assist Prof Department of Biology College of Education For Woman University of Anbar/Iraq

Abstract

The current study was conducted to study the synergistic effect of AgNPs with anti-biotics on Klebsiella pneumonia isolates, where 50 samples were collected from different pathological conditions (wounds - burns - urine - ear - and excretion) on the Maconkey Agar. To the Klebsiella, it was diagnosed microscopically and biochemically and was also diagnosed by the Vitek2 device, whose results were identical with the biochemical diagnosis, and the isolates were also subjected to molecular diagnosis for confirmation by using the PCR technique based on the diagnostic gene 16SrRNA, which is characterized by being good, stable and low heterogeneity for a long time in the bacterial type. Ten types of antibiotics were selected and immersed in silver nanoparticles at a concentration of 20Mm, a test was conducted for the synergy between the silver nanoparticles AgNPs and the antagonists towards 12 isolates of Klebsiella bacteria, as they were applied to Muller-Hunton medium and after they dried up, the anti-saturated tablets were fixed with a silver particle solution, and after the expiration of the incubation period, the diameter of the inhibition area around the tablets was measured in mm. According to the standard results, the results of the statistical analysis showed that the interaction of silver nanoparticles with the antibiotics had a significant effect on increasing the sensitivity of the isolates towards the synergistic antagonists, as the general rate of inhibition diameters of the antagonists (Impenem, Gentamicin, Amikacin Ciprofloxacillin) was (32.59, 21.6, 18.98, 36.18), respectively. This activity is due to the ability of the nanoparticles to bind to the surface of the bacterial cell. The binding causes structural damage and changes such as permeability, which facilitates the entry of the antagonist into the cell and thus its death. As for the synergies with the antagonists Tetracycline, Vancomycin, Erythromycin did not show efficacy in the direction of Klebsiella isolates did not give good results of synergy as it has a weak effect towards negative bacteria and it is as a non-synergistic antagonist because it does not have effective binding sites for silver nanoparticles.

Keywords: molecular methods; health; death; toxicity; synergy

Introduction

Nanotechnology or nanoparticle technology, which is the science that studies the treatment of matter on the atomic and molecular scale. Nanotechnology is interested in creating new techniques and means whose dimensions are measured in nanometers, which is a thousandth of a micrometer, i.e. a part of a millionth of a millimeter, nanotechnology deals with measurements between 100-1 nanometer, i.e. it deals with atomic assemblies ranging from five to one thousand atoms, which are of dimensions much less than those of bacteria and a living cell (1). Silver nanoparticles are one of the most common types of nanoparticles known, with multiple applications, lower costs, and have antibacterial efficacy for a wide range of pathogenic and antibiotic resistance. As some antibiotics have become useless in the treatment of diseases, especially acute and pathogenic diseases, as increasing the dose beyond the required limit or the frequent use of antibiotics or cutting treatment without completing it allows the bacteria to become more resistant to those antibiotics, so it has become necessary to find alternative treatment methods to produce effective active substances. Health benefit without side effects, and these alternative solutions
include nanoparticles, including silver nanoparticles. One of the characteristics of *Klebsiella* is that it has a capsule, so its colonies are mucous and sticky, and it is also able to release large quantities of the mucous gel on the surface of the core of the components in it, where a strand of mucus can be observed when touching the colony with the object. One of the common diseases that this bacteria cause to humans are burn and wound. It also causes pneumonia, which causes inflammation of the air sacs in the lungs, if these sacs or vesicles are filled with liquid waste and pus and cause bronchitis and severe pneumonia. The genus *Klebsiella* spreads in humans in the form of throwing organisms in the nasopharynx and intestinal tract, as the bacteria are swallowed with food and drinks contaminated with bacteria or by contact and transmitted through the spray of respiratory patients from one patient to another. The ability of the bacterium to cause infection is due to its possession of many virulence factors that play an important role in causing disease and enable it to reduce and multiply within the host’s body, including the capsule and the adhesion factors that enable it to attach to the surface of the host cell. *Klebsiella* possesses virulence genes that have the ability to evade and mask the process of phagocytosis by large phages to enable them to resist antibiotics. Bacterial resistance to antibiotics is a real threat to patients, as some antibiotics have become preferred food for some types of bacteria and their activity increases. In addition to the agricultural environment, the reason for this resistance is due to the widespread and frequent use of antibiotics, and this problem is one of the most medical problems facing the world, which leads to lack of control of diseases, and from a genetic point of view there are genetic elements called plasmids, which are an important vector for genes responsible for factors Virulence in bacteria, such as biofilm and hemolysin production, is the main cause of bacterial cysts events.

**Materials and Methods**

**Sample collection**

30 samples were collected from different pathologies. The samples included 10 samples from urine, 5 samples from wounds, 6 samples from burns, 5 samples from the ears, and 4 samples from the exit. The samples were planted directly in the planning method on suitable agricultural environments such as the environment Acids for 24 hours at a temperature of 37 °C, and after the first transplantation of samples and purification of colonies, the samples were then diagnosed by biochemical and microscopic diagnosis, and *Klebsiella* was negative for the oxidase test, the indol test, the methyl red test, and positive for the Catalase test, and the Fox-Proscor test. Proskauer-Voges, also has the ability to ferment the sugar lactose.

Diagnosis was made based on the culture, microscopic and biochemical characteristics.

Molecularly diagnosed by PCR method

DNA was extracted and purified using a ready-made DNA extraction kit and according to the instructions of the American manufacturer Promega The concentration and purity of the DNA genetic material was measured using the Fluor meter Quintus device, which has high accuracy and sensitivity compared to the Nano drop, and then set and programmed the PCR device according to the optimum temperature of the mutant and correlation Prefixes and number of cycles. Then, 5 μL of the product of gene duplication was transferred to electrophoresis on an acarose gel. Where DNA loading was carried out in the gel pits and electrocuted on a horizontal surface. Then, 5 μl of the product of gene duplication was transferred to electrophoresis on an acarose gel. The antibiotics were used (Ciprofloxacin, Gentamicin, Tetracycline, Amikacin, Imipenem, Vancomycin, PiPeraciclin, Chloramphenicol is, Erythromycin) where these antibiotics were placed in sterile boxes of 8 boxes and the name of the antagonist was recorded on each carton containing a solution of silver nanoparticles at a concentration of 20 mM.

To test the synergy between silver nanoparticles and antibodies

The synergy test between AgNPS silver nanoparticles was tested against 12 isolates of *Klebsiella* pneumonia, as they were transferred (5-3) a pure colony into tubes containing5ml) of a physiological salt solution and the resulting suspension turbidity was compared.
with a standard McFarland turbidity of 0.5, after which a sterile cotton swab was submerged with the bacterial suspension and spread over Muller-Hunton’s medium. The plates were incubated at a temperature of 37 ° C for 24 hours, and then the diameter of the inhibition zone around the disks was measured in mm (mm) for the silver saturated nanoparticles, and then compared with the standard results.

Results and Discussion

After conducting biochemical tests for bacterial isolates isolated from Ramadi Teaching Hospital, 30 isolates of *Klebsiella pneumonia* were diagnosed out of 50 samples, which were isolated from different pathological conditions that included (wounds, burns, diuresis, ears, excretions) and diagnosed based on the culture, microscopic and biochemical characteristics as well as Use a Vitek2 device. As between the microscopy and staining with the Cram stain, they are negative bacilli of the Gram stain organized in the form of short chains, and the *Klebsiella* was characterized by rather large colonies and mucous in consistency because they contain the capsule fermented for lactose sugar, and the biochemical examination showed that it is negative for the indole test and negative for the Proaskaure Vogas test. Citrate utilization And it is positive for the Uerase test, and it was confirmed by the Vitek2 device, as it gave results identical to the results of the biochemical examination Sanders and Sanders, 1997 The number of *Klebsiella pneumonia* bacteria in urine samples was the highest rate of 33%, while the rates of wounds and burns were 20, 16% (16%) respectively, and this result is close to what the world reached (9) as the percentage of *Klebsiella pneumonia* in urine samples was 21.8. The reason for their prevalence in hospital environments is due to their ability to develop different methods of resistance to disinfectants and their possession of virulence factors that resist antibiotics, and the results obtained by the two researchers are also converging (10) Molecular identification, whereby all bacterial isolates were subjected to molecular diagnosis by using PCR technology based on the 16SrRNA diagnostic gene, which is good, stable and has little heterogeneity for a long time in the bacterial species. It is also the main key to identifying among several thousand genes inside the cell. Bacterial (11) The results of DNA extraction of *Klebsiella* isolates that were isolated from different pathological conditions, as 12 isolates were selected for molecular diagnosis, showed that there are clearly packages of the isolates after the DNA transposition extracted on the acarose gel, as the molecular diagnosis matched the biochemical diagnosis. The results of the current study are consistent with the findings of the researcher (12) who showed that the percentage of identical molecular diagnosis of *Klebsiella* bacteria was 98%.
The effect of overlapping silver nanoparticles with antibiotics against *Klebsiella pneumonia*

The results of the statistical analysis showed that the interaction of AgNPs silver nanoparticles with antibiotics had a significant effect on increasing the sensitivity of isolates towards antibiotics, as the general rate of antagonist inhibition diameters with AgNPs was reached (Cip-Ag, Imp-Ag, AK-Ag, GN-Ag) (32.59, 36.18, 21.6, 18.98 respectively)
While there was no significant effect of the interaction of AgNPs with the antagonists (Tet-Ag, Er-Ag,) the general rate of inhibition diameters was (Er-Ag 8.4 VA-Ag, 9.1 Tet-Ag, 8.5) as these antagonists did not show efficacy against isolates, because It has a weak effect against negative bacteria and is non-synergistic as it does not have effective binding sites that bind with nanoparticles. Many researches indicate the synergism between nanoparticles and antibiotics, and the increased sensitivity of bacteria to the effect of antibiotics mixed with nanoparticles. As the antibiotic molecules have many active groups such as hydroxyl and amide groups that easily interact with silver nanoparticles by chelation. The silver nanoparticles bind to the surface of the bacterial cell and this association causes damage and structural changes that affect the biological functions of the bacterial cell, such as permeability, causing gaps and pits that allow the antibiotic to penetrate the bacterial cell, leading to its death (13). As the researcher (2014) explained, The role of nanoparticles in enhancing antibodies), Amikacin Imipenem, towards Gram-negative bacteria, as it is one of the modern strategies in treating multiple bacteria resistant to antibiotics, as he found that the use of the antibiotic with silver nanoparticles is more efficient in killing bacteria, the particles also act as a carrier medium for antibiotics. The anti-synergistic antagonists with Imipenem Ciprofloxacin silver nanoparticles showed the highest significant effect ratio (32.50, 36.00), respectively, in the direction of K. pneumonia bacteria isolates. This study

<table>
<thead>
<tr>
<th>No.</th>
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<th>CN-Ag</th>
<th>CIP-Ag</th>
<th>ER-Ag</th>
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<td>32.59</td>
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<td>17.43</td>
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<td>1.798</td>
<td>1.665</td>
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agrees with the findings of (14) indicating strong efficacy. Particles that are synergistic in inhibiting the growth of a group of multiple antibiotic-resistant bacteria, including (Salmonella typhi, Pseudomonas aeruginosa Klebsiella pneumonia), and he explained that the particles mixed with the antagonists behave an anti-bacterial behavior towards many types of negative bacteria by causing a defect in the cellular membrane correlation. Bigger for nanoparticles in bacterial cells. The synergistic effect of Chloramphenicol with AgNPs increased the susceptibility to infection of bacteria and its effect was clear against Pneumoniae K, as the study agrees with the results reported before. (15) who confirmed the increasing efficacy of synergistic antibiotics and explained this to the efficiency of silver nanoparticles in transporting the antibiotic to the inside of the bacterial cell to interfere with the protein synthesis mechanism to prevent bacterial growth.

Figure 3 inhibition zone of Antibiotic inhibition with silver nanoparticles

Figure 4 inhibition zone of Antibiotic inhibition with silver nanoparticles
Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References

Celiac Disease among Adults and Children in AL-Anbar Province West of Iraq: a Comparative Cross-Sectional Study

Yasin. Hamad Majeed
Assistant Professor University of Anbar, College of Medicine, Department of Internal Medicine/Iraq

Abstract

Background: gluten-sensitive Enteropathy is a public non-communicable, immune-associated disease which is activated by wheat (gluten) ingesting that occur in hereditarily susceptible individuals in initial childhood & elderly individuals with varied presentations whereas several studies have shown CD are predominant in adults. The purpose of this study was to compare the clinical presentation, serological, endoscopic, and histopathological features in adults and children with celiac disease at Al-Anbar province, west of Iraq. A total of 170 consecutive newly confirmed CD patients in AL-Ramadi teaching hospital, Gastroenterology Clinic during a period between August 2017 & Jun 2020 had been involved in comparative cross-sectional design research. Patients were divided into two groups: adults and children and each of them was also divided into two groups- classical and non-classical celiac disease based upon the clinical presentation. Clinical presentation, endoscopic findings were recorded in all cases gastrointestinal endoscopy & duodenal biopsy with Anti -TTG IgA had been determined & tested for the CD diagnosis. Histopathology results had been divided using modified Marsh taxonomy. Results had been examined through a suitable statistical test. Of 170 CD patients that include 97 (57.1%) adults CD and 73(42.9) children. Classical symptoms were predominant among 85.56% of adults while non-classical symptoms were more common among 45.2% of children. Bloating with abdominal pain and anemia were predominant among adults and children respectively. No significant differences in the Levels of anti –tTG among each of adults and a pediatric group of CD patients (P-Value 0.072). The most common endoscopic finding was scalloping 60.3%, 40.2% among children and adults respectively. Marsh grade G3A were predominant among adults [83 (89.2%)] versus [67 (95.7%)] among children of CD patients with anti-tTG IgA U/ml 10+1 and more levels. No statistical difference regarding marsh grade G3 frequency among adults and children CD patients (p 0.191).

Introduction

Celiac disease (CD), also named gluten–sensitive enteropathy, is a general chronic, the autoimmune disease that occurs due to long-lasting gluten intolerance in hereditarily sensitive persons [1].

CD distributes in 1-2% of the overall people of totally races & countries [2-4] CD was usually supposed to be a disease of children, unusual in adults. But, several reports in the previous decades have exposed that adults are also infected with CD some time more frequently than children as it could occur at every age in a lifetime & about 20% of patients are older than 56 years of life [5-7] In adult CD typically in a larvae mode with less intestinal appearances & nonappearance of Mal-absorption, & exhibition atypical forms described by public extra- digestive complaint’s & numerous associated environments, which make detection more challenging [8-10].

Classical CD in children is usually confirmed in the 2nd year of life, it is described by mal-absorption signs like permanent diarrhea, Steatorrhea, failure to thrive & loss of weight, stunted-growth, muscle wasting, loss of appetite, nausea & vomiting linked with lethargy & emotional stress [11-13]. Additional slighter abdominal signs are abdominal-pain, abdominal− distention, flatulence, irregular bowel habits, & perminant constipation [14].
The non-classical CD is frequently designated in young children & adolescents offering with extra-intestinal signs linked CD, as delay in puberty, unexplained permanent or ferrous deficiency anemia, non-responsive to supplementation, reduced bone mineralization (osteopenia/osteoporosis, dental enamel defects, irritability, chronic fatigue, neuropathy, arthritis/arthralgia, amenorrhea, unexplained elevated liver enzymes & repeated Aphthous stomatitis[12-14]. Also, monitoring & celiac prognosis can diverge dependent on the pediatric or adult stage. Aims of the Present study is to compare the clinical presentation, serological & endoscopical findings in adults & children suffering from CD at Al-Anbar province, west- of Iraq.

Patients and Methods

A total of 170 consecutive new confirmed CD cases at AL-Ramadi teaching hospital - Gastroenterology Clinic during a period from August 2017 & Jun 2020 had been comprised in a comparative cross-sectional study. Patients were divided into two groups: adults and children and each of them was also divided into two groups- classical and non-classical celiac disease based upon the clinical presentation. Clinical presentation, endoscopic findings were recorded. Four duodenal biopsies were taken from each case. Anti-TTG IgA were also taken from each patient.

In total cases, gastrointestinal biopsies in addition to serum anti-tissue transglutaminase (anti-tTG) were firm &confirmed for diagnosis of CD, histopathological results were divided through the use of "modified marsh taxonomy"[15][16]. Anti-tTG levels were determined by ELISA test. All Patients with IgA anti -tTG less than 10 iu /ml were considered as negative cases & IgA anti > 10+1 positive and it had a calibration range of 3 to 100 iu/ml. Gastroduodenoscopy was performed in suspicion of CD patients. Four duodenal biopsies from D2 had been obtaining, with duodenal endoscopic markers valuation such as a reduction in duodenal folds, visible mucosal vessels & fissures, scalloping, & mucosal nodularity. The specimen has been tested by an-experienced pathologist. Diagnosis of CD frequently depending on the sero-positivity of anti tTG antibodies [17] plus anti-tTG histopathological findings of intestinal mucosa [18]. From every patient. Family and patients who have written consent have been taken & from Anbar medical college, Iraq- ethics approval committee, the ethical approval had been taken.

Statistical Analysis

Data had been examined through IBM SPSS software version 22 using. The results had been offered in tables as descriptive statisticians results (mean, SD, frequency, percentage), Chi-square test had been used for comparing the CD frequency between groups. Means of scales of adults and pediatric groups had been compared using the independent sample T-test. A two-tailed P value has been used in overall analyses & a P value of less than 0.05 has been used to decide statistical significance.

Results

A total of 170 CD patients, 97 (57.1%) of them in the adult’s CD group of consist of ( 39 (22.94%) male and 58(34.12%) female with a mean age of 35.38 ±11.129 years and 73(42.9) of them in the pediatric group consisted of 42 (24.71%) males and 31 (22.24) females with a mean age of 10.81±4.020 years. As reported in Table 1A and Table 1B, Classical symptoms were predominant among adults 85.56% versus 54.79% among children which was statistically significant (p 0.000), while non-classical symptoms were more common among children 45.2% versus adults 14.5% which was statistically significant (p 0.000). Bloating and abdominal pain with a percent (34.0%) and (22.7%) respectively were more common among adults which were statistically significant (p 0.001), whereas anemia with a percent 24 (32.9%) was predominant among children’s which was statistically significant (p 0.046).
Table 1A. Classical forms of CD among adults and children

<table>
<thead>
<tr>
<th>Presentation (Symptoms) classical</th>
<th>Group</th>
<th>P. Value</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Adult(n = 97 )</td>
<td>Children(n = 73 )</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>BLOATING</td>
<td>33(34.0%)</td>
<td>3(4.1%)</td>
</tr>
<tr>
<td>ABDOMINAL PAIN</td>
<td>23 (23.7%)</td>
<td>5(6.8%)</td>
</tr>
<tr>
<td>DIARRHEA</td>
<td>21(21.6%)</td>
<td>24(32.9%)</td>
</tr>
<tr>
<td>CONSTIPATION</td>
<td>5(5.2%)</td>
<td>2(2.7%)</td>
</tr>
<tr>
<td>VOMITING</td>
<td>1(1.0%)</td>
<td>6(8.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 1B. Non-classical forms of CD among adults and children.

<table>
<thead>
<tr>
<th>Presentation (Symptoms) Non-classical</th>
<th>Group</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult(n = 97 )</td>
<td>Children(n = 73 )</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>ANEMIA</td>
<td>12(12.4%)</td>
<td>24(32.9%)</td>
</tr>
<tr>
<td>MIGRAINE</td>
<td>2(2.1%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>SHORT STATURE</td>
<td>0(0.0%)</td>
<td>9(12.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>37</td>
</tr>
</tbody>
</table>

Levels of anti–tTG ranged from <10.0 to >10 in CD patients. There were no significant differences in the Levels of anti–tTG among each of adults and a pediatric group of CD patients (P-Value 0.072) with Anti-tTG IgA IU/ml level >10 as shown in table 2.

Table 2. Anti-tTG level in each of adults and children with celiac disease

<table>
<thead>
<tr>
<th>Anti-tTG IgA IU/ml</th>
<th>Group</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult(n = 97 )</td>
<td>Children(n = 73 )</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>93 (95.9%)</td>
<td>70 (95.9%)</td>
</tr>
<tr>
<td>&lt;10.0</td>
<td>4 (4.1%)</td>
<td>3 (4.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>97 (100.0%)</td>
<td>73 (100.0%)</td>
</tr>
</tbody>
</table>
The most common endoscopic finding was scalloping 60.3%, 40.2% among children and adults respectively. No statistically, a significant difference of scalloping between children and adults followed by a reduction in duodenal folds 24.7%, 9.6% among adults and children respectively. There was a statistically significant difference in reduction in duodenal folds between adults and children (p 0.002), whereas Nodularity, Visible vessels, and mucosal fissures and Atrophy with visible vessels were less frequently among adults and children with CD. The normal endoscopic finding has significantly higher in the adult group [32 (33.0%)] compare with a pediatric group [15 (20.5%)] (P 0.013) as shown in table 3.

**Table 3. Endoscopic finding in adults and children with celiac disease**

<table>
<thead>
<tr>
<th>Endoscopic finding</th>
<th>Group</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult (n = 9) n (%)</td>
<td>Children (n = 73 ) n (%)</td>
</tr>
<tr>
<td>Scalloping</td>
<td>39 (40.2%)</td>
<td>44 (60.3%)</td>
</tr>
<tr>
<td>Normal</td>
<td>32 (33.0%)</td>
<td>15 (20.5%)</td>
</tr>
<tr>
<td>Reduction in duodenal folds</td>
<td>24 (24.7%)</td>
<td>7 (9.6%)</td>
</tr>
<tr>
<td>Nodularity</td>
<td>1 (1.0%)</td>
<td>2 (2.7%)</td>
</tr>
<tr>
<td>Visible vessels and mucosal fissures</td>
<td>1 (1.0%)</td>
<td>2 (2.7%)</td>
</tr>
<tr>
<td>Atrophy with visible vessels</td>
<td>0 (0.0%)</td>
<td>3 (4.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>97 (100.0%)</td>
<td>73 (100.0%)</td>
</tr>
</tbody>
</table>

Histopathological evaluation of intestinal biopsies revealed marsh grade G3 were predominant among adults [83 (89.2%)] versus [67 (95.7%)] among children of CD patients with anti-tTG IgA U/ml 10+1 and more levels. No statistical difference regarding marsh grade G3 frequency among adults and children CD patients with anti-tTG IgA U/ml 10+1 and more levels (p 0.191). G3A (40.2%) were more common in adults while G3B (42.5%) and G3C (19.2%) were more common among children. There were 7 adults with marsh G2 as shown in Table 4.

**Table 4-Correlation of anti-tTG IgA U/ml group with level 10+1 and marsh grade 3 between adults and children with CD**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Group</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult(n = 97 ) n (%)</td>
<td>Children(n = 73 ) n (%)</td>
</tr>
<tr>
<td>G3A</td>
<td>36</td>
<td>23</td>
</tr>
<tr>
<td>G3B</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>G3C</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Marsh grade 3</td>
<td>83 (89.2%)</td>
<td>67 (95.7%)</td>
</tr>
<tr>
<td>G2</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>G1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>70</td>
</tr>
</tbody>
</table>
Discussion

Our study shows the predominance of females in adult patients. This predominance was not significantly significant, this consistent with Oberhuber G et al. [16], maybe due to females may seek health advice more than males.

Regarding CD children there is boy predominance, this is similar to the study done by Rana et al. [19], this may be explained by better care and attention given for boys for that reason they brought earlier for health advice while in the western study there is no sex predominance [20].

Our study shows that the classical form of CD was predominant among adults 85.56% versus 54.79% among children, which was statistically significant (p 0.000), this is inconsistent with the study done by Poddar, 2013 [21] which shows the predominant of children. While non-classical symptoms are more common among children 45.2% versus adults 14.5%. which was statistically significant (p 0.000), this is inconsistent with the research prepared by Pooni et al. [20] that found the predominance of CD among adults.

The current study shows that clinical differences of a classical presentation at diagnosis in pediatric and adult groups, as bloating and abdominal pain being the most common symptoms in adults which were statistically significant (p < 0.001); while diarrhea was the predominant complaint in children this consistent with the study done by Sez-Rodrigo Luis et al. [22].

Regarding non-classical presentations, anemia was the most common findings among children 32.9% versus 12.3% among adults which was statistically significant (p 0.046), this inconsistent with Sez-Rodrigo Luis et al. [22].

Regarding endoscopic findings, scalloping was the most common finding in both age groups this consistent with Pooja Semwal et al. [23]. Our study shows no correlation between anti-tTG levels and marsh 3, this is similar to a study done by Ganji et al. [24] and inconsistent with another study that showed a statistically significant relation P < 0.001 [25]; this may be attributed to environmental and genetic variation.

Our study shows marsh 2 among adults 7.52% and 1.4% among children while marsh 1 1.07% only among adults, regarding Marsh G1 and G2 Type these lesions are well-matched with but nonspecific for CD, minimal intestinal lesions plus sero-positivity of anti-tTG point to CD diagnosis. whoever, least lesions can lead to additional causes, such as food allergies (such as cattle milk proteins), Crohn’s syndrome, lymphocytic colitis, bacterial infection & helminthic gastro-intestinal manifestation, like Giardia, public immunodeficiency, small intestinal microbial growth, NSAID, & Helicobacter pylori infection [26-28].

Normal histology [marsh 0] with positive serology was found in 2.15% Among adults versus 2.85% among children, this may be a latent type of CD [30]. Conclusion: Classical symptoms and bloating with abdominal pain were more common among adults whereas non-classical symptoms and anemia were predominant among children of CD patients. No difference in levels of anti –tTG in both groups. Scalloping is more common among both groups. marsh grade G3A more common in adults while G3B anG3C is more common among children.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References


22. Rodrigo-Sáez L, Fuentes-Álvarez D, Pérez-Martínez


Antibacterial Effect of *Lactobacillus acidophilus* and Silver Nanoparticles on Antibiotics Resistance *Klebsiella pneumonia* Isolates from Burn Infection

Maryam Hayder¹, Baydaa A. Hassan²

¹Mater Studern; ²Assist Prof. Dr./ University of Kufa-Faculty of Science-Department of Biology/Iraq

Abstract

This study was completed in laboratories of Biology Department in Faculty of Science. It explains the antibacterial effect of *lactobacillus acidophilus* and silver nanoparticles on antibiotics resistance *Klebsiella pneumonia* that isolated from burn infection patients in the province of Najaf. A total number of 130 samples were collected from patients with burn infection from Burn Center AL-Najaf Governorate, the results showed that inhibition zone of *K. pneumonia* increased progressively with increase the silver nanoparticles and *Lactobacillus acidophilus* concentrations in reaching a optimum inhibition in 80 µg/ml,0.7mg/ml respectively , also antibacterial activities of some resistance antibiotics included Amoxicillin, Ceftriaxone , ceftazidime and tetracycline exhibited that the presence of silver nanoparticles with *L. acidophilus* together with the resistance antibiotic resulting in decrease in the ability of the antibiotics to inhibit the *K. pneumonia* resulting in the occurrence of inhibition zone of *K. pneumonia* and insignificant compared with the presence of silver nanoparticles alone as well as less than the case of the presence of *Lacidophilus* alone with these antibiotic . The results showed that the *bla oxa* was detected in from the 30 (100%) isolates of *K. pneumonia* have *bla oxa* gene 22 (73.33 %) ,and 17 (56.66%) were have *ctx-m*

**Key words**: nanoparticles, inhibition, silver, *Lactobacillus acidophilus*

Introduction

Burn wounds are susceptible to an extensive array of colonization and infection by microorganisms, the granulation tissue in burn wound makes a rich culture medium for the growth of different organisms as the exposed skin is exchanged by a protein rich, avascular environment that generates an appropriate niche for microbial colonization ,the movement of immune cells is also hampered contributing to the septic process (1). The depression of local and systemic host cellular as well as humoral immune responses show an essential role in infectious complications of severe burn patients (2). Antibiotic resistance remained informed toward occur when a treatment misses its capability to prevent microbial growing efficiently, microbes change resistant’ also remain ‘to grow in the occurrence of healing stages for antibiotics, bacteria, after duplicates even in the occurrence of the antibiotics are known as resistant, bacteria, antibiotics become are typically active in contradiction of them, however when microorganism fewer sensitive or resistant, it needs a greater from the ordinary concentration of the identical treatment to need an influence (3)

Materials and Methods

Samples Collection and bacterial identification

A total number of 130 were collected from patients with burn infection (October , 2020 to January , 2021 ) . Sample collection after removal of old plaster of burns patients, the superficial of the burns wound was cleaned with normal saline to avoid contamination. All sample was collected by swabbing the wound by a disinfected cotton- tip swab staff. Circling the swab stick amongst the toes, the swab stick was moved across the whole wound external, the swab staff was rapidly place in carriage middle, Swabs were taken and close it until transported to advanced test center of Science College.
in Kufa University and culturing on diverse media for 24 hours at cultivate 37 °C for bacterial diagnosis. silver nanoparticles and lactobacillus acidophilus as antibacterial agent preparation of silver nanoparticles: according of (4) preparation of lactobacillus acidophilus: according of (5)

**Evaluation of the silver nanoparticles efficiency in the inhibition of the growth of antibiotic resistance bacteria that causes infection**

The preparation of Muller Hinton medium , it is sterilized in the autoclave and poured in petri dishes , then antibiotic resistance *K. pneumonia* isolates were streaked by sterilized swab on petri dish , antibacterial activity of the silver nanoparticles was determined using the agar well diffusion assay method , all the dishes were incubated at a temperature 37 °C for 24 h, and the plates were studied to indication of zone of inhibition, it seem as a pure zone about the pits , the width of inhibition regions was determined by a meter leader (6)

**Evaluation of the lactobacillus acidophilus in the inhibition of the growth of antibiotic resistance bacteria that causes infection :** This test is agreed in the same method described in paragraph excluding the use of *Lactobacillus acidophilus* (5)

**The relationship between silver nanoparticles, lactobacillus acidophilus and resistance antibiotics**

Muller Hinton Agar was poured in petri dishes , antibiotic resistance *K. pneumonia* isolates were streaked by sterile swab on petri dish ; then, the optimum concentration of silver nanoparticles and *lactobacillus acidophilus* were added , also, the resistance antibiotic powders were placed in each dish , then, all the dishes were incubated at 37 °C for 24 h and the diameter of inhibition zones were measured using a meter ruler

**DNA Extraction**

Bacterial DNA was detached through using a process of (7).

**Molecular Identification**

Gel electrophoresis was used to determine of DNA via UV trans illuminator , the primer was planned by Alpha DNA company, Canada .

**Results and Discussion**

The effect of different concentrations of sliver nanoparticle antibiotics resistance *K.pneumonia* isolates growth :

The results also directed that inhibition zone of *K.pneumonia* bigger progressively with increase the silver nanoparticles concentrations in attainment a finest inhibition in 80 µg/ml figure (1). A additional of science reports suggests that the antibacterial mode of action of silver nanoparticles is similar to the antimicrobial effects of silver ions, due to the life cycle of silver nanoparticles and their change to silver ions (10). Silver ions have ability connection for the specific transporter proteins and enzymes residing in the microbial plasma membrane, the sequence of proteins and enzymes carriage electrons also concurrently transfer protons from the cytoplasm for the periplasmic area making a concentration decline called the PMF, the electron transport scheme above the plasma membrane is the main producer of ATP through aerobic breathing in microbes, in addition the manner is named chemiosmosis, the one place protons have ability diffuse into the cytoplasm again is through the ATP formation compound, which effects in the creation of ATP in a redox response amid ADP and inorganic phosphate, also the electrons reaching the ending electron acceptor place, the last electron acceptor is melted oxygen in the situation of aerobic respiration, also mainly results in water or in minor concentrations of cytotoxic ROSs, which specific enzymes method (11;12).
Figure (1): Effect of Different Concentrations of silver nanoparticles in *K. pneumonia* growth

The effect of different concentrations of *lactobacillus acidophilus* in antibiotics resistance *K. pneumonia* isolates growth:

The results indicated that inhibition zone of *K. pneumonia* increased increasingly with rise the *lactobacillus acidophilus* concentrations in attainment a finest inhibition in 0.7 mg/ml figure (2). Specific investigators from advanced nations must reported significant in vitro inhibition of Gram negative and Gram-positive pathogenic microbes via *Lactobacillus acidophilus* class, *L. acidophilus* can display antimicrobial effect by many processes such as the making of antibacterial materials or substance (hydrogen peroxide, lactic acid, Benzoic acid, Hydroxy fatty acids, bacteriocin), competition to nutrients, inhibition of microbial, and improvement of the immune response (13). Certain in vitro research have too exposed that *Lactobacillus* strains have ability display antibacterial act in contradiction of *K. pneumonia, E. coli, Shigella spp., S. mutans, P. aeruginosa, and S. aureus* (14-17).

Figure (2): Effect of Different Concentrations of *L. acidophilus* in *K. pneumonia* growth

The mixture effect of silver nanoparticles and *L. acidophilus* with most resistance antibiotics:

The results proved that the antibacterial activities of most resistance antibiotics were include Amoxicillin (AMC) Ceftriaxone(CRO) Ceftazidime (CAZ) Tetracycline (TE) increased in the presence of silver nanoparticles as well as *L.acidophilus* to causes the inhibition of multi-resistance isolates *K. pneumonia* growth.
Furthermore, *L. acidophilus* is amongst a several *lactobacilli* this must been research to the antimicrobial properties in previous studies (18). as of the therapeutic effect of probiotic lactobacilli to MDR bacterial infection, they are one such microbes of special for use as the remedial agents in mixture with antibiotics (14). and has been recounted through (19) the *L. acidophilus*-ciprofloxacin combined therapy against several gram negative pathogenic bacteria of the intestine and stomach. Silver nanoparticles (AgNPs) are the most promising nano antibiotics now adays , lately, an development in antibacterial action–synergistic effect- it studies however AgNPs are synergism with many antibiotics, example ampicillin, amoxicillin, and chloramphenicol (20).

AgNPs with antibiotics shared actions have been studied previously but never mechanism of effect has been established since varied phenotypes have been observed among studies (21). Furthermore, the action of AgNPs-antibiotics activity may be alteration in diverse organisms, these discrepancies create on interaction AgNPs-antibiotics may be effect with diverse features: this stabilizing factor used also its comparative concentration, it can effect the capability of contact between AgNPs and antibiotics (22).

The mixture influence of *L. acidophilus* and silver nanoparticles together with most resistance antibiotics

The results displayed that the presence of *L. acidophilus* with silver nanoparticles together with the resistance antibiotic resulting in decrease in the ability of the antibiotics to inhibit the *K. pneumonia* resulting in the occurrence of inhibition zone of *K. pneumonia* and insignificant compared with the presence of silver nanoparticles alone as well as less than the case of the presence of *L. acidophilus* alone with these antibiotic.

The decrease in the size of the inhibition zones in the case of the interaction of Ag-NPs *L. acidophilus* with resistance antibiotics means the antagonists effect between them, the cause may be the effect of the silver nanoparticles on the inhibition of *L. acidophilus*, so the sizes of the inhibition zones were less than the case of the presence of *L. acidophilus* with resistance antibiotics alone, lately, an development in antibacterial activity–synergistic effect- may be reported after AgNPs are joint with many antibiotics, such as ampicillin, amoxicillin, and chloramphenicol ,enoxacin, kanamycin, neomycin, and tetracycline (20;22). The interaction between AgNPs with antibiotics has been also credited previously for chemical binding between the sulphur cluster of antibiotics with AgNPs (23;24). Therefore, *L. acidophilus* can be fermented the sugars resulting in lactic acid, hydrogen peroxide, enzymes and vitamin (B) complex, as well as antibacterial materials that inhibit or kill many pathogenic bacteria (25). its effect on pathogenic bacteria isolates by adhesion agents and their secondary metabolic products such as bacteriocins, it’s have the inhibitory activity through association with special receptors found on membranes, the plasmatic of pathogenic bacteria and this association leads to an uncontrollable flow of charged ions Cation and a flow of amino acids causing in the explosion of cellular membranes and to kill bacteria and decrease bacterial infections (26;27).

Detection of genes that responsible for β-lactamase-resistance:

The results showed that the bla *oxa* and *ctxm* was detected in *K. pneumonia* isolates, from the 30 (100%) isolates of *K. pneumonia* 17 (56.66%) were have bla *ctx-m* gene, and 22 (73.33 %) were have bla *oxa* gene Figure (3 and 4). Beta-lactam antibiotics are it one of the greatest usually prescribed treatment classes with many therapeutic indications, these advent initial from the 30s of the twentieth century drastically different the scenario of the fight in contradiction of bacterial infectious diseases, the mechanism of action for this antibiotics comprise, the peptidoglycan or murein is a vital basic of the bacterial cell wall this gives mechanical constancy for it, that is an very conserved constituent of within the gram-positive and gram-negative covers, the beta-lactam antibiotics prevent the latter stage in peptidoglycan creation via acylating the transpeptidase involved in cross-linking peptides to make peptidoglycan (28)(29). The goals for activities of beta-lactam antibiotics it called as penicillin-binding proteins (PBPs), the binding, in turn, interrupts the terminal transpeptidation method and gives failure of viability and lysis, and by
autolytic methods with the bacterial cell, β-Lactamases are by far the greatest significant resistant process in Gram-negative bacilli, with the popularization of genetic techniques, an increasing number of this enzymes have been categorized different in amino acid series and hydrolytic activity for β-lactam antibiotics (30).

Gram-negative bacteria, inducible appearance of β-lactamases is ordinarily originate in chromosomal β-lactamases whereas plasmid-mediated enzymes are usually constitutively expressed, improvement appearance for this hydrolytic action is frequently controlled within promoters appear in upstream genes (31). This findings of it current study revealed that the rate of bla oxa gene, and bla ctx-m gene are excessive in K. pneumonia isolates, and it is accordance with previous studies (32-34), when they found the bla ctx–m gene and bla oxa gene are the most frequently detected gene encoding in clinical isolates of K. pneumoniae and Pseudomonas spp at different rates.

**Figure (3) Agarose gel with ethidium bromide stained of mono-plex amplified product from extract DNA of K. pneumoniae isolates with bla bla ctx-m gene primers, Lane (L) DNA molecular size marker (100-bp ladder), Lane (4,6,7,8, 11,12,13,14,18,19,20,22,24,25,27,28,30) show positive results bla ctx-m gene**

**Figure (4) Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of K. pneumoniae isolates with bla oxa gene primers, Lane (L) DNA molecular size marker (100-bpladder), Lane (1, 4, 5, 6,7,8, 9,11, 12,13, 14, 15,17, 18,19,20,22, 24, 25, 27, 28, 30) show positive results with bla oxa gene**

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

**References**


2. Ghai S, Sachdeva A, Mahajan R, Dogra S, Soodan


Accuracy of Different Apex Locaters. (A Comparative in Vitro Study)

Mohammad Munthir Abdulrazzaq¹, Emad Farhan Alkhalidi²

¹Lecturer Dr. PhD, Conservative Dentistry/Al-Iraqia University College of Dentistry/Department of Conservative Dentistry, ²Assistant Professor, Dr. PhD Conservative Dentistry/University of Mosul/College of Dentistry/Department of conservative Dentistry/Iraq

Abstract

Aims: The objective of this in vitro study was to compare the accuracy of the Root-ZX II, Root ZX Mini, i Pex II, Kerr apex ID, Root PI and YD2 electronic apex locators (EALs) in locating the apical foramen.

Materials and Methods: Fifty extracted human teeth with mature apices were used. Access cavities were prepared. In order to make sure that we have an accurate working length of 21 mm the crown was sectioned with diamond disc (control). The teeth were then embedded in an alginate model to simulate the periodontium after that the Canals were irrigated with 2.5% sodium hypochlorite. The actual length and electronic length measurements were made on each specimen separately by apex locator devices with an aid of a K-type file.

Results: Statistical results showed no significant difference between the 6 devices although concerning locating the apical foramen, Root-ZX II, Root ZX Mini, were accurate 96% of the time to ±0.5 mm, where as I pex II and Kerr apex ID were accurate 94% of the time to 0.5 mm from the apical foramen, 44% ±0.5 mm for the Root PI and 44% ±0.5 mm for the YD2. Conclusions: All the apex locators were able to determined the position of the apical foramen but consequently the Root-ZX II, Root ZX Mini, i Pex II, Kerr apex ID were more accurate than Root PI and YD2 apex locators.

Key word: Apex Locator, Root-ZX II, Root ZX Mini, i Pex II, Kerr apex ID , Root PI apex locator and YD2 apex locator.

Introduction

The determination of an accurate working length is one of the most critical steps of endodontic therapy. The cleaning, shaping, and obturation of the root canal system cannot be accomplished accurately unless the working length is determined precisely. Most operators attempt to terminate instrumentation 0.5-1 mm short of the radiographic apex. Although radiography is the most commonly used diagnostic aid in endodontics, clinically root morphology and radiographic distortion may cause the location of the radiographic apex to vary from the anatomic apex which could lead to over or under filling (1). Furthermore, radiographic interpretation of the apices of some teeth (e.g. maxillary molars) may be so difficult that radiographic working length determination is not accurate (2).

Recently, electronic methods for tooth length determination have gained popularity. The latest generation of apex locators has many advantages when compared to earlier devices. Unfortunately, many devices are inaccurate in root canals that contain moisture, vital pulp tissue, blood, and other exudates (3).

On the other hand many studies report on the accuracy achieved by the new generation of electronic apex locators as well as their extended measurement capabilities, which include accurate measurements in the presence of electrolytes (4).

Many types of EALs were manufactured but most studies on electronic apex locators using two frequencies (the third generation) report accuracy rates of 85–95% (5).
The objectives of the present study were to test the accuracy of six types of apex locators in an in vitro model and to compare its accuracy to the actual working length.

Materials and Methods

This study was approved by Research Ethics Committee at College of dentistry, University of Mosul (Approval number UoM.Den/H.L.34/21).

The research involved 50, extracted, single-rooted, human lower premolar teeth with mature apices, preserved in Thymol solution and kept refrigerated.

In order to make sure that we have an accurate working length of 21 mm the crown was sectioned with diamond disc to a level so that all the teeth have a constant working length of about 21 mm (control) and establish a level surface to serve as a stable reference for all measurements and also for statistical purposes.

The access cavities were prepared (Diamond burs, 10541M, Technical & General Ltd). The actual root canal length is the distance from the coronal reference point to the apical foramen. It was measured by inserting a #20 file into the root canal until the file tip was just visible at the level of the apical foramen and this procedure was done under a stereo microscope (Heerbrugg, Switzerland) at this point the stopper adjusted to the coronal reference point. This procedure was done to make sure that all the teeth have constant working length of about 21 mm.

Each measurement was repeated three times and if there was a difference in the measurements from the 21 mm the working length of the tooth was adjusted to be 21 mm.

Teeth were then embedded in an alginate model to simulate the periodontium specially developed to test apex locators (6). The alginate (Kromopan Lascod, Italy) was poured into the mold then each tooth was embedded into the alginate and kept in position until the alginate had set completely. The model was used immediately so that to keep it humid and when not in use; it was wrapped with a wet paper and refrigerated to keep it in a moist environment throughout the experiment. Previous studies have shown that keeping the model in such an environment was satisfactory (Kaufman & Katz 1993) (6).

Measurements were taken after irrigation with normal saline into the root canals. Cotton tips were used to dry the tooth surface and eliminate the excess irrigating solution.

The file was placed in to the canal while attached to the appropriate electrode of the apex locator and the other electrode (lip clip) was attached to the alginate (figure 1).

Figure 1 show tooth inside alginate with apex locator.
The file size was 20 Flexo file (Dentsply,Maillefer), it was inserted slowly inside the canal till the signal of the apex locator reached the '0' mark (apical foramen), at this point the stopper of the file was moved to the coronal reference point, then the file was removed from the canal and working length was measured with an endodontic ruler and its length registered as the electronic length (EL). On each tooth this procedure was repeated for each one of the six types of apex locators, in this study the apex locators used are

1. Root ZX II (Morita, Japan).
2. Root ZX Mini (Morita, Japan)
3. ipex II (NSK, Japan)
4. Kerr apex ID (Kerr, USA)
5. Root PI apex locator (Osakadental, China)
6. YD2 apex locator (Shanghai S&D Dental International Co., China).

The results obtained (in millimeters) for each electronic apex locator was recorded in independent tables. In each case, we subtracted the corresponding reference measurement (i.e. actual length) from the electronically determined distance, recording the result in tabular form as positive form (measurements exceeding the apical foramen), negative (measurements short of the apical foramen), or correct (measurement coinciding with the actual length) with a ±0.5 mm acceptable range.

Percentage was used to statistically analyze the significance of the mean differences between electronic length and actual length.

**Results**

Each tooth served as its own control. Statistical results showed Root-ZX II, Root ZX Mini were accurate 96% of the time to ±0.5mm, where as I pex II and Kerr apex ID were accurate 94% of the time to 0.5 mm from the apical foramen, 44% ±0.5mm for the Root PI apex locator and 44% ±0.5mm for the YD2 apex locator Table(1).

<table>
<thead>
<tr>
<th>Distance from apical foramen (mm)</th>
<th>Root ZXII N=50</th>
<th>Root ZX Mini N=50</th>
<th>I Pex II N=50</th>
<th>Kerr apex ID N=50</th>
<th>Root PI N=50</th>
<th>YD2 N=50</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; – 0.5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>– 0.5 to 0.5</td>
<td>48</td>
<td>96%</td>
<td>47</td>
<td>94%</td>
<td>47</td>
<td>44%</td>
</tr>
<tr>
<td>&gt; 0.5</td>
<td>2</td>
<td>4%</td>
<td>2</td>
<td>4%</td>
<td>2</td>
<td>4%</td>
</tr>
</tbody>
</table>

**Discussion**

The main purpose of this study was to evaluate the accuracy of electronic apex locators most widely used in clinical practice that are available in the local markets. An in vitro study was developed in comparing electronic measurements with a control.

No individual technique is truly satisfactory in determining endodontic working length. Modern electronic apex locators can determine this position with accuracies of greater than 90% but still have some
Two operators measured the actual working length (control) with the use of a stereo microscope to be sure that all the samples having a working length of 21mm in order to get a precise measurement and the same operators measured the electronic measurements. All the teeth used were single rooted teeth with mature apices; several studies have demonstrated that EALs can accurately determine the working length in between 75 to 96.5% of the root canals with mature apices \( (8-11) \), therefore any tooth with wide or opened apex was neglected because it gave inaccurate readings.

Study carried out by Lucena-Martin et al. \( (12) \) showed that the Root ZX gave a precise measure in 85% of the cases. Shabahang et al. \( (13) \) produced values to a precision of 96.2% for the Root ZXII which is in general agreement with our study. It was not possible to compare the results of the other five apex locators with other existent studies because of the lack of research on these devices however there was no significant difference in the accuracy between the root ZX II, Root Zx Mini and i pex II and the kerr but all of them significantly differ from the Root PI and YD2 in which there was no significant difference between them.

The accuracy of the Root-ZXII , Root ZX Mini, were accurate 96%, where as i pex II and Kerr apex ID were accurate 94% , 44% for the Root PI and 44% for the YD2. these variations showed a difference in the accuracy of the measurements between the devices.

It is important to mention that these measurements were made in vitro and in ideal situations but clinically there are more problems related in working length measurement like the presence of blood and the use of different irrigate solutions so more studies are needed to evaluate the accuracy of EAL under different situations and also in vitro studies are needed to evaluate the real accuracy of the EAL clinically.

**Conclusions**

Within the limitations of this in vitro study the following conclusions could be drawn:

All the apex locators were able to determine the position of the apical foramen but consequently the (Root-ZX II, Root ZX Mini, i Pex II, Kerr apex ID) were more accurate for the determination of the root canal length than (Root PI apex locator and YD2 apex locator).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest**

The authors declare that they have no conflict of interest.

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**References**


Assessment of Enzymatic Bioremediation for Catechol in Water by the Role of Immobilized Catechol 1,2-dioxygenase on Biosilica of Diatoms Frustule and other Polymers

Khalid Dheyaa Abdulwahid¹, Najm Abdulla Al-Zubaidy²

¹Post Graduate/ Department of Biology, College of Science, University of Diyala, Iraq.
²Prof., Department of Biology, College of Education for Pure Science, University of Diyala, Iraq

Abstract

The highest specific activity for (C12O) enzyme purification was within the second stage of the purification steps represented by precipitation with ammonium sulfate AS 2% (weight/ volume), reaching about 14.56 U. mg⁻¹ after achieving the purification by 6.6 folds with a recovery rate of 78.3%. The optimum catalytic activity of the enzyme (C12O) was within the pH of 6.5-8.5 and the temperature of 25 °C. Immobilization of (C12O) enzyme by biosilica of diatom frustules DFS/G GA gave a double shifting of 20 °C from the optimum temperature for enzyme activity, within all the purification stages, as the percentage of catechol degradation through it at the temperature of 45 °C reached about 93% and 87.2% and 85% were in the precipitation stage with ammonium sulfate AS and the precipitation with streptomycin sulfate SS and the enzymatic crude extract CEE respectively. While the shifting was only 10 °C in the case of immobilization on polymeric supports types: PAN / G and PAA / G. In contrast, the soluble (C12O) enzyme which did not show any shifting by its optimum temperature , and its highest activity values were within the optimum temperature of 25 °C. While the method of immobilization for (C12O) enzyme by biosilica of diatom frustules DFS/G gave a 1.5-degree shifting for the optimum pH of the enzyme activity, within all the purification stages, the highest rates of catechol degradation were reached through it at the base pH 9-10 about 91.8% and 86.5. % And 85.0% in the AS, CEE and SS stages, respectively. Whereas, the enzyme immobilization methods of (C12O) by PAN / G and PAA / G did not show such displacement during the enzymatic purification stage with streptomycin sulfate SS but were limited to the primarily stages of enzymatic purification. , As it was able to shift the optimal pH of the (C12O) enzyme by 1.5 degrees also within the purification stages of AS and CEE. The rates of catechol biodegradation through it at the base pH 9-10 were about 79.0% and 71.8%, respectively, in the case of immobilization by PAN /G and PAA / G with percentages of 71.8% and 62.0%, respectively. In contrast, soluble (C12O) enzyme did not show any shifting by its optimum pH, and its highest activity values were within the optimum pH of 6.5-8.5. The reason for these results may be attributed to the role of the large surface area ready for covalent immobilization of (C12O) enzyme by biosilica of diatom frustules compared with the surface area of pores of the two types of polymeric membranes used in current study.

Keywords: Bioremediation; Biosilica; Feustule; Diatoms; Catechol; catechol 1,2-dioxygenase; immobilization; polymers

Introduction

Many extensive studies have been conducted during the last decades on both types of aromatic ring-degrading enzymes, external and internal effects, due to their important role in the field of biological treatment (¹). The aerobic biodegradation of phenolic compounds takes place through Certain pathways, with catechol or its derivatives playing a major role. These intermediates are then metabolized by the intradiol or extradiol cleavage of the aromatic core ring by enzymes dioxygenases (²). In the case of phenol, the first step of biodegradation involves converting phenol in to catechol via specific enzymes including the enzyme phenol hydroxylases, whose action involves binding of a hydroxyl group at the
ortho-position of the aromatic ring \(^{(3,4)}\). Immobilization of enzymes to different carriers or stents is an important challenge in the field of biotechnology. To date, a large variety of systems designed in this area have been examined and discussed. The main goal of enzyme immobilization is to obtain stable and reusable enzymes with resistance to various environmental factors \(^{(5,6,7)}\). The immobilization improves the control of a reaction through the possibility of using different forms of interaction. Moreover, the immobilization enzymes exhibit a high degree of selectivity and specificity \(^{(8,7)}\). The immobilization of dioxygenases, which has a low stability of action, has been shown to allow an increase in its value in terms of biotechnology \(^{(9,10,7)}\). In contrast, polyphenol oxidases and peroxidases are enzymes that most often require electron carriers to effectively complete their activity. Therefore, fixing these enzymes on insoluble carriers facilitates the construction of stable electron transport chain systems \(^{(11)}\). The activity of dioxygenases has been found in *Candida tropicalis* \(^{(12)}\), and *Candida albicans* \(^{(13)}\). In another study conducted by Tsai and Li, the enzyme catechol 1,2-dioxygenase was purified from the yeast strain TL3 *Candida albicans*, and they found that the homodimer has a molecular weight of 32,000 Dalton and each subunit was mono iron. And the optimum temperature and pH were 25 °C and 8.0, respectively \(^{(14)}\).

Diatoms are widely distributed unicellular algae capable of carrying out photosynthesis and which can deposit a silicon cell wall in a highly precise and unique arrangement called the Frustule cap, which is characterized by its porous structure that includes the presence of micro- to nano porous structure, which has an important role. In increasing the surface area of the wall with the potential to be easily modified \(^{(15,16)}\). The use of diatom biosilica as a support for restriction of enzymes came due to its various advantages such as natural origin, low cost and presence of hydroxyl groups as well as being non-toxic, these things are very important because the use of polymers of petroleum origin as restriction supports is not desirable because they are mostly toxic, which makes them a major cause of pollution \(^{(17)}\). Listed as a priority pollutant by the US Environmental Protection Agency \(^{(18)}\), phenol is the most hazardous industrial liquid waste with minimal toxicity at 1 mg. L\(^{-1}\) was developed by the World Health Organization (WHO) relatively to control the concentration of phenol in drinking water \(^{(19)}\). Thus, treatment of wastewater containing phenolic liquid wastes is essential \(^{(20)}\). Therefore current study aims to assessment of enzymatic bioremediation for catechol in water as phenolic compound by the role of immobilize catechol 1,2-dioxygenase on diatoms frustule and other some polymers.

### Materials and Methods

#### Extraction and purification of catechol 1,2-dioxygenase enzyme

Biodegradation potential of *Candida albicans* for phenolic compounds were identified, a qualitative examination was performed to determine the presence of the activities of Catechol 1,2-dioxygenase and Catechol 2,3-dioxygenase according to the approach followed by \(^{(4)}\), yeast strains were selected from the genus *Candida ssp*. Yeast cultures, with the biodegradation capacity for catechol, were grown in yeast cultures, in 8 Erlenmeyer flasks 2 liter at 30 °C, each flask containing 800 ml of MSM or YNB 0.67% (w / v) and 10 mM of phenol \(^{(13)}\), and inoculated with an initial cell density (ICD) of *Candida albicans* which isolated and purified in previous other study, had about 0.02 absorbance-induced OD optical density using a spectrophotometer at the wavelength of 600 nm. Harvesting of the yeast cells was started when the growth of the strain reached the stationary phase, that is, when the optical density due to the absorption reached approximately 1.4 on the wavelength of 600 nm \(^{(14)}\). The harvested yeast cells were transferred to freezing at a low temperature of below zero degrees Celsius. Freeze was used for this purpose in preparation for the enzymatic purification steps. The enzyme catechol 1,2-dioxygenase was extracted and purified from *Candida albicans* and the enzyme activity was assessed according to the method described by \(^{(14)}\). All the following enzyme purifications steps were carried out at low temperatures (0 to 6) degrees Celsius.
**Determination of the total enzymatic protein**

Protein concentration of the enzymatic extracts was determined by the Bradford method \(^{(21)}\), so that one unit of the enzymatic activity or activity of the enzymes under study was defined as the amount of enzyme that formed 1 µmol of the product per minute at 40 °C. The specific activity was expressed as one unit (U) per mg protein \(^{(1)}\). Although some researchers argue and adhere to the fact that the enzymes present in the crude extract are more stable and useful in building biosensors \(^{(22)}\), a dissolving and cleaning step of enzymes has been suggested according to the approach followed by \(^{(23)}\) with the aim of eliminating potential interactions, such as salts, sugars, and phenol.

**Preparation of biosilica from diatomaceous frustule**

Standard method was used to remove the organic and carbon materials from the diatoms samples obtained from the river according to modified method followed by \(^{(24,25)}\), briefly it was done through the following steps: 2 grams of the diatom samples collected in the previous study were passed in a baker with a Teflon beaker and heated to 60 °C in a 30% \(\text{H}_2\text{O}_2\) solution (w/w) for 2 hours, diatomaceous sample was washed three times using distilled water and centrifuge at 2000 rpm for 5 minutes, then the precipitate formed was transferred to a baker with a Teflon film and 20 ml of \(\text{H}_2\text{SO}_4\) solution 30% (v/v) was added and placed in an incubator at a temperature of 105 °C for 12 hours. After the incubation period had elapsed, the diatom biosilica particles were washed with pure water and dried at 40 °C.

**Preparation of immobilization membranes**

Current study included enzymatic immobilization on three types of immobilized materials: Diatom Frustule Silicon (DFS) membrane as Nano filtration with pore size (micro - nano), and polyacrylamide membrane (PAA) as microfiltration with an average pore size of 0.2 micron , and a polyacrylonitrile (PAN) ultrafiltration (PAN) with an ultrafiltration of 0.01 micron pore size .Phase inversion technique was used to prepare the immobilize film membranes that included a diatom frustule silicon (DFS), polyacrylamide (PAA), and a polyacrylonitrile (PAN) membrane, by following the modified method according to \(^{(26)}\), which is briefly as following: 2g of the immobilized materials (DFS, PAN, or PAN) was immersed in a solution containing 8g of DMF( N, N-dimethylformamid) and under continuous stirring at a temperature of 60 °C for 4 hours to form a solution of a homogeneous gel. Then the homogeneous gel solution was placed in a vacuum pump at 37 °C for a period of 12 hours to ensure complete exit of air bubbles.

**Fixing of immobilized material films on glass support**

Diatom Frustule Silicon / Glass (DFS / G) films, Polyacrylamide / Glass (PAA / G) and Polyacrylonitrile / Glass (PAN / G) films have been fixed by following the modified method according to \(^{(27, 26)}\), which is briefly as following: Mercaptol ester (NOA 61) was deposited as a heat-malleable polymer on a thin layer of 1.5 cm² from a glass slide and this was done with the help of centrifugation by a spin-coater device with a thermo adaptive procedure \(^{(27)}\). Then 10-20 µL of the homogeneous gel solution of the immobilized membrane material (DMF- DFS) or (DMF-PAA) or (DMF-PAN) was applied in a thin layer over the glass slide that was pre-coated with mercaptol ester with a weight of not more than 250 mg. Distributing it over the entire slide with the help of centrifugation by means of a spin-coater device with a thermoplastic process, and this treatment process was repeated again to obtain optimal adhesion. After fixing the immobilized membranes, the glass slides were placed in a petri dish containing deionized water with gentle stirring to ensure complete conversion of the homogeneous gel material to a solid state according to the phase inversion technique \(^{(26)}\). Slides which have regular immobilized films were picked up and discarded irregular ones, then washed with distilled water for 3-4 times, then the obtained slides were transferred to a beaker to storage for further use.

**Amine modification and chemical activation of immobilized films**

Chemical amine modification for films of the immobilized membranes was carried out by modified
procedure according to (28,26,27) as follows: Immobilized films on the glass slides, which included: (DFS / G), (PAA / G), and (PAN / G) were treated with 10% aqueous solution of NaOH at 50 °C for 60 minutes. After complete washing of the Immobilized films with distilled water, they were treated with 10% of aqueous solution 1,2-diaminohexane for 60 minutes at 40 °C and allowed to interact with continuous stirring in a magnetic stirrer at about (200) rpm. Then, they were cooling at room temperature. After that washed with acetone and ethanol to remove residual reagents. Then they were dried at 40 °C for 12 hours to a constant weight of approximately 250 mg. Finally, the glass slides with DFS, PAA, and PAN which are amino-modified immobilized films were transferred to a 5% aqueous solution of glutaraldehyde prepared in a phosphate buffer 0.1 M; pH: 7.0 to complete glutaraldehyde bonding reaction at 35 °C for a period of 3 hours. After the reaction period, the activated immobilized films by glutaraldehyde were sequentially washed with 0.1 M of acetic acid solution and 0.1 M phosphate buffer.

**Immobilization of (C12O) enzyme on activated immobilized films**

After completing the amine modification and chemical activation procedure with glutaraldehyde for immobilized films that fixed on the glass slides, the immobilization of catechol 1,2-dioxygenase enzyme (C12O) on these films were carried out through the following procedure according to (28,26). Slides of DFS/G, PAA/G and PAN/G immobilized films which were previously modified and activated were immediately treated with C12O enzyme solution, prepared in 4 mL of phosphate buffer 0.1 M; pH: 7.0 containing enzymatic protein as concentration (20.8, 16.7,20.2) mg. ml⁻¹ in cases of purification steps (CEE, AS 30%, SS 2%) as respectively, which are equivalent to activity as (71.6, 256.3,126.2) U.ml⁻¹ in cases of purification steps( CEE, AS 30%, SS 2%) as respectively. And then slides were placed in an incubator at 22 °C for 8 hours to complete the reaction. After reaction period, the membranes were washed with phosphate buffer 0.1 M and pH: 7.0. the same buffer was used to store glass slides which are containing immobilized catechol 1,2-dioxygenase enzyme for further use. Immobilized enzymatic protein on DFS, PAA and PAN membranes was quantified by Bradford method (21).

**Efficacy of enzymatic immobilization**

Enzyme activity for immobilized Catechol-1,2-dioxygenase (EC1.14.13.1) was determined by estimating the residual catechol by the Fujita et al colorimetric method (29) with a modification procedure consistent with the current study by using specimen cup plastic tube for each test equipped with a screw cap (QT) type with a capacity of 60 ml, provided that the total volume of additives in test tube is only 60 ml. Modification procedure and additives for each test tube could be illustrated as following: For the purpose of testing the effect of the pH difference on enzyme activity of catechol 1,2-dioxygenase, 32 ml of certain different buffers at a concentration of 50 mM were added to provide a medium (acidic, neutral, and basic), so that a sodium acetate buffer (pH: 5.0--6.0) was used when performing an activity test in a medium with acid characteristics and use a buffer of potassium phosphate (pH: 6.5--8.5) when performing an activity test in a medium with neutral specifications, while Tris–acetate buffer (pH: 9.0-10) was used when performing an activity test in a medium with basic specifications. 4 ml of free (soluble) C12O enzyme or immobilized C12O enzyme on immobilized films as weight of 250 mg, provided that the concentration added in both cases was about 17.6 mg. ml⁻¹ or equivalent to the specific activity of 256.3 U.ml⁻¹. The enzymatic addition was carried out depended on testing the different enzyme status, which included: soluble enzyme, and immobilized enzyme. Three types of enzymatic immobilization were tested as follows: immobilized on (Diatom Frustule / Glass) encoded DFS / G, immobilized on (Polyacrylamide / Glass) encoded PAA / G, and immobilized on (Polyacrylonitrile / Glass) encoded PAN / G. As well as testing activity of soluble and immobilized C12O enzyme were performed on different purification stages, including: crude extract enzyme and CEE encoded, precipitation with streptomycin sulphate encoded SS, and precipitation with ammonium sulphate encoded as AS. 24 ml of catechol substrate at 40 mM was added to the reaction mixture, and as soon as the
substrate was added the enzymatic reaction began. The reaction solutions were well mixed in each test tube and left under different incubation conditions in a Shaking water-bath at different temperature conditions: 15, 25, 35, 45 and 55 °C for 3 days. After the incubation period had elapsed, 1.6 ml of H₂O₂ hydrogen peroxide was added at a concentration of 5% (v/v), in order to inhibit the activity of the enzyme catechol 1,2-dioxygenase. Portions of the reaction solution (aliquots) were withdrawn and the same procedure of colorimetric method described by (29) were performed by ELIZA microplate reader for determination of residual catechol.

**Statistical Analysis**

The statistical analysis was done according to Completely Randomized Blok Design (CRBD) and Completely Randomized Design (CRD) with significance level 0.05 and 0.01, using SPSS version 22 of 2019.

**Results and Discussion**

**Characterization of (C12O) enzyme**

Catechol 1,2-dioxygenase (C12O) was extracted purified from *Candida albicans* strain which gave a positive result of qualitative primarily test (4) towards intradiol or ortho-cleavage of aromatic ring in catechol. Purification data for (C12O) enzyme were calculated under its optimal conditions, which are pH: 8 and a temperature of 25 °C, as indicated earlier (14,30) and as shown in Table1.

<table>
<thead>
<tr>
<th>Purification stage</th>
<th>Volume ml</th>
<th>Protein</th>
<th>Activity*</th>
<th>Sp.activity U.mg-1</th>
<th>purif. fold</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. mg.ml⁻¹</td>
<td>Total mg</td>
<td>Conc. U.ml⁻¹</td>
<td>Total U</td>
<td>Recovery %</td>
</tr>
<tr>
<td>CEE</td>
<td>21.6</td>
<td>20.87</td>
<td>3090</td>
<td>71.6</td>
<td>10601</td>
</tr>
<tr>
<td>30% AS</td>
<td>16.4</td>
<td>17.6</td>
<td>2164</td>
<td>256.3</td>
<td>31513</td>
</tr>
<tr>
<td>SS 2%</td>
<td>3.4</td>
<td>20.2</td>
<td>444</td>
<td>126.2</td>
<td>2774</td>
</tr>
</tbody>
</table>

*:One unit of catechol 1,2-dioxygenase (C12O) enzyme activity represents 1 µmol of substrate per mg of protein per minute.

It is evident from the data of Table1. that the highest specific efficacy of the enzyme purification (C12O) was within the second stage of the purification steps represented by precipitation with AS 30% (w/v) ammonium sulfate, reaching about 14.56 U.ml⁻¹ after completion the purification was 6.6 times, with a recovery rate of 78.3%, in order to reach the amount of enzyme protein close to 20 mg.ml⁻¹. Indeed, the enzymatic protein was purified by 17.6 mg. mg.ml⁻¹, which is equivalent to an enzymatic activity of 256.3 U.ml⁻¹. Whereas, (C12O) enzyme was purified by 15.3 times during the third stage of the purification steps by precipitation with streptomycin sulfate SS 2% (w/v) with an activity recovery rate of 70.3%, and enzyme protein was purified by 20.2 mg.ml⁻¹, which is equivalent to an enzymatic activity of 126.2 U.ml⁻¹ and specific enzyme activity of 6.25 U.mg⁻¹. The optimal catalytic activity of the enzyme (C12O) was within a pH of 6.5-8.5 and a temperature of 25 °C. This result is consistent with (14,30) in their studies on characterization of this enzyme, as they indicated its optimum catalytic conditions are pH 8 and a temperature of 25 °C.

**Biodegradation of catechol by (C12O) enzyme**

The percentages of biodegradation of catechol were calculated based on the concentrations of the catechol...
residues that were extracted with the concentration mM according to the conversion of the OD optical density readings from micro plate reader and on the basis of the tendency equation inferred from the standard curve of catechol shown in Annex (2-4), which was made.

**Optimum thermal limit of (C12O) enzyme**

As shown in Table 2. It was found that there are high significant differences in biodegradation ratio of catechol according to the variation in the temperature of the reactant mixture on the one hand and the degree of purity of (C12O) enzyme on the other hand, and that highest rates of biodegradation for catechol in moderate acid conditions were within the stage of precipitation with ammonium sulfate AS30% (w/v), specifically at temperatures 35, 25 and 45 Celsius degrees, as its rates were about 73.6 ± 15.0%, 72.6 ± 13.2%, and 70.0 ± 17.7%, respectively, which indicates that the peak activity of the enzyme (C12O) is in the neutral condition pH level was at 35 °C, meaning that there is a shift in the optimum temperature of the enzyme activity, perhaps due to the role of enzymatic immobilization. This result may not agree with the study conducted by (30), to assess the environmental conditions for the activity of the two enzymes (C12O) and (C23O) produced by the Gordonia polyisoprenivorans strain of actinomycetes, as they tested cell free enzymes in their soluble and immobilization states. As previously, the optimum conditions for pH, temperature, time path and the effect of ions on enzyme activity were determined, and they found that the peak of the enzyme activity for (C12O) was at a pH of 8.0.

<table>
<thead>
<tr>
<th>Enzyme status Purification stages</th>
<th>Insoluble enzyme (immobilized)</th>
<th>Soluble enzyme (free)</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DFS/G</td>
<td>PAA/G</td>
<td>PAN/G</td>
</tr>
<tr>
<td>Crude Enzyme Extract CEE</td>
<td>15</td>
<td>72.5</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>78.7</td>
<td>36.2</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>81.2</td>
<td>41.5</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>85.0</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>65.0</td>
<td>16.2</td>
</tr>
<tr>
<td>Precipitation with ammonium sulfate 30% AS</td>
<td>15</td>
<td>88.5</td>
<td>62.0</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>89.5</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>91.2</td>
<td>69.5</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>93.0</td>
<td>63.5</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>83.5</td>
<td>56.7</td>
</tr>
<tr>
<td>Precipitation with streptomycin sulfate 2% SS</td>
<td>15</td>
<td>79.0</td>
<td>41.0</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>82.2</td>
<td>44.7</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>84.2</td>
<td>49.0</td>
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<td>45</td>
<td>87.2</td>
<td>37.2</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>71.7</td>
<td>29.2</td>
</tr>
</tbody>
</table>

Mean ± S.D. | 82.2±7.8 | 44.7±16.2 | 57.3±13.7 | 30.2±18.3 | 53.6±23.9 |
While the lowest biodegradation ratio of catechol were within the crude enzyme extract stage of CEE, specifically at temperatures 55, 45 and 15 Celsius degrees, as their rates were about 28.6 ± 27.3%, 39.2 ± 33.5% and 41.6 ± 24.9%, respectively, as it seems clear the role of temperature extremes in enzymatic inhibition, especially in degrees above 25 Celsius degrees, and that the best ratios of catechol degradation within the crude enzyme extract stage as moderate acid conditions were at temperatures 35°C and 25°C, reaching about 49.7 ± 25.9% and 47.9 ± 23.2. It also shows in Table 2, the displacement amount of 10 degrees from the optimum temperature limit, which may also be due to the role of enzyme immobilization in increasing the stability of the enzyme and the permanence of its activity towards high temperatures.

As for the precipitation stage of streptomycin sulfate SS 2%, it was observed that the highest rates of biodegradation for catechol as moderate acid conditions were at temperatures 35, 25 and 15°C, reaching 56.2 ± 23.0%, 54.8 ± 20.7% and 49.9 ± 23.2% as respectively, through which the effect of the different kinds of enzyme immobilization under current study was also shown on the displacement of the optimum thermal limit of (C12O) enzyme action to include the temperature of 35 °C, while the low values of the rates of biodegradation ratios of catechol during the precipitation stage of streptomycin sulfate in moderate acid conditions were about 39.8 ± 24.0% and 49.1 ± 28.3% at high temperatures (55 °C) and 45 °C, respectively, and this may be due to the role of high temperatures in inhibition of (C12O) enzyme activity (31). And as shown in Figure 1. The increase in the optimum temperature may be due to the improvement in the stiffness and stability of the enzyme action due to immobilization method by covalent bonding method using the glutaraldehyde. These results may be in agreement with the study conducted by (25), in which they found that immobilization of tyrosinase to diatoms’ modified biosilica had a role in its activity within a wider temperature range compared to the free enzyme. A similar displacement to the optimum temperature of the immobilized enzymes has been observed in many studies, but the extent of the displacement varied with the different immobilization methods, the type of binding stent, as well as the type of binding or interaction between the immobilized enzyme and the immobilized support (32,31). It was found that the thermostability in the organic solvents of HabB enzyme was much higher in the case of the enzyme being included genetically within the biosilica of the diatom compared with the enzyme in its free state. Also, the stability of this enzyme was equal or even better if it was used within silica envelopes in laboratory experiments (in vitro). Examination by electron microscopy proved that genetic manipulation did not affect the silica architecture in Thalassiosira pseudonana (33).
Figure 1. biodegradation ratio of catechol (%) as an initial concentration of 40 mM by catechol 1,2-dioxygenase in incubation conditions: constant pH:(6.5-8.5) and different temperatures, for a period of 3 days

On the other hand, the statistical analysis showed high significant differences in biodegradation ratio for catechol according to the variation in the temperature of the reactant mixture on the one hand and the state of the enzyme on the other hand, and that the highest rates of biodegradation for catechol under moderate acid conditions were within the enzyme immobilization method by covalent glutaraldehyde on the modified biosilica support as DFS / G diatoms, with a rate of about 82.2 ± 7.8%, followed by polymeric support of the type of PAN / G at 57.3 ± 13.7%, while the lowest rates of biodegradation of catechol in the moderate acid conditions were in the case of the free enzyme (soluble) as about 30.2 ± 18.3%, followed by immobilization on the polymeric support of the type of PAA / G at 44.7 ± 16.2%, as is Shown in Figure 1. Which indicates that the used enzymatic immobilization method has given good results in all types of the immobilization supports used under this study in maintaining the thermal stability of the enzyme (C12O) and making it stable under different temperature conditions under the current study compared to the use of the enzyme in its free or soluble form.

The method of immobilization of the enzyme (C12O) on the biosilica supports of the diatom plate gave a double displacement of 20 °C from the optimum thermal limit for the enzyme activity, within all the purification stages. 87.2% and 85% were in the precipitation stages of ammonium sulfate AS and the precipitation with streptomycin sulfate SS and the enzymatic crude extract CEE respectively. While also the methods of immobilization of the enzyme (C12O) on the polymeric supports of the type of PAN / G and PAA / G gave a displacement of 10 Celsius degrees from the optimum thermal limit of the enzyme activity, within all the purification stages, the rates of breakdown rates reached Catechol with it at 35 °C is about 78.0%, 62.2% and 56.5% in the case of PAN / G immobilization, followed by the PAA / G immobilization condition with ratio of 69.5%, 49.0% and 41.5%, respectively, in the enzymatic purification stages AS SS. And CEE. On the other hand, the free enzyme (C12O) did not show
a displacement from its optimum thermal limit, and the highest values of its activity were within the temperature of 25 °C in all the purification steps under current study. This may be due to the fact that the pores in the polymeric immobilization supports are of microscopic sizes and are relatively few in terms of quality and quantity compared to the large number of micro and nanoscale pores and nano silicon in the biosilica of the diatomatum (16). The sizes of the polyacrylamide microfiltration (PAA) had a microfiltration rate of 0.2 m while the sizes of the polyacrylonitrile (PAN) were ultrafiltration with the average pore size of 0.01 m. This means that the surface area ready for covalent immobilization of the enzyme (C12O) using the glutaraldehyde method was higher in the organic silica of the diatom frustule compared with the two types of polymeric films used under current study, followed by the surface area ready for enzymatic binding in the polymeric membrane PAN and then the polymeric membrane of PAA. This, in turn, leads to a variation in the number of units of the bound (C12O) enzyme. It appears that the amine modification using 10% diaminohexane solution 1,2 in order to increase the amount of amine groups on the surface of the carriers had a role in making the surface carboxyl groups on the surface of the polymeric restraint supports on the one hand and the carboxylic end of the silaffin groups, which represent one of the most important organic components of diatom frustule, on the other hand (33), binds to one of the amine groups, leaving the other free and predisposed to binding with glutaraldehyde, which in turn binds to the enzyme units. This indicates the possibility that the biosilicate of the diatom layer was modified to a greater degree compared to the PAN films which were also to a greater degree than the PAA films. This result may be consistent with what was indicated by (28).

Optimum pH of the (C12O) enzyme

Table 3. shows biodegradation ratio for catechol with an initial concentration of 40 mM, resulting from the activity of the enzyme catechol 1,2-dioxygenase (C12O), whose efficacy was tested under reaction conditions that included different acidic conditions, as solutions were used during enzymatic purification. PH (various pH) such as sodium acetate buffer pH: 5.0-6.0; Potassium phosphate buffer pH 6.5--8.5; Tris-acetate pH 9.0--10 buffer, for the purpose of assessing the optimum pH of the enzyme activity (C12O) under conditions of its free and immobilization state on the biosilica of the DFS / G diatomaceous frustule on the one hand and on the polymeric films PAA / G and PAN / G, which is in turn fixed on glass struts on the other side.

After conducting a statistical analysis of the values shown in Table 3., it was found that there are high significant differences in the percentages ratios of biodegradation for catechol. According to the variation in the pH conditions of the reaction mixture on the one hand and the purity of the enzyme on the other hand, and the highest percentages of biodegradation ratios of catechol under constant temperature conditions, it was within the precipitating stage of ammonium sulfate AS 30% (w / v), specifically at the acidic functions 6.5-8.5 and 9. - 10, as the biodegradation rates of catechol reached 72.6 ± 13.2% and 63.0 ± 36.6%, respectively, while the lowest rates in all the purification stage were at the acidic function 5-6 and were about 28.5 ± 29.4. The reason for this may be due to the role of acidity in inhibiting the denaturation of the enzyme protein (34), especially the free unconstrained enzyme and thus its catalytic activity decreases in breaking down the catechol substrate. The enzyme is within the acid and base levels.

On the other hand, the statistical analysis showed high significant differences in the percentages of biodegradation for catechol according to the variation in the acidic function of the reactive mixture on the one hand and the state of the enzyme on the other hand, and that the highest rates of percentages of biodegradation of catechol under constant temperature conditions of 25 °C were within the method immobilization of the covalent enzyme with glutaraldehyde on the modified biosilica support of the DFS / G diatoms, reaching a rate of about 80.3 ± 9.3%, followed by the method of immobilization of the covalent enzyme with glutaraldehyde on the modified biosilica support of the DFS / G diatoms, reaching a rate of about 80.3 ± 9.3%, followed by the method of immobilization of the covalent enzyme with glutaraldehyde on the modified biosilica support of the DFS / G diatoms, reaching a rate of about 80.3 ± 9.3%, followed by the method of immobilization of the covalent enzyme with glutaraldehyde on the modified biosilica support of the DFS / G diatoms, reaching a rate of about 80.3 ± 9.3%, followed by the method of immobilization of the covalent enzyme with glutaraldehyde on the modified biosilica support of the DFS / G diatoms, reaching a rate of about 80.3 ± 9.3%, followed by the method of immobilization of the covalent enzyme with glutaraldehyde on the modified biosilica support of the DFS / G diatoms, reaching a rate of about 80.3 ± 9.3%, followed by the method of immobilization of the covalent enzyme with glutaraldehyde on the modified biosilica support of the DFS / G diatoms, reaching a rate of about 80.3 ± 9.3%, followed by the method of immobilization of the covalent enzyme with glutaraldehyde on the modified biosilica support of the DFS / G diatoms, reaching a rate of about 80.3 ± 9.3%.
method of immobilization of the covalent enzyme with glutaraldehyde on the polymeric support of the type of PAA / G with a rate of $42.7 \pm 20.7\%$, and as shown in Figure 2., which indicates that the used enzymatic immobilization method gave good results in all types of immobilization supports used for current study in maintaining the stability of the enzyme (C12O), the extreme values of the acidic function and making it stable, especially under the basic pH conditions, compared with the instability of the enzyme in its free or soluble form under such basic conditions.

<table>
<thead>
<tr>
<th>Enzyme status Purification stages</th>
<th>Insoluble enzyme (immobilized)</th>
<th>Soluble enzyme (free)</th>
<th>Mean ± S.D.</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>DFS/G</td>
<td>PAA/G</td>
<td>PAN/G</td>
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<tr>
<td>Crude Enzyme Extract CEE</td>
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<tr>
<td>Precipitation with ammonium sulfate 30% AS</td>
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<td>Precipitation with streptomycin sulfate 2% SS</td>
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<tr>
<td>Mean ± S.D.</td>
<td>80.3±9.3</td>
<td>42.7±20.7</td>
<td>55.8±17.3</td>
</tr>
</tbody>
</table>

RCBD ANOVA-TABLE

- $P\text{.value (} \alpha \text{ 0.05)( 8, 27)} = 2.30$
- $P\text{.value (} \alpha \text{ 0.01)( 8,27)} = 3.26$

<table>
<thead>
<tr>
<th>DFS/G: Diatom Frustule Silica / glass</th>
<th>PAA/G: Polyacryl amide / glass</th>
<th>PAN/G: Polyacryl Nitrile / Glass</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P\text{.value (} \alpha \text{ 0.05)}(3, 32) = 2.92$</td>
<td>** $F = 11.48$ **</td>
<td>L.S.D.( $\alpha \text{ 0.05)}(27) = 38.66$</td>
</tr>
<tr>
<td>$P\text{.value (} \alpha \text{ 0.01)}(3,32) = 4.51$</td>
<td>** $F = 19.23$ **</td>
<td>L.S.D.( $\alpha \text{ 0.05)}(32) = 16.29$</td>
</tr>
</tbody>
</table>
The method of immobilization of (C12O) enzyme on the biosilica supports of the diatom frustule gave a displacement of 1.5 degrees to the optimal pH of the enzyme activity, within all the purification stages, the highest rates of catechol degradation were reached through it at the basal pH 9-10, about 91.8% and 86.5%. And 85.0% in the precipitation stages with ammonium sulfate AS and the crude enzyme extract CEE and precipitation with streptomycin sulfate SS respectively, and the reason for this may be attributed to the water absorption, if it exceeds the retention capacity (i.e. saturation) of the ionic liquids at room temperature, to facilitate denaturation. The enzyme was found at the interface between the solvent and the water, thus reducing the enzyme activity (31), whereas the C12O immobilization methods did not appear such as those displacements on the PAN / G and PAN / G as polymeric supports. The optimal pH of the enzyme activity during the enzymatic purification stage with streptomycin sulfate SS, but it was limited to the initial stages of enzymatic purification, as it was able to deflect the optimal pH of the enzyme (C12O) by 1.5 A score also within the AS and CEE purification stages, the rates of catechol degradation through it at a base pH 9-10 were about 79.0% and 71.8%, respectively, in the case of PAN / G immobilization, followed by the case of PAA / G immobilization with percentages of 71.8% and 62.0%, respectively. On the other hand, the free (C12O) enzyme did not show a displacement from the optimum pH, and the highest values of its activity were within the neutral pH 6.5-8.5 in all the purification steps under current study, with the highest rates of biodegradation of catechol about 58.8%, 34.3% and 25.3 in enzymatic purification stages AS, SS and CEE, respectively. Although the results obtained may not agree with the opinion of (35) and (36) who have demonstrated that immobilization of enzymes does not fundamentally change the properties of the pH response, they noted in their studies of tyrosinase immobilization, that the limit maximum stimulatory response to the immobilization enzyme was in the pH range of 5.6 (35) and at pH 7 (36), so now this may not apply to the features that the biosilica
possesses in the diatom frustule that he found \(^{(25)}\), in enhancing the properties of the immobilized tyrosinase compared to the free form of the same enzyme. It has been found that the optimum pH of polyphenol oxidase extracted from mushrooms is about 6.2 \(^{(37)}\) and according to many scientific studies including \(^{(38,39)}\), the optimal pH of this enzyme may differ when it is immobilized and dependent on the type of immobilization prop and the method used for immobilization. There are many studies that are consistent with the results of our current study related to determining the optimum temperature and pH of the enzyme (C12O) from \textit{C. albicans} which is of 25 °C and pH 8.0, respectively, including isolation and characterization of the enzyme (C12O) among the \textit{Pseudomonas} bacteria. By \(^{(40,41)}\), but less if compared to those isolated from different species such as \textit{Rhizobium leguminosarum} \(^{(42)}\) and \textit{Rhizobium trifolii} before \(^{(43)}\), as they found that it was optimally active at the pH was 9.0-9.5 and the enzyme purified from it was stable and maintained its activity at a rate of >85% for at least 30 minutes in the pH range of 7.0-9.0, while the stability of the enzyme was significantly reduced at pH outside this range.

**Conclusions**

It was observed that the surface area ready for covalent immobilization of the (C12O) enzyme was higher in the basilica of the diatom frustule compared with the two types of polymeric films used under study, followed by the surface area ready for enzymatic binding in the polymeric membrane (PAN) and then the polymeric membrane membrane (PAA). This, in turn, leads to a variation in the number of units of the bound (C12O) enzyme. As the method of immobilization of the (C12O) enzyme on the biosilica supports of the diatom frustule gave a double displacement of 20 °C from the optimum thermal limit for the enzyme activity, within all the purification stages. Whereas, the enzyme immobilization (C12O) methods on the polymeric supports of the type PAN / G and polyacryl amide PAA / G gave a displacement of 10 Celsius degrees from the optimum thermal limit of the enzyme activity, within all the purification stages. In contrast, the free, unbound (C12O) enzyme did not show a displacement from its optimum thermal limit, and the highest values of its activity were within the temperature of 25 °C in all the purification steps under study.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

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Comparison Between 3 Different Types of Mouthwash on the Healing Process of Periodontal Diseases

Ammar Alwan Ali1, Zainab Kasim Mohammad1, Abeer Isam Abdulhameed1
1 B.D.S., H.D.D. Researcher, High Diploma in Periodontics / Dental Health Specialist Center in Sheikh Omar / Baghdad, Iraq

Abstract
Dental plaque is the principal etiological agent for the development and progression of gingival and periodontal diseases. Three different mouthwash types were compared in this study for the periodontal healing process based on the results. For all bacterial strains, the culture type collection is used. The serial dilution process is used for preparing every mouthwash and adding it to the tubes with a specific dilution. In certain microorganisms of the respective mouthwash, the last tube dilution that does not show any turbidity will be considered MIC. The three types of mouthwashes were compared in terms of the ability to inhibit microbial growth. Twenty-four hours after the agar was solidified and the colony forms (CFU) units counted, the plates were incubated. This study showed that bacterial growth inhibition might occur in the three types of mouthwash. Boht, Behsa and Kin-gingival mouthwashes had a significant difference in their antimicrobial effect. This study showed that they were unable to perform s after 24 hours of in vitro incubation while the bacterial count was lowered after two weeks of in vivo use in the boht washings. Continuous exposure to bacteria may be necessary to wash the mouth, especially for Behsa and Boht. It is difficult to remove all bacteria from the mechanical plaque controls; antibacterial mouthwash can cause additive. In the current study, three types of mouthwash have compared antimicrobial effects. The results showed only that bacterial counts are affected by mouthwash. Based on this study’s results, Boht mouthwash is more effective than Kin Gingival and Behsa mouthwash for oral microorganisms, but further clinical studies are necessary to confirm our findings.

Keywords: Dental plaque, Periodontal diseases, Mouthwash, Bacterial, Boht, Behsa, Kin-gingival

Introduction
A variety of microorganisms, mainly bacteria, can be colonised and developed through the mouth; one influence factor in teeth and periodontal tissue destruction is bacterial plaque. The primary etiological factor for tooth decay, gingivitis, and periodontal disease is dental plaque. Dental and periodontal conditions are diseases associated with the prevention of biofilm. Dental plaque is the primary etiological agent in gingival and periodontal disease development and progression. Dental and periodontal susceptibility varies according to risk factors, including genetics, systemic factors and oral hygiene.

The primarily mechanical removal of plastic plaque through regular tooth brushing is used to prevent different periodontal diseases’ development and progression. The use of mouthwash to control plaque bacteria is about 5000 years long when the Chinese suggested that urine for children be used to prevent gingivitis. Various bacterial and fungal communities integrated into a highly specialised extracellular matrix are present in oral biofilms. The decrease in oral biofilm accumulation (dental plates) and the control/reduction of dental disease risks are essential for good oral care practices.

The primary effect of Mouth rinses on the supragingival and marginal plaque is limited due to tight gingival contact with the tooth in healthy persons. It must always be used in conjunction with mechanical plaque control measures but should never be used
solely as a means of oral hygiene \(^8\). Mouth washing can help prevent oral infections, reduce inflammation, decrease halitosis and local fluoride prevent caries. Mouth washing has several preventative and therapeutic purposes. Usually, mouthwash is based on anecdotal evidence instead of on-the-counter (OTC) scientific evidence \(^9\).

The use of mouth washing and chemical cleansing of teeth is typically to reduce dental accumulation \(^10\). It can even be used as oral care only for patients who cannot brush their teeth either after surgery or due to the motor or cognitive constraints \(^11\). Chlorhexidine (CHX) is long considered a short-term gold standard of action for bacteria, spores and fungi, including many antiseptic components of oral mouth washing. Mouthwash is a safe and effective anti-plaque and antimicrobial agent, which prevents adhesion, colonisation, metabolic activity and bacterial proliferation \(^12\). Due to the variety of antibacterial efficacy, cytotoxicity and kinetics of different solutions, it’s not easy to decide whether to use a particular mouthwash. In decreasing oral microbial counts, CHX is regarded as the gold standard \(^13\).

In preventing periodontal illnesses, mechanical plaque control measures (tooth brushing and flossing) are essential. Benzylamine chloride can also help avoid periodontal diseases by chemical plaque control measures, such as utilising only 0.12 or 0.2 per cent mouthwashes preparations with chlorhexidine \(^14\). If there is unevenness between the host defence and the bacterial niche, periodontitis may occur. The primary aetiology of the gingival tissues and the paradental attachment is the bacterial plaque \(^7\). Mechanic removals of the plaque or calculus and topical antimicrobial agents that can inhibit periodontal pathogens are commonly used in periodontal therapy. Moreover, in current periodontal and other applications, extracts from plants have been offered as anti-plaque agents \(^15\).

Body rinse can produce a therapeutic effect on the tooth’s entire surface, including interproximal areas where toothpaste is very effective \(^16\). Although it is effective in plaque control, chlorhexidine cannot be used for a long time because of some of its unpleasant side effects after a long time. The use of mouthwash in oral hygiene is ancient, in contrast to the popular notion. The use of mouthwash originates from religious codes of behaviour such as the outdated Manu laws of India that must rinse their mouths after a meal \(^11\). This study aimed to compare three different mouthwash types for the periodontal healing process based on the findings.

**Methodology**

This study aimed to determine the effect of three types of mouthwash on the treatment of periodontal disease. The impact on Kin Gingival, Behsa and Boht are assessed on the selected bacterial. Thus, minimum inhibiting concentrations (MIC) were used. The collection of culture type is used for all bacterial strains. The serial dilution method has been used to prepare every mouthwash with specified dilution and added to the tubes \(^3\).

The last tube or the last mouthwash dilution not showing turbidity is considered the MIC in certain microorganisms of the respective mouthwash. In terms of the ability to inhibit microbial growth, the three mouthwash types’ MIC was compared \(^17\). The tubes without turbidity (transparent) were then transferred to a solid media after 24 hours of incubation, which showed bacterial growth inhibition with the respective mouthwash and evaluated for the microbial growth to determine the MBC of mouthwash. In terms of the solid medium culture, the last tube, which was negative, indicated the minimum concentration (MBC) of bactericidal fluid \(^17\).

For all bacterial strains, this procedure was done. 0.5 ml diluted samples have been transferred in empty plates for counting bacterial colonies. The agar was cooled to 50°C and poured into each plate. The pouring of the agar was done into each plate. The plates were incubated 24 hours after the agar had solidified and the colony forms (CFU) units counted. On dilute samples collected before the patients’ used mouthwashes or water, the zone of growth inhibition test was done in vitro \(^8\). On the agar surface with the swab, bacteria were streaked. The filter paper impregnated disks were then placed at the centre of each section and slightly pressed to the agar with the
water and mouthwash. The plates were then incubated for 24 hours at 37°C inverted positions. The inhibition zone was measured after 24 hours \(^{(10)}\).

**Results**

![MIC and MBC of the Mouthwashes](image1)

*Figure 1: The MIC and MBC effect of the three mouthwash on the selected bacteria*

![Zone of Inhibition](image2)

*Figure 2: The effect of the three mouthwash on the zone of inhibition in selected bacteria*
Discussion

This study showed that the three types of mouthwash could cause bacterial growth inhibition. The antimicrobial effect of Boht, Behsa and Kin, gingival washes of the gum, were significantly different (Figure 1). The SM growth inhibition areas in the three study groups are shown in Figure 2. Two-week use of Boht, Behsa and Kin gingival mouthwash, before and after periodontal patients, is summarised in Figure 3. In patients with periodontal disease, the summary of bacterial counts is also presented in Figure 3 before and after two weeks.

Mueller Hinton Agar was the artistic medium of this study, used as a growth speciality for those bacteria in procedures commonly conducted for aerobic and optional anaerobic bacteria (neogen.com). Therefore, it would appear logical to assume that both aerobic and optional bacteria cultivated on agar plates were actual. The results of this study demonstrated a difference in the antibacterial effects shown by Behsa and Boht, as they were unable to make s after 24 hours of in-vitro incubation, while boht washings showed a reduced bacterial count after two weeks of in-vivo use. The culture medium was treated only once and then after 24 hours in inhibition tests with mouth washing. At the same time, bacteria were repeatedly exposed to the effect of mouth washing for the in vivo testing for two weeks. The results were obtained after 24 hours only. It may require continuous exposure to mouth washing, especially for the Behsa and Boht, to reduce bacterial counts. Mechanical plaque controls are difficult to remove all bacteria; antibacterial mouthwashes may help additives to this effect. Antimicrobial effects were compared in three types of mouthwash in the current study.

The role of bacterial plaques was demonstrated by dental caries aetiology and by periodontal diseases.
The mechanical methods of plaque inhibition are limited; this problem is proposed to chemical methods for plaque inhibition (6). Therefore, the utilisation of mouth washing as disinfectants can help mechanically reduce plaques. Mouth washing takes place in few ways, including apoptosis, bacterial growth inhibition, metabolic inhibition of cells, and bactericidal inhibitions based on their concentration (11).

A great deal of study has shown that washing chlorhexidine’s mouth is the best way to wash the mouth. The supremacy of most studies comparing mouthwashes, with only a few studied products competing in antibacterial characteristics with chlorhexidine, has been demonstrated. Streptococci are the primary etiologic agent for tooth decay (18).

Removal of streptococci prevents plaque formation and disease spreading. In the study of S. mutans susceptibility to mouthwashes, Jarvinen et al. showed that S. mutans is resistant to antimicrobial agents. S. mutans have the highest mouthwash strength and even greater varnish resistances (19).

We also have confirmed that S. mutans are somewhat resistant to chlorhexidine. The most significant anti-caries effect of fluoride chlorhexidine was achieved using a study comparing Behsa polyphenol extracts with fluoride washes, showing its synergistic effect on microorganisms (20).

A study comparing oral-B with other mouthwashes has demonstrated greater efficacy in reducing the S. mutans by washing Boht in plaques around orthodontic brackets, indicating high antimicrobial activity mouth (14). Kin Chlorhexidine Gingspace effectively eliminates Streptococci causing decay and beneficial antimicrobial and anti-gingival effects, as they support initial plaques in these microorganisms. Studies have shown that a higher concentration of the antimicrobial effect. The main impact on the microorganism concentration is Kin gingival mouthwash (20).

The first microorganisms to develop dental caries are lactobacilli and chemical or mechanical removal. The differences between the chlorhexidine compound may lead to previous studies since different combinations have other products (21).

Streptococcus mutans are the primary etiologic agent for dental caries. It may adhere to the acquired film as the first step in plaque formation. By eliminating this bacterial species, I prevent plaque formation and caries development (7). Mechanical methods of plaque inhibition have several limitations; dental plaque inhibition techniques have therefore been suggested. The use of disinfectants to wash the mouth can help to reduce plaque. The effect of Behsa mouthwash on SM inhibition has been evaluated and compared with Boht and Kin Gingival mouthwash’s effectiveness (22).

The results showed that all three washers could inhibit SM’s spread with the highest Boht-inhibiting effect. Many oral studies in microorganisms have shown that Boht is the most appropriate gold standard for chemical treatment with SM and dental cavities. The study showed the inhibition by Boht containing mouthwash and Total Care Kin gingival of the formation of the plaques by various Streptococci species (23). They also say Boht’s mouthwash is more efficient than Total Care Kin’s gingiva mouthwash. Dental caries and pathology decreased, damaging the innocent bacterial species that competed with SM effectively inhibited by washed-in (1).

Boht’s positive effect on reducing the SM and Lactobacillus colonies has been described in the literature. Boht is a large anode and adsorbs the tooth, plaque and mucus surface and increases adsorption to the above characters by its cationic nature. The extracellular polysaccharides cause the absorption of this antibacterial mouth washing (24).

However, previous authors, contrary to this study, have shown a better effect on the Kin gingival mouthwash plankton and biofilm bacteria than Boht (diluted). Boht also decreased the number of plaques and gingivitis, but no antibacterial activity existed when Boht was diluted. In its antimicrobial activity, Boht concentration appears to play a vital role (2).
Another discovery of this study was Behsa mouthwash’s best, higher, antibacterial effect than Kin Gingival mouthwash. The essential oils available are kin gingival. The antimicrobial activity of Kin gingival against oral microorganisms has already been evaluated and has been confirmed (2).

The main flavonoids in tea are epigallocatechin-3-Gallate and epicatechin. The catechin’s anti-cancer activity may be attributed to a direct anti-bactericidal effect on SM inhibition adherence to tooth surfaces. Behsa purifies the oral cavity, and reduced dental caries affects people who drink large quantities of Behsa (8). The combination of several antibacterial agents in a single product is one way of increasing anti-plaque efficiency. In Behsa mouthwashes, the higher antibacterial effect is higher than that of Kin gingival, given confirmations from various studies on the impact of Behsa extract and Kin Gingival mouthwashes in SM (25).

The positive effect of Behsa extract on the decrease in SM and Lactobacillus colony figures has previously been demonstrated in line with this study. Contrary to current research, the use of Behsa extract mouthwash has been shown to reduce the number of oral Kin Gingival-like micronutrients and have similar implications for both mouthwash types (17). The differences in the concentration of active agents in the formulation of mouth washing can be due to the study results. Unfortunately, the manufacturer’s Behsa concentrations in the Kin gingiva mouthwash have not been reported, and studies’ attention is not similar (26).

Therefore, Boht may be used every day as the natural component is present in Behsa and the tooth’s lack of dental colour potentials (5).

**Conclusion**

The results showed just that mouthwash affects bacterial counts. Since the oral cavity for commensal species has nevertheless played a positive part, there is still discussion of the need to maintain a constantly low number of bacteria within the mouth. This study demonstrated that three types of mouthwash could reduce the number of bacteria in the oral cavity. The S. mutans proliferation was more efficient than Kin gingival due to the Behsa containing a mouthwash. But Boht was less potent than both types of mouthwash. This study helps doctors to select the best antimicrobial agent on the market. Based on this study’s findings, boht mouthwash is more effective for oral microorganisms than Kin Gingival and Behsa mouthwashes, but further clinical studies are required to confirm our findings.

**Conflict of Interest:** No

**Source of Funding:** Self funded

**Ethical Clearance:** Not Required

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Induced Hyperlipidemia in Adult Male Rats and Treated by using *Rosmarinus officinalis* Aqueous Extract

Marwa Karim Taha¹, Mohammed Salman Dalas², Ahmed Hamad Saleh³

¹Assist Lec. M.S.C. in Physiology/ Radiology Department/University of Al-Qalam College, Iraq, ²Assist. Lect M.S.C. in Cell Biology/ Dental Industry Department/University of Al-Qalam College, Iraq, ³Lect. Ph.D. in Histology/Medical Laboratory Department/University of Al-Qalam College, Iraq

Abstract

The current work was goaled to revealed anti-hyperlipidemia activity of *R. officinalis* extract. This work utilized 20 male rats that divided to negative group; positive group was given drinking water containing 0.5% H₂O₂ and (1%) cholesterol for 60 days. Third group: hyperlipidemia rats were treated with (50mg/kg/daily) extract. Fourth group: hyperlipidemia rats were treated with (100mg/kg/daily) extract. The outcomes revealed significant (P < 0.05) rise in cholesterol and triglyceride and reduce the HDL in positive group compare with negative group. Malonedialdehyied (MDA) was increased with reduce in glutathione (GSH) compare with negative group. Otherwise, diameters of aorta artery show significant (P < 0.05) rise compare with negative group. *R. officinalis* aqueous extract when used in treatment, the outcomes exhibited improvement in lipid profile, MDA, GSH and diameters of aorta artery compare with negative group. It was concluded that *R. officinalis* extract possess anti-hyperlipidemia activity.

Keywords: *Rosmarinus officinalis*; hyperlipidemia; lipid profile; malonedialdehyied; glutathione.

Introduction

*R. officinalis*, arising from the Mediterranean region is describe as aromatic plant (family: Lamiaceae)¹¹, and is called rosemary [²]. As well as to utilize in culinary side due to the feature aroma, *R. officinalis* is widely used by indigenous people [³]. The products of *R. officinalis* are utilized in testing resulting in their low toxicity and major medicinal properties [⁴]. *R. officinalis* extract composed of various types of polyphenols inclusive phenolic acids and flavonoids. These molecules show strong antioxidant properties which decrease lipid peroxidation, prevent reactive oxygen species production and suppress inflammation, and comprise from terpenoids with flavonoids, phenols and volatile oil [⁵]. *R. officinalis* is important for its therapeutic in folk medicine utilized for example like antidepressant activity, hepatoprotector activity, antidiabetic activity, antiangiogenic activity, anti-inflammatory activity and antitumor activity [⁶-⁹]. Hyperlipidemia is defined as a heterogeneous cluster of troubles characterized by an increase of lipids profile in the blood, hyperlipidemia also refers to elevate in concentrations triglycerides, and cholesterol in the serum [¹⁰]. Hypercholesterolemia may be occurring through high-fat diet or in persons with physiological defects like decreased of receptors number functioning LDL-cholesterol [¹¹].

Materials and Methods

Animal model

In this work 20 rats, (wt 140-180 gm with age 2-4 month) were used and kept on a standard diet and normal saline until beginning the experiment.

Aqueous extraction

The solution of 20% extract was prepared by utilizing boiling water (100 ml) with leaf powder (10 gm), then the mixture was centrifuged at 10000 rpm for 10 min, then supernatant of mixture was put in oven at 45ºC to dry, after that dried powder was collected and stored until use [¹²].
Experimental design

20 rats were utilized in this work and divided in four groups (each group consist five rats):

- Negative group: received normal saline only.
- Positive group: drinking water containing 0.5% \( \text{H}_2\text{O}_2 \) and (1%) cholesterol for 60 days
- Third group: hyperlipidemia rats were treated with (50mg/kg/daily) extract for 30 days.
- Fourth group: hyperlipidemia rats were treated with (100mg/kg/daily) extract for 30 days.

Biochemical measurements

Total cholesterol, triglycerides and high density lipoprotein (HDL) levels were determined using standard kits of biomereiux kit, France. Malondialdehyde (MDA) is determination based on formation of colored complex upon reaction with thiobarbutyric acid. The detection was recorded at (500 nm). Glutathione was measured according to method of [13].

Histological study

Aorta artery from all studied groups were fixed by using formalin 10% , then embedded by using paraffin. After the routine processing, paraffin sections of aorta tissue were cut into 7 μm and stained by using haematoxylin and eosin [14].

Statistical analysis

All data of present work were collected and analyzed by utilizing the SPSS version 12. Descriptive data was summarized using mean, standard error (SE). P values < 0.05 were considered statistically significant.

Results

Lipid profile

The levels of TC (283.1 ± 11.15), TG (243.83 ± 9.35) and HDL (25.92 ± 3.6) in second group show high significant changes (P < 0.05) compare with control group (94.1 ± 5.72; 78.03 ± 4.1 and 32.9 ± 2.62 respectively). The levels of TC (98.2 ± 8.63; 91.61 ± 6.51 respectively), TG (81.4 ± 4.8; 74.3 ± 5.17 respectively) and HDL (30.1 ± 4.2; 34.6 ± 5.39 respectively) in third and fourth groups show non-significant changes (P < 0.05) compared with control group as shown in figure (1).

![Figure (1): levels of lipid profile in studied groups.](image-url)
MDA and GSH

The levels of MDA (2.38 ± 0.32) and GSH (0.235 ± 0.028) in positive group show high significant changes (P < 0.05) compare with control group (1.48 ± 0.24 and 0.362 ± 0.038 respectively). The levels of MDA (1.61 ± 0.38; 1.52 ± 0.19 respectively) and GSH (0.378 ± 0.028; 0.366 ± 0.026 respectively) in third and fourth groups show non-significant changes (P < 0.05) compared with control group as shown in figure (2).

![Figure (2): levels of MDA and GSH in studied groups.](image)

Aorta diameters

Aorta diameter (72.2 ± 6.1) in positive group show high significant changes (P < 0.05) compare with control group (34.3 ± 3.5). Aorta diameter (38.17 ± 5.63; 33.28 ± 4.82 respectively) in third and fourth groups show non-significant changes (P < 0.05) compared with control group as shown in figure (3).

![Figure (3): Aorta diameter in studied groups.](image)
Discussion

The current work exhibit the role of *R. officinalis* extract to reduce the levels of cholestrel and triglyceride, aorta diameters and oxidative/antioxidant factors. In study carried out by [15] referred that *R. officinalis* extract lead to reduce the elevated cholesterol in rats that feeding on the high-fat diet (HFD) that is in agreement with outcomes of present work. Al-Sheyab et al. [16] demonstrated clearly the hypolipidemic activity of *R. officinalis* species. The lipid profile (TC, HDL, LDL and TG) showed reduction in rosemary-fed mice as compared to the HC mice. Otherwise, significant elevation of the HDL was observed in rosemary-fed mice as compared to the HC mice. Otherwise, *R. officinalis* extracts have the ability to decompose free radicals by quenching active singlet oxygen and by trapping and quenching radicals before they reach a cellular target [17] that explains the role of *R. officinalis* extract to regulate the oxidative/antioxidant factors. Also, Rosemary is also capable preventing of lipid peroxidation process that is caused by oxidative stress [18]. In addition to reducing the amount of reactive species in the body, rosemary has been found to increase the activity of antioxidant enzymes [19-20]. About the thickness of aorta and the role of *R. officinalis* extract, rosemary extract prevented weight gain by limiting lipid absorption process in the intestine. This was made possible through the prevent of pancreatic lipase activity [21]. Finally, the third study found rosemary extract to inhibit and prevent lipid synthesis via the suppression of diacylglycerol acyltransferase (DGAT) [22] that may explain the ability of rosemary extract to prevent the accumulation of lipid in tissues.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

Reference


Combined Effect of Physical and Psychological Stress Exposure during Pregnancy on the Expression of Caspase-3 Cerebrum and Cerebellum of Newborn Mus musculus

Binta Dwi Novitasari¹, Hermanto Tri Joewono², Widjiati³

¹Postgraduate Student of Reproductive Health Science, Airlangga University, Surabaya, Indonesia, ²Lecturer at Department of Obstetrics and Gynecology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia, ³Professor at Department of Embryology, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia

Abstract

Background: Prenatal stress affects fetal development including brain development. When a stressor is felt, the brain as the main target for stress will release a hormone that stimulates the release of pro-apoptotic proteins and activate caspase-3 which acts as an executioner caspase in the cell death process. The aim of the study was to analyze the effect of combined stress during pregnancy on the expression of caspase-3 cerebrum and cerebellum of newborn Mus musculus. Methods: An experimental study using 24 pregnant mice (Mus musculus). Subjects were randomized into four groups, consisting of physical stress exposure group (forced swimming) (G1), psychological stress exposure group (noise) (G2), combination stress exposure group (forced swimming + noise) (G3), and control group (G4). Stress exposure was given on 6th-15th days of pregnancy. From each mother, three Newborn of Mus musculus were taken to make preparations from brain tissue. Immunohistochemical examination was performed to assess caspase-3 expression. Results: The study shows that the mean and standard deviation of the expression of caspase-3 cerebrum and cerebellum in the physical stress exposure group is 5.70 ± 0.99 and 5.80 ± 1.35, the psychological stress exposure group is 7.23 ± 1.39 and 7.40 ± 1.24, the combined stress exposure group is 8.67 ± 1.09 and 9.30 ± 1.12, and the control group 4.17 ± 1.18 and 3.90 ± 1.06. ANOVAs statistical test results show significant differences among groups with a value of p = 0.000 in the cerebrum and p = 0.000 in the cerebellum. Conclusion: Exposure to physical and psychological stress during pregnancy increases the expression of caspase-3 in the cerebrum and cerebellum of newborn mice.

Keywords: Caspase-3, stress, pregnancy, cerebrum and cerebellum

Introduction

Pregnancy stress not only has a negative impact on the survival of the pregnancy, it can also affect fetal development and maternal well-being. Prenatal stress affects the fetus resulting in low birth weight, prematurity, and impaired brain development (5, 21). Studies report that the prevalence of stress during pregnancy in the world ranges from 5.5 to 78%, while in developing countries it ranges from 6% to 52.9% (4, 15).

The brain is the main organ that interprets, responds to, and becomes the target of stress marker hormones (14). When a stressor is felt, the hormone (CRH) which acts on the anterior pituitary to promote the secretion of adrenocortico-tropic hormone (ACTH). This hormone then stimulates the adrenal cortex to release glucocorticoids (GCs) into the bloodstream (8). High glucocorticoids will reduce BDNF expression and stimulate cell apoptosis (18).

Caspase-3 acts as the executioner caspase in the cell apoptosis process (12). Caspase-3 when activated has the function of controlling cell death (20), causing cleavage of protein kinases, cytoskeletal proteins, DNA repair proteins, endonuclease inhibitory subunits and ultimately to deterioration of cellular function (9).
Increased apoptotic activity of brain cells will reduce the number of cells making up the central nervous system in the fetal brain where there are two main types of cells that make up the central nervous system, namely neurons and glial cells (9). The cerebellum is a part of the brain that contains more neurons than other parts of the brain (7), the cerebrum and cerebellum are interconnected by means of polysynaptics, forming a system related to cognitive function and neuropsychiatric disorders (1). There is little literature and researches on the impact of stress and neurobehavioral studies on the impact of stress on the cerebrum or cerebellum.

This study identifies differences in caspase-3 expression in the cerebrum and cerebellum of newborn mice (Mus musculus) whose mothers are exposed to physical and psychological stress during pregnancy.

Materials and Methods

This research is an experimental study on mice (Mus musculus) which was conducted from January to March 2021 at the Laboratory of the Faculty of Veterinary Medicine, UNAIR, Surabaya. This study used 24 adult female mice (Mus musculus) aged 2-2.5 months of pregnancy which were exposed to stress during pregnancy on the 6th until 15th days of pregnancy. The research subjects were divided into 4 groups which were randomly selected (G1, G2, G3, and G4) with 6 mice in each group.

First group: Exposure to physical stress in the form of forced swimming for 5 minutes once a day in a special box measuring 50x30x25 cm filled with water with a height of 18 cm with a water temperature of 24 °-28°C and put in a dark box cage, exposure time is 09.00 am.

Second group: The group of exposure to psychological stress by giving noise with an intensity of 90 dB once a day for 1 hour successively in a dark and soundproof enclosure measuring 1x1x2 m, TrueRTA software (real time audio analyzer) was used to produce noise. Noise intensity was measured with a real time sound analyzer (TES 1358) each day prior to exposure to experimental animals, by placing the analyzer in animal cages at several locations, and taking the average of the different readings.

The third group: The stress exposure group, a combination of physical and psychological, was given a noise of 90 dB for 1 hour, then given a break of 5 minutes and then be swam for 5 minutes per day, exposure time starts at 09.00 am.

Fourth group: Control group with standard treatment without stress exposure.

Sampling Inspection

Mus musculus mothers were anesthetized then the pups were born by sectio caesarea (SC) on the 16th day of pregnancy. The pups of Mus musculus which were to be sacrificed were anesthetized first, and then the cranium was cut in the sagittal direction from caudal (occipital) to rostral (frontal), right between the two hamisters of their brain. Furthermore, the brain was released. The separated brain was weighed, and then put in a 10% formalin solution for organ preservation; the cerebrum and cerebellum were taken. Furthermore, immunohistochemical preparations and Hematoxylin-Eosin (HE) staining were made.

Data Analysis

To see the normality of the data, the Shapiro-Wilk test was used. If the data obtained are normally distributed, then the ANOVA test is used followed by LSD (Least Significant Difference) to see the differences in all groups. If the data obtained are not normally distributed, the Kruskall Wallis test and the Mann Whitney test are used. This study uses a significance level of P<0.05. To simplify statistical calculations, researchers used the SPPS tool version 21.

Results and Discussion

Results

The results show the highest expression of caspase-3 in the cerebrum and cerebellum in the combination of physical and psychological stress exposure group compared to the physical stress exposure group, psychological stress exposure group, and the control group (Table 1). The results of the normality test using
the Shapiro-Wilk test on the treatment group obtained a significance value (p-value)> 0.05, which means that the data distribution is normally distributed, so the Analysis of Variance (ANOVA) test was used to test whether there were differences in the treatment groups on the expression of caspase-3 in the cerebrum and cerebellum.

Based on the ANOVAs test results in table (2), it is known that there are significant differences among the groups in the expression of caspase-3 in cerebrum and cerebellum of newborn *Mus musculus*.

Table (1) Mean and standard deviation of caspase-3 expression in cerebrum and cerebellum of newborn *Mus musculus*.

<table>
<thead>
<tr>
<th>Group of Treatment</th>
<th>Mean ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cerebrum</td>
</tr>
<tr>
<td>G.1</td>
<td>5.70 ± 0.99</td>
</tr>
<tr>
<td>G.2</td>
<td>7.23 ± 1.39</td>
</tr>
<tr>
<td>G.3</td>
<td>8.67 ± 1.09</td>
</tr>
<tr>
<td>G.4</td>
<td>4.17 ± 1.18</td>
</tr>
</tbody>
</table>

Table (2) Anova test results on Caspase-3 Expression in Cerebrum and Cerebellum of newborn *Mus musculus*.

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression of Caspase-3 in Cerebrum</td>
<td>0.000*</td>
</tr>
<tr>
<td>Expression of Caspase-3 in Cerebellum</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

* Significantly different <0.05

Figure (1) The differences in the description of caspase-3 expression in the cerebrum tissue of newborn mice. The red arrow indicates the presence of Caspase-3 expression which is indicated by the presence of brown chromogen. The combination of physical and psychological stress exposure group (G3) is the strongest among the physical stress group (G1), psychological stress group (G2), and the control group (G4). The expression of caspase-3 in the control group is the weakest. IHC is with a magnification of 400 times.

Figure (2) The differences in the description of caspase-3 expression in the cerebellum tissue of newborn mice. The red arrow indicates the presence of Caspase-3 expression which is indicated by the presence of brown chromogen. The combination of physical and psychological stress exposure group (G3) is the strongest among the physical stress group (G1), psychological stress group (G2), and the control group (G4). The expression of caspase-3 in the control group is the weakest. IHC is with a magnification of 400 times.

Discussion

The developing brain is the most sensitive organ to the effects of stress during the prenatal period, due to
substantial changes in structural growth and connectivity during fetal life (2). During the gestational period, there are processes of proliferation, differentiation, migration, and aggregation of fetal neurons which are strongly influenced by environmental factors (2). Prenatal stress exposure shows a long-term effect that includes both behavioral and molecular changes (6, 13).

When a stressor is felt, the hypothalamus will release the hormone cortisol. During pregnancy, fetal exposure to maternal cortisol is limited by the placental enzyme 11β-HSD-2 which functions to convert cortisol to cortisone, an inactive glucocorticoid (17). High glucocorticoids under stress reduce placental expression of 11βHSD2 which is associated with intrauterine growth restriction (19).

In acute stress, the binding of glucocorticoids and glucocorticoid receptors increases the tissue-plasminogen activator (tPA) which helps in the process of converting proBDNF to mature BDNF (mBDNF) and increases proteolytic processing of proBDNF in mBDNF which can increase BDNF levels during stress, but in chronic stress due to decreased tPA hence the process of proBDNF to mBDNF is inhibited and BDNF expression is reduced. If proBDNF is not processed into mBDNF then proBDNF will bind more highly to the p75NTR receptor which induces a pro-apoptotic signaling pathway (3). The proBDNF binding to the p75NTR receptor will activate the apoptotic pathway through the co-receptor bond, namely sortilin. Sortilin will activate jun-N terminal kinase (JNK) which will then phosphorylate C-Jun which will activate pro-apoptotic proteins such as p53, Bad, BIM, BAX so that it will stimulate mitochondria to release cytochrome-C which then activates caspase (12).

It is known that there are two types of caspases that have been identified, namely the initiator caspase and the effector / executioner caspase. Caspase 8 and 9 are the initiator caspases, while caspase-3 is the effector / executioner caspase (22). Caspase executioner mediates cell death during apoptosis, caspase-3 has the ability to partially cleave caspase substrates and its activity is required to induce cell death (10).

The results of our study indicate that there are significant differences in the expression of caspase-3 in cerebrum and cerebellum of newborn mice among treatment groups. Combined stress exposure show the strongest expression of caspase-3 compared to the physical, psychological, and control stress exposure groups. This finding is supported by a study by Qiao Y et al in 2020, where the combination of stress exposure caused significantly more hyper-activity of the HPA system as indicated by increased serum cortisol, CRH and ACTH levels (16).

High caspase-3 expression can cause an increase in apoptotic activity of brain cells and will decrease the number of cells making up the central nervous system in the fetal brain (9). A study conducted by Kinsella et al in 2009 shows that chronic stress during the prenatal period interferes with fetal neurodevelopment. Sandman et al found that prenatal stress impaired cognitive performance during infancy and decreased brain volume (11).

**Conclusion**

Stress exposure during pregnancy increases the expression of caspase-3 in cerebrum and cerebellum of newborn *Mus musculus*.

**Sources of Funding:** This research was self-funded by the author.

**Conflict of Interest:** There is no conflict of interest in this study.

**Ethical Approval:** This study has obtained ethical eligibility permit based on the Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University No: 2.KE.001.01.2021.

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Correlation between *Cryptosporidium parvum* and *Helicobacter Pylori* Infections in Gastrointestinal Patients at Wasit Province

Abdulsada A. Rahi¹, Safa A. Fadhil²

¹Prof, Post Doctorate, ²Research Scholar, Department of Biology, College of Science, Wasit University, Kut, Iraq

Abstract

The present study aimed to identify the relationship between *Cryptosporidium parvum* parasite and *Helicobacter pylori* in patients at Wasit province and its environs. One hundred stool samples were collected (male and female) from suspected diarrheal patients of parasitic and bacterial infection during the period October 2020 to April 2021 who attended to Al-Karamah Teaching Hospital at Wasit province and General Hospital of Martyr Fairuz at Hay district.

The investigation of the oocysts of the *Cryptosporidium parvum* parasite was carried out by staining with Modified Ziehl Neelson method and also detecting of *Helicobacter pylori* by using dipstick. The results of our study revealed that 55(55%) was positive for *C. parvum* and 48 (48%) was positive for *Helicobacter pylori* and the co- infections between the parasite and bacteria reached to 32(32%). The age group(1-20 years) showed the highest11 (11%) prevalence rate while the lowest prevalence was in patient with age group (21-40 years) reached to 6(6%) interplay infections between the parasite and bacteria.

Keywords: Direct smear, Modified Ziehl Neelson, Cryptosporidium parvum, Helicobacter pylori, dipstick.

Introduction

*Cryptosporidium* is a small protozoan parasite that infects microvillus region of epithelial cells in the digestive tract of vertebrates. It is an obligate intracellular parasite of man and other mammals. Environmentally robust oocysts are shed by infected hosts into the environment, these oocysts can survive the adverse conditions on the environment for months until it is using resources of the host [1]. Cryptosporidiosis is considered as emerging pathogen by CDC, the disease is mostly asymptomatic and not noticed but might be presented with fever and mild to severe diarrhea which is self-limiting in immunocompetent individuals [2].

*Helicobacter pylori* is a Gram-negative bacterium that can infect the human stomach. Its significance for human disease was first recognized in 1983. The bacterium lives in the lining of the stomach, and the chemicals it produces causes inflammation of the stomach lining. Infection appears to be lifelong unless treated with medications[3]. Among the commonest prevalent diseases worldwide were intestinal parasitosis [4]. These infections included intestinal protozoa; *Giardia lamblia* and *Cryptosporidium spp*. that proved to have a serious impact on children [5]. In addition, *H. pylori* may support *Cryptosporidium spp*. and *G. intestinalis* colonization in human gastro-intestinal tract by producing urease enzyme to overcome gastric acidity [6]. On the other hand, gastrointestinal parasitic infection may affect inflammatory response to *H. pylori*. This significant co-existence may suggest that *H. pylori* could be a risk factor for intestinal parasitic infection or vice versa[7]. This study aimed to evaluate co-infection prevalence of *Helioabacter pylori* in symptomatic patients with intestinal protozoa causing diarrhea *C. parvum* of Al-karamah Teaching Hospital at Wasit province and General Hospital of Martyr Fairuz at Hay district.

Corresponding author:
Abdulsada A. Rahi
Professor, Wasit University, Iraq
Email: abdulsadah1966@yahoo.com

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Materials and Methods

A total of 100 stool samples collected from suspected patients suffering from Cryptosporidiosis (strong to moderate diarrhea) and *H. pylori* symptoms like burping feeling of bloating nausea heartburn loss of appetite fever unexplained weight loss difficulty swallowing. The samples were collected from suspected patients attended to Al-karamah Teaching Hospital at Wasit province and General Hospital of Martyr Fairuz at Hay district during the period October 2020 to April 2021. The stool samples were collected in clean and label plastic containers and examined by Modified Ziehl-Neelsen stain for staining fecal smear has been the gold standard for detecting *Cryptosporidium* oocysts. This method is commonly used in clinical microbiology laboratories to easily identify *Cryptosporidium* oocysts. Although the concentration and staining procedures are time-consuming [8], Fecal antigen test (FAT) (SAT - stool antigen test) detects *H. pylori* antigen in feces using an immunoassay sensitivity: ~95% [9].

Statistical analysis

Statistical analysis: The statistical analysis was performed using SAS (Statistical Analysis System-version 9.1) [10].

Results:

A total of 100 stool samples collected from suspected patients suffering from Cryptosporidiosis (strong to moderate diarrhea) and *Helicobacter pylori* symptoms which collected from patients attended to Al-karamah Teaching Hospital at Wasit province and General Hospital of Martyr Fairuz at Hay district. Samples of feces were stained using Modified Ziehl-Neelsen and examined under microscope for detection of *C. parvum*. Also, the stool was examined by dipstick to detect the presence of *Helicobacter pylori* antigen. In (table-1) show out of 100 samples were detected 55% (55/100) of stool samples which considered as a positive result for *C. parvum*, while 45 (45/100) were negative also 48% (48/100) which considered as a positive result for *H. pylori* while 52(52/100) was negative. While the rate of co-infections between the *C. parvum* parasite and the *H. pylori* bacterium was 32(32/100).

Table 1. Modified Ziehl-Neelsen staining test and dipstick (SAT - stool antigen test)

<table>
<thead>
<tr>
<th>Result</th>
<th>Samples</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive <em>C. parvum</em></td>
<td>55</td>
<td>55%</td>
</tr>
<tr>
<td>Negative <em>C. parvum</em></td>
<td>45</td>
<td>45%</td>
</tr>
<tr>
<td>Positive <em>H. pylori</em></td>
<td>48</td>
<td>48%</td>
</tr>
<tr>
<td>Negative <em>H. pylori</em></td>
<td>52</td>
<td>52%</td>
</tr>
<tr>
<td>Co-infections between <em>C.parvum</em> and <em>H. Pylori</em></td>
<td>32</td>
<td>32%</td>
</tr>
</tbody>
</table>

In table-2 explain the rate of *C. parvum* infection and *H. pylori* according to the age and gender. The highest infection of *C. parvum* rate was recorded in female 15(15%) in age group(1-20) and the lowest infection was recorded also in female 3 (3%) in both age groups (21-40) and (41-60) years old. The highest *H. pylori* and co-infections were also recorded among females, and in the same age group, the highest rate of *Cryptosporidium* infection was recorded.

Table 2. Patients with Cryptosporidiosis and *H. pylori* infection in Relation of Age and Gender.

<table>
<thead>
<tr>
<th>Age group year</th>
<th>C. parvum male +ve %</th>
<th>C. parvum female +ve %</th>
<th>C. parvum male -ve %</th>
<th>C. parvum female -ve %</th>
<th>H. pylori male +ve %</th>
<th>H. pylori female +ve %</th>
<th>H. pylori male -ve %</th>
<th>H. pylori female -ve %</th>
<th>co-infection male %</th>
<th>co-infection female %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>11</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1-20</td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>8</td>
<td>6</td>
<td>11</td>
<td>11</td>
<td>13</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>21-40</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>41-60</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Sub-total</td>
<td>35</td>
<td>16</td>
<td>26</td>
<td>23</td>
<td>26</td>
<td>26</td>
<td>22</td>
<td>26</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>100 %</td>
<td>100 %</td>
<td>32 %</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Discussion

*Cryptosporidium parvum* is a zoonotic protozoan parasite that mainly affects the ileum of humans and livestock, with the potential to cause severe enteric disease [11]. And when staining with modified Ziehl–Neelsen stain digested *Cryptosporidium* spp. oocysts appear as bright red sphere (4 to 6 mm) containing four crescent-shaped sporozoites (which may or may not be seen) in all oocysts [12]. Cryptosporidiosis remains a debilitating disease in third world countries, particularly in infants and young children, in whom it is associated with chronic diarrhea and malnutrition [13]. There was a strong correlation between age distribution of diarrhea and the occurrence of *C. parvum*, between the ages of 3 and 36 months [14]. This agreement with our study that documented prevalence of *C. parvum* infections among people living in Wasit province, Iraq which was the rate of infections higher among patients with age group (1-20). And disagreement with study of *C. parvum* dispersal in children in Ramadi province which showed more infection were at age (1-12) months [15]. Worldwide more than 50% population is infected by *H. Pylori* gram-negative bacterium, the infection rate is much higher in developing countries due to the lack of hygiene and sanitation [16]. The prevalence of the bacterium is higher among middle-aged adults [17]. This study agreement with our study which indicated that the largest infection rate was recorded in the age group (1-20) years old. The prevalence of *H. pylori* shows more than 80% of the population is *H. pylori* positive, generally it is consider ably lower in children and adolescents than in adults and elderly people [13]. This is not in line with our study, as the results showed that the age group most affected by is the *H. pylori* category of adults and children, and this is due to the lack of hygiene and health awareness among many of them. The current study also revealed the existence of a relationship between the presence of *C. parvum* and *H. pylori* in patients suffering from diarrhea, and their percentage was about 32 out of a total of 100 samples, this agreed with study which revealed that more than half of cryptosporidiosis (60%) and giardiasis (58%) cases coexisted and showed a duplicated risk for *H. pylori* [18], which was among its statistical results found a rate of 5.3% with a significant statistical association between *H. pylori* infection and *Cryptosporidium* [19]. This is also consistent with our current study. While there are studies that do not agree with our current study, in Modifaid Ziehl-Neelsen staining, acid-fast parasites were never observed in patients infected with *H. pylori* [20]. There are some studies suggest that interaction between resident bacteria and invading *C. parvum* is not pathogenic, but rather synergistic [21]. And it was among the studies that did not correspond to our study is diagnosis of intestinal protozoan infections in patients in Cuba where confirmed the absence of information about co-infections between protozoa and bacterial, viral pathogens [22]. As for the relationship between gender and the rate of infection with the *C. parvum*, the current study showed that males are the most infected, as the percentage is 35(35%), while females were 26(26%), and our study corresponds to the study of Zhang which showed *Cryptosporidium* infection in males more than females [21]. While Salman mentioned in his study marked by prevalence of *Cryptosporidium parvum* among Iraqi displaced people in Kirkuk that there are no significant differences in infection for both genders as the infection rate higher frequency of males and females, respectively, is 22.66%, 21.2 by using MZN method [24], this is inconsistent with the current study. A study was also conducted in Nigeria on *Cryptosporidium* infection, which showed the rate of infection was higher among females (27%) than males (17%) among adults [25]. And this does not correspond with our current study, which showed that the rate of infection in males is higher than that of females. As for *H. pylori*, the results do not differ much in this study from the results of infection with *C. parvum*, as it has also shown that the rate of infection of males with *H. pylori* infection is higher 26(26%) than that of females 22(22%), this agreed with Rodrigues study about *H. pylori* infection which showed the frequency of infection was higher among men (33.4%) rather than women (30.7%) [26]. Whereas, other studies showed that the infection rate for females is higher than males, like the study Şeyda, which was among its results *H. pylori* positivity was 67.7% in males and 68.2% in females [27] and Almadi studies that showed the prevalence rate between male and female students (34 % and 38% respectively) [28]. While other studies showed that no differences were found between
males and females\textsuperscript{29}. These studies are not compatible with this study currently in force.

### Conclusion

Our study revealed that the prevalence rate of infection with \textit{C. parvum}, \textit{H. pylori} and co-infections with bacteria and parasite was respectively 55 (55\%), 48 (48\%) and 32 (32\%) and higher infection found in age group (1-20) years in patients at Wasit Province.

### Ethical Clearance:

Taken from College of Science Committee

### Source of Funding:

Self

### Conflict of Interest:

Nil

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Relationship between *Cryptosporidium parvum* in Water And Diarrheal Infections at Wasit Province

Abdulsada A. Rahi¹, Safa K. Alwan²

¹Prof, Post Doctorate, ²Research Scholar; Department of Biology, College of Science, Wasit University, Kut, Iraq

Abstract

The present study aimed to identify the relationship between *Cryptosporidium parvum* in water and diarrheal infections at Wasit province. One hundred stool samples were collected (male and female) from suspected diarrheal patients of parasitic infection who attended to Al-kut hospital and Al-karamah teaching hospital at Wasit province and fifty water samples were collected from different points at Dijlah river; Household water supply and Kut Liquid water during the period from October 2020 to April 2021. The investigation of oocysts of the *Cryptosporidium parvum* parasite carried out by staining with modified Ziel Neelson method. The results of our study revealed that 60(60%) was positive for *C.paravum*. The age group (1-5) months showed the highest 12(12%) prevalence rate while the lowest prevalence was in patient with age group (>20) months reached to 1(1%).

Keywords: Water ,Stool, Modified Ziehl Neelson ,Cryptosporidium parvum, Human

Introduction

*Cryptosporidium* is a coccidial protozoan parasite Which can infect a widely variety of vertebrates and not seem to be host specific [¹]. *Cryptosporidium parvum* is a common enteric protozoan parasite of humans and other species that contract young farm animals (calves, lambs, goats) and pets (kittens, puppies). It is can be found worldwide in virtually every human Society. The prevalence of infection varies widely but seems to be highest in young children and immunocompromised persons [²]. In the other hand the infection in immunocompetent persons is self-limited[³]. *Cryptosporidium* oocysts are spherical (in *C. parvum* about 3 to 5 lm in diameter) and are subtract in numbers of up to 105 to 107 oocysts per g in hurry feces. It particularly oversensitive to cryptosporidiosis. In children younger than 5 year [⁴] developing countries, *Cryptosporidium* is cause of diarrheal disease in humans, and several groups of humans are infections occur frequently Cryptosporidiosis is an opportunistic parasitosis. It is characterized by self-limiting gastroenteritis in otherwise healthy humans, but it is more severe in immunocompromised in HIV-infected patients and constitutes a serious life threatening leading to chronic or fulminant disease, wasting and death [⁵]. The present study aimed to identify the relationship between *Cryptosporidium parvum* in water and diarrheal infections at Wasit province.

Materials and Methods

A total of 100 stool samples were collected from suspected patients suffering from Cryptosporidiosis (strong to moderate diarrhea). The samples were collected from suspected patients attended to Al-kut and Al-karama teaching hospital and fifty water samples were collected from different points at Dijlah river; Household water supply and kut Liquid water at Wasit province during the period from October 2020 to April 2021.

The stool and water samples were collected in clean and label plastic containers and examined by Modified Ziel Neelson stain for staining fecal and water smear has been the gold standard for detecting *Cryptosporidium* oocysts. This method is commonly used in clinical microbiology laboratories to easily identify *Cryptosporidium* oocyst.
Result

A total of 100 stool samples were collected from suspected patients suffering from Cryptosporidiosis (strong to moderate diarrhea). The samples were collected from suspected patients attending Al-kut and Al-karama teaching hospital and fifty water samples were collected from different points at Dijlah river; Household water supply and Kut Liquid water at Wasit province were stained using Modified Ziel Neelson stain and examined under microscope for detection of *C. parvum*. The result shows that 60 samples were positive for *C. parvum* which means 60% while the rest of the samples (40) were negative which means 40% as shown in table 1.

<table>
<thead>
<tr>
<th>Result</th>
<th>Samples</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive C. parvum</td>
<td>60</td>
<td>60%</td>
</tr>
<tr>
<td>Negative C. parvum</td>
<td>40</td>
<td>40%</td>
</tr>
</tbody>
</table>

Table 1. Modified Ziel Neelson staining test

Table 2 shows the rate of *C. parvum* infection in human according to the age. The highest infection rate was recorded in male 32 (32 %) and the lowest rate of infection with *C. parvum* were recorded in female 28 (28%). The age group (more than 20 months) appeared higher infection 26 (26%) than other age groups.

<table>
<thead>
<tr>
<th>Age group</th>
<th>C. parvum Male +ve %</th>
<th>C. parvum Male –ve %</th>
<th>C. parvum Female +ve%</th>
<th>C. parvum Female –ve%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1_5 months</td>
<td>12</td>
<td>12</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>5-10 months</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>10-15 months</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>15-20 months</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>months &lt;20</td>
<td>11</td>
<td>0</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Total%</td>
<td>32</td>
<td>22</td>
<td>28</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 2. Distribution of positive and negative cases with *C. parvum* infection in relation of age and gender

Table -3 shows the rate of *C. parvum* in water samples that had been taken from different areas of Kut city. Our results revealed that the highest rate was recorded in tap water 28% while the lowest rate was recorded in rivers 4%.

<table>
<thead>
<tr>
<th>Area</th>
<th>No. of samples</th>
<th>C. parvum +ve</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rivers</td>
<td>16</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>Tap water</td>
<td>24</td>
<td>14</td>
<td>28%</td>
</tr>
<tr>
<td>Raw water</td>
<td>5</td>
<td>5</td>
<td>10%</td>
</tr>
<tr>
<td>Treated water</td>
<td>5</td>
<td>5</td>
<td>10%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>26</td>
<td>52%</td>
</tr>
</tbody>
</table>

Table 3. Distribution of positive water samples with *C. parvum* infection
Discussion

The results of the present study recorded the prevalence rate of Cryptosporidium parvum collected from suspected diarrheal patients of parasitic infection who attended to Al-kut hospital and Al-karamah teaching hospital at Wasit province.

Table -2 showed that males are more likely to be affected than females by the rate 32% while females were 28%. This may be due to that males at this age possibly are more exposed to C. parvum oocysts because of their more activities than the females. Our results were disagreed with Fatimah, (2013) in Sulaimani which were 15.5%, 14.8% respectively[6]. Abdulsadah, (2013) showed the rate of male that infected with C.parvum was 33.74% which is somehow agreed with our data 32% [7]. Also, the results of the current study were disagreed with Tumwine, (2003) in Uganda [8], Abdel-Messih, (2005) in Egypt [9], and Ali, (2014) in Iraq [10].

The results of present study revealed that the hight rate of C. parvum infection 26(26%) was recorded in the age group ( more than 20 months) This result agreed with the results of Mumtaz et al., (2010) [11] which was(27.8%) and El-Helaly et al., (2012) in Egypt[12], but it disagreed with the results of the studies that were carried out in Iran by Khalili and Mardani (2009) [13] which was (43.3%) and Abdulsadah et al., (2013) (30.83%) [7]. There was a strong correlation between age distribution of diarrhea and the occurrence of C. parvum, between the ages (6 month to 3 years) at a rate of (15.6%) by Ali et al., (2013) [14] with our study 16% age group (1-5 months). Also In the age group (1-5months) agreed with Saneian et al., (2010) [15] Cryptosporidium oocysts were detected in 12.5%. Mismatch of readings could be due to the number of samples or geographical location.

In the other hand water samples were collected from various areas of Kut city and C. parvum infection was recorded (28%) in tap water which quite agreed with ul Akbar,(2015) in Pakistan which was 25% [16]. Rivers samples were recorded 4% which disagreed with Franco, (2001)which reported 30% [17] and Yang,(2008) [18]. There are some many factors affecting the prevalence competing Interests: location of sampling, the diversity of animals in the areas, season, climatic condition, volume of sample, the population of the animals in the areas and rainy seasons etc. So the transmission of C. parvum is different in emerging countries than that of developed countries [19].

Conclusion

Our study revealed that the prevalence rate of infection with C.parvum was 60 (60%), and higher infection found in age group (months <20) in patients at Wasit Province. While the prevalence rate of C.parvum infection in water sources was 26 (52%).

Ethical Clearance: Taken from College of Science Committee

Source of Funding: Self

Conflict of Interest: Nil

References


Detection of *Cyclospora cayetanensis* by PCR in Wasit Province, Iraq

Abdulsada A. Rahi¹, Israa N. Abed Al-Saadi², Yoosra N. Abed Al-Saadi²

¹Prof, Post Doctorate, ²Research scholar; Department of Biology, College of Science, Wasit University, Kut, Iraq

Abstract

The present study aimed to detect the *Cyclospora cayetanensis* among patients that attended in Al-karama Teaching Hospital and General Hospital of Martyr Fairuz in Wasit City. The sample were collected from October 2015 to April 2016. Thirty stool samples were collected from patients that suffering from diarrhea, their aged between 1 year to 50 years old from both genders who attended the hospitals at Wasit province. Data was collected using a questionnaire form including information about gender, age, location. Stool samples were examined by PCR technique to detect the presence of *Cyclospora cayetanensis*. The result showed that 8 of 30 (26.7%) samples were positive while 22 of 30 (73.3%) were negative. Our study is considered as a first study that detect the *Cyclospora cayetanensis* in Wasit province, Iraq. This study was aimed to determine the prevalence of *C. cayetanensis* in Wasit Province, Iraq.

Key words: *Cyclospora cayetanensis*, Stool, PCR, Human.

Introduction

The risk of exposure to exotic and uncommon tropical diseases has increased in parallel with the globalization of the food supply, increased consumption of fresh foods, and increased travel. The rapid transport of fresh fruits and produce from developing countries has increased the chance that endemic parasites from other regions may come into contact with consumers from industrialized nations. Changes in nutritional habits have resulted in increased consumption of undercooked or raw foods, thus potentially exposing consumers to parasites that proper food processing would otherwise reduce or eliminate (¹). *Cyclospora* is obligate intracellular apicomplexan protozoan parasites that infect the mucosal epithelium of the small intestine or bile duct of a variety of hosts, mostly vertebrates (²). *Cyclospora* was first identified as a human pathogen in three patients from Papua, New Guinea and concluded that they could be a coccidian of the genus *Isospora* (³). In recent years, *Cyclospora cayetanensis* has consider as an important human pathogen that causes diarrhea in both immuno-compromised and immunocompetent hosts. In the later, diarrhea is usually prolonged, but self-limited, while in immuno-compromised, it may be prolonged and severe (⁴). It differs significantly from all other *Cyclospora* species not only in its host but also in its oocyst stage, which is much smaller and spherical rather than oblong (⁵,⁶). The route of transmission is by ingestion of contaminated water and food products with sporulated oocysts, especially vegetables that are the most implicated source for the spread of cyclosporiasis (²). It is unlikely transmitted directly between individuals. The infection dose is presumed to be low (⁷). In view of this, the present study was designed for the detection of *Cyclospora cayetanensis* by PCR in Wasit province, Iraq.

Materials and Methods

**Stool Collection.**

A total of 30 stool samples were collected from
patients that suffering from diarrhea in Al-karama Teaching Hospital and General Hospital of Martyr Fairuz in Wasit city. The sample were collected from October 2015 to April 2016. The fecal sample was placed to a clean, dry plastic container and transported to the laboratory for analysis.

**Stool DNA Exteraction**

Genomic DNA was extracted from stool samples by using (Stool DNA extraction Kit, Bioneer. Korea). The extraction was done according to company instructions by using stool lysis protocol method with Proteinase K (8). After that, the extracted gDNA was checked by Nanodrop spectrophotometer, and then stored at -20°C until used in PCR amplification.

**Convention PCR**

PCR technique was performed for specific detection *Cyclospora cayetanensis* based on subunit ribosomal RNA gene (ITS1 region) from human stool samples. This method was carried out according to (9). The primers were provided by (Bioneer company.Korea) (Table-1). PCR reaction solution was prepared by using (AccuPower PCR PreMix Kit) according to company instructions with the volumes (Table -2).

**Table (1): The primers of *C. cayetanensis*.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Amplicon</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. cayetanensis</em> F</td>
<td>GAGAGGGAGCCTGAGAAACG</td>
<td>575bp</td>
</tr>
<tr>
<td>R</td>
<td>TCCTTGCAATGCTTTCGC</td>
<td></td>
</tr>
</tbody>
</table>

**Table (2): The quantities of prepared PCR reaction solution.**

<table>
<thead>
<tr>
<th>Components of PCR reaction solution</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA template</td>
<td>5µL</td>
</tr>
<tr>
<td>ITS Forward primer (10pmol)</td>
<td>1.5 µL</td>
</tr>
<tr>
<td>ITS Reveres primer (10pmol)</td>
<td>1.5 µL</td>
</tr>
<tr>
<td>PCR master mix</td>
<td>12.5 µL</td>
</tr>
<tr>
<td>D.D. water</td>
<td>4.5 µL</td>
</tr>
<tr>
<td>Total volume</td>
<td>25 µL</td>
</tr>
</tbody>
</table>

After preparing the PCR reaction solution, its components above mentioned were placed in standard Accu Power PCR Pre Mix Kit that contained (Taq DNA polymerase, dNTPs, Tris-HCL pH: 9.0, KCL, MgCL2, stabilizer, and tracking dye) all the components including DNA template and two pair of primer in final total volum 25 µL was transferring to PCR tubes into Exispin vortex centrifuge at 3000rpm for 3 minutes, then placed in PCR Thermo cycler (My Gene. Bioneer/ Korea). PCR thermo cycler conditions were accomplished by using traditional PCR thermocycler system as shown in the following (Table -3). The PCR products (575bp) were examined by electrophoresis in a 1% agarose gel, stained with ethidium bromide, and visualized under UV
illuminat.

**Table (3): PCR Thermocycler Conditions.**

<table>
<thead>
<tr>
<th>PCR step</th>
<th>Temp.</th>
<th>Time</th>
<th>Repeat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>95°C</td>
<td>5 min.</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95°C</td>
<td>30 sec.</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>58°C</td>
<td>30 sec.</td>
<td>30 cycles</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>1 min.</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>5 min.</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Hold</td>
<td>4°C</td>
<td>Forever</td>
<td>-</td>
</tr>
</tbody>
</table>

**Statistical Analysis**

Statistical analysis: The statistical analysis was performed using SAS (Statistical Analysis System - version 9.1) (10).

**Results**

3.1 Polymerase chain reaction.

Out of 30 stool samples were examined by using Conventional PCR test. Eight samples revealed the presence of *Cyclospora cayetanensis* giving rate of infection (26.7%), as shown in (Table - 4) (Figure -3).

**Table (4): The results of PCR test according to age groups of Cyclosporiasis patients.**

<table>
<thead>
<tr>
<th>Age Year</th>
<th>PCR Positive</th>
<th>Percent</th>
<th>PCR Negative</th>
<th>Percent</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1-10</td>
<td>4</td>
<td>13.4%</td>
<td>10</td>
<td>33.3%</td>
<td>14</td>
<td>46.7%</td>
</tr>
<tr>
<td>11-20</td>
<td>0</td>
<td>0%</td>
<td>3</td>
<td>10%</td>
<td>3</td>
<td>10%</td>
</tr>
<tr>
<td>21-30</td>
<td>1</td>
<td>3.3%</td>
<td>0</td>
<td>0%</td>
<td>1</td>
<td>3.3%</td>
</tr>
<tr>
<td>31-40</td>
<td>1</td>
<td>3.3%</td>
<td>3</td>
<td>10%</td>
<td>4</td>
<td>13.4%</td>
</tr>
<tr>
<td>41-&gt;50</td>
<td>2</td>
<td>6.7%</td>
<td>6</td>
<td>20%</td>
<td>8</td>
<td>26.6%</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>26.7%</td>
<td>22</td>
<td>73.3%</td>
<td>30</td>
<td>100%</td>
</tr>
</tbody>
</table>

P-Value=0.052 significant
Discussion

The first record of *Cyclospora* in Arabic countries was in Basra/Iraq where three diarrheic cases have been observed by (11). The laboratory diagnosis of recently recognized infectious agents, such as *Cyclospora cayetanensis*, is frequently problematic because of lacking of required detection techniques (12). Microscopy is mostly a monotonous procedure and take long time that may lead to extreme tiredness for the analyst and incorrect negative results, but PCR assay is more valuable for identification of *Cyclospora oocysts* (5). Therefore, the PCR assay for 18S rRNA gene is highly sensitive and specific, it was also capable of overcoming many errors of microscopic diagnosis (13). Infection rate of *Cyclospora cayetanensis* obtained in this study was agreed with other previous studies reported in Egypt (14) and (15) that were (24.5%) and (25%) respectively using PCR test. The results of this study were approximate to (16) who recorded (38%) positive results in the PCR *Cyclospora* test in India. The prevalence of Cyclosporiasis were reported in India (13) who found the prevalence rate of *Cyclospora cayetanensis* was (89%), while Mundaca’s study (17) recoded 57.1%in Lima and Peru this results observing that the prevalence was highest in India because *Cyclospora cayetanensis* endemic in India , and it appears to be the more common disease in tropical and sub-tropical countries whereas this protozoan is endemic in Haiti, Guatemala, Peru, and Nepal. The prevalence of *Cyclospora cayetanensis* is higher in developing countries than in Europe and North America, this parasite cause diarrhea infection in voyagers who provide the more extensive facts about *Cyclospora* infection (18,13). Typically, the Cyclosporiasis infection has been related to international consumption of imported fruit and vegetable, which may be the reason that led to existence of 26.7% Cyclosporiasis infection in our current study. The investigations of Cyclosporiasis prevalence in developing countries, which the studied area is one of them, have listed some reasons of infection including numerous variables such as region of study, sanitary conditions, season, and individual properties (age, term of stay in the territory, socioeconomic status, probability of prior *Cyclospora* infection, and immunocompetence) (19).

Conclusion

PCR is considered as an alternative tool in epidemiological studies and the diagnosis of *C. cayetanensis*. The results showed that the PCR is sensitive
test to obtained *C. cayetanensis* in stool samples.

**Acknowledgement**: Authors sincerely wish to acknowledge the members of the Laboratory of the College of Science, Wasit University for their supporting.

**Ethical Clearance**: Taken from College of Science Committee

**Source of Funding**: Self

**Conflict of Interest**: Nil

**References**


Epidemiological Study of Cutaneous Leishmaniasis in Wasit Province

Abdulsada A. Rahi 1, Zainab K. Hashim 2, Ali Mohsin Al-Jamea 3
1 Prof, Post Doctorate, Department of Biology, College of Science, Wasit University, Iraq
2 Research scholar, Department of Biology, College of Science, Wasit University, Iraq
3 Research Scholar, Dermatologist, Al- Karamah Teaching Hospital, Directorate of Wasit Health, Iraq

Abstract

The present study was conducted to detect the cutaneous Leishmaniasis from a total of suspected cases that were collected from dermatology center of AL-Karamah Teaching Hospital in Al-Kut city from 1st October 2019 to the end of February 2020. Totally 60 patients were included 36 males and 24 females. The collected skin specimens were smeared and stained with Giemsa stain, then examined by microscope under oil immersion lenses. The results of current study appeared that 56(93.3%) were positive amastigotes, The prevalence of CL were 36 (60%) in males and 24(40%) in females, while the highest infection 36(60%) in rural area and lowest 24(40%) in urban area. The percent of infection with Cutaneous leishmaniasis according to type of lesions recorded were equal in dry and wet ulcers type (50 %) for each one.

Keywords: cutaneous leishmania, Giemsa, Leishmania major, Leishmania tropica

Introduction

Leishmaniasis is an old protozoan parasitic disease that is caused by Leishmania species. The infection is widespread in approximately 100 countries, and more than 10 million people are infected with the parasite. Additionally, 350 million people are at risk of acquiring the infection (1,2). Leishmaniasis can present itself in three forms: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL) (3). The spread of Cutaneous leishmaniasis is linked to multiple economic and environmental factors such as population growth, population migration, population expansion, and agricultural activity. Population movement and rapid urbanization projects and the invasion of disease-endemic areas are the most important factors in the emergence and survival of the disease (4).

The disease is endemic in 88 countries of 5 continents with a total of 350 -million people at risk and 12 million cases. Among the 88 endemic countries, 22 are in the New World and 66 in the Old World with an estimated incidence of 1- 1.5 million cases of cutaneous leishmaniasis (CL) and 500, 000 cases of visceral leishmaniasis (VL) (5).

Cutaneous leishmaniosis in Iraq has 2 forms, zoonotic cutaneous leishmaniasis (ZCL), which is mainly caused by Leishmania major, and anthroponotic cutaneous leishmaniasis (ACL), which is mainly caused by Leishmania tropica (6).

In recent decades conventional identification and taxonomic procedures for Leishmania microscopic examination slides, geographic distribution, clinical manifestations, pathogenic features, and culturing patterns were identified and categorized. These lack the necessary precision because there is a high similarity among species that makes the morphologic identification difficult, and also there are epidemiologic distributions for multiple Leishmania species coexisting in both nonendemic and endemic areas (7).
The study aimed to identify some epidemiological factors accompanying the spread of Cutaneous leishmaniasis in Wasit province which includes gender, types of skin lesion and residence of patients.

**Material and Methods**

**Population study**

This study was carried out during the period from 1st October 2019 to February 2020 in Al-Karamah teaching hospital of Kut city, Iraq. A total of 60 skin samples were taken from suspected patients with CL. Samples were taken from the skin leishmanial lesion, smear samples are taken from the active edge of the leishmanial lesion, presumably due to the maximum number of parasites in the active edge (8). After the smears dried completely, they were fixed with 100% methanol, allowed to dry again, and stained with Geimsa stain for microscopic examination under oil immersion lenses for presence of amastigotes (9).

**Results and Discussion**

**Table (1): Microscopic examination of Leishmania parasite in skin lesions by Giemsa stain**

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Total number of samples</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Microscope examination</td>
<td>60</td>
<td>56</td>
<td>93.3</td>
</tr>
</tbody>
</table>

Microscopy was positive in 56 (93.3) samples for amastigotes of *Leishmania* presence. Our result was in agreement with what was reported by (16) that found (80.7%) was positive, also agreed with another study that found (83.3%) was positive in the direct microscopic examination (17).

**Table (2): Gender characteristics of patients enrolled in the present study**

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>36 (60%)</td>
</tr>
<tr>
<td>Female</td>
<td>24 (40%)</td>
</tr>
</tbody>
</table>

Male: Female ratio 1.5

The results of this study showed the percent of infection in males were about (60%), while female was (40%), the percent of infection in males were higher than females as shown in table (2). The reason of higher infection take out in males more than females, possibly, due to the high incidence of working or sleeping males in open areas (surfaces of houses) with less coverage of body as well as more exposure to infected vectors compared with the females (10). Some studies have hypothesized that the gender difference observed in some parasitic disease can be attributed to hormonal effects (11). However, controversy still exists regarding the role of sex hormones in the cellular immune response (12,13). On the contrary of other studies that found the higher incidence of infection among females than males (14-16).
Table (3) : Residency of patients enrolled in the present study

<table>
<thead>
<tr>
<th>Residency</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural</td>
<td>36 (60%)</td>
</tr>
<tr>
<td>Urban</td>
<td>24 (40%)</td>
</tr>
<tr>
<td>Total</td>
<td>60 (100%)</td>
</tr>
</tbody>
</table>

This study showed that 60% of CL infection were appeared in rural region while in urban region 40%, this study reported the significant prevalence of CL in urban region more than in rural region. This study agreed with Wisam and Yisnnh, (2007) who finding the rate of infection in urban region was lower than rural region (17). Also, our results disagreed with Abdulsada, (2014) who finding that the higher infection in urban areas than rural areas (18).

Table (4) : The distribution of patients according to the type of CL lesions

<table>
<thead>
<tr>
<th>Type of CL Lesion</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>30</td>
<td>50%</td>
</tr>
<tr>
<td>Wet</td>
<td>30</td>
<td>50%</td>
</tr>
</tbody>
</table>

Nodule dry type lesions (ACL) were present in (50%), while the ulcerative wet type lesions (ZCL) were present in (50%). The results of study showed that percentage of dry type are equal to wet type. This result was disagreement with the results of (19-23) who mentioned that wet type more finding than the dry type.

Conclusions

Cutaneous leishmaniasis lesions affecting both sexes, but male is more prone to infection than female, patients from rural areas appeared high infection of cutaneous leishmaniasis.

Ethical Clearance: Taken from College of Science Committee

Source of Funding: Self

Conflict of Interest: Nil

References


Co-infection between *Entamoeba histolytica* and *Helicobacter Pylori* in Patients at Wasit Province

Abdulsada A. Rahi¹, Sima’a H. Mohammed², Magda A. Ali³
¹Prof, Post Doctorate, ²Research Scholar, ³Research Scholar, PhD, Department of Biology, College of Science, Wasit University, Iraq

Abstract

The current study was conducted at Wasit province, Iraq during the period from 28 October 2020 to 28 December 2020. The study included one hundred stool samples taken from questionable patients of *Entamoeba histolytica* and *Helicobacter pylori*. Samples were collected from patient attended to General Hospital of Martyr Fairuz at Hay district, Saeed Health center and Hospital of Al-Kut. All samples have been checked using direct smear lugol’s iodine stain for *Entamoeba histolytica* and dipstick for *Helicobacter pylori*. The result showed that 40 samples (40%) out of 100 patients were gave positive by rapid test cassette; females 23 (23%) positive samples and males 17(17%). While 14 samples (14%) out of 100 samples were positive for *Entamoeba histolytica*. The highest infection in females 8(8%) and the lowest in males 6(6%). The result showed 10 samples (10%) out of 100 patients positive for both *E. histolytica* and *H. pylori*. The highest infection was recorded in females 6(6%) and the lowest in males 4(4%).

The highest infection of *H. pylori* in age group (39 and more) years ; females recorded 6(6%) and males recorded 6(6%), while the lowest infection in group (>1) years old ; females recorded 1(1%) and males recorded 1(1%). The infection rate of *Entamoeba histolytica* was highest in age group (1-13) years old ; females was recorded 1(1%) and males was recorded 4(4%) , while the lowest in age group (26-39) years old ; females recorded 1(1%) while males without infection. Infection with both *E.histolytica* and *H.pylori* was recorded the highest in group (39 and more) years old ; females was recorded 3(3%) and males was recorded 2(2%) while the group (>1) years old do not recorded infections.

Keywords: *Entamoeba histolytica*, *H. pylori*, Dipstick, Lugol’s iodine stain, Human, Stool

Introduction

A parasite is an organism that cause harm in its host. *E.histolytica* was first described by Fedor Losch in 1875 in St. Peters burg, Russia. cause anemia in pregnant and children. The spread of intestinal parasites differ from one region to another and its spread related to many factors such as geographical factors, climate, poorness, malnutrition, personal clean less and other factors. One trophozoites of *Entamoeba histolytica* reach the host intestine can damage the mucosa epithelial layer and prevalence through the sub-mucosa and the lamina propia and other tissues. Infection of the gastrointestinal tract include two distinct clinical entities: *Helicobacter pylori* the causative of gastric infection this bacteria was first isolated by Warren and Marshal in 1983. And *Entamoeba histolytica* the causative agent of inflammation of the gastric and intestinal mucosa. *Entamoeba histolytica* cause the human amoebiasis is endemic in most tropical and sub-tropical countries and it the causative agent of the dysentery. *Helicobacter pylori* (*H.pylori*) are gram-negative bacilli responsible for chronic gastritis. It has been known for more than a century are present in human’s stomach. The epidemiology of the *Helicobacter pylori* changing with...
parallel decline in peptic ulcer and gastric cancer (10). Poor socioeconomic circumstances is a risk for H. pylori infection and parasite infection.

**Materials and Methods**

**Materials:** glass slides, cover slides, microscope, normal saline, lugol’s iodine stain, pipette, stick, eppendorf tube, dipstick for H. pylori.

**Samples collection:** A total of one hundred stool specimens collected from suspected persons with Entamoeba histolytica and H. pylori in general Hospital of Martyr Fairuz at Hay district, Saeed health center and Al-Kut Hospital. Stool samples were collected during the period from 28 October 2020 to 28 December 2020. These specimens were collected in sterile plastic containers in morning (11) and from different ages and both sexes.

**Methods**

For examination with microscope used two slides for each specimen, one drop of Iodine and added small amount of stool on one slide and mix well and cover slip, then one drop of normal saline (0.9%) on another slide with added small amount of stool, mix well and cover slip, then examination with microscope at 40 magnification 40X (12). Each sample checked H. pylori by dipstick (13).

**Results**

A total of 100 samples of stool collected from patients with solid, semisolid and diarrhea.

Table 1 shows the rate of Helicobacter pylori infection 40 positive samples out of 100 patients. The highest infection in females 23 (23%) positive samples and the lowest infection in males 17 (17%). The rate of Entamoeba histolytica infection 14 (14%) positive samples out of 100 patients. The highest infection in females 8 (8%) and the lowest in males 6 (6%). The rate of infection association between E. histolytica and H. pylori 10 (10%) positive samples out of 100 patients. The highest infection was recorded in females 6 (6%) and the lowest in males 4 (4%).

<table>
<thead>
<tr>
<th>H. pylori + ve %</th>
<th>Direct &amp; Iodine</th>
<th>E. histolytica &amp; H. pylori + ve %</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 (40%)</td>
<td>14 (14%)</td>
<td>10 (10%)</td>
<td>36 (36%)</td>
<td>100 (100%)</td>
</tr>
</tbody>
</table>

Table 2 shows the infection rate according to the gender and the age. The highest infection of H. pylori in age group (39 and more) years old; females recorded 6 (6%) and males recorded 6 (6%), while the lowest infection in age group (>1) years old; females recorded 1 (1%) and males recorded 1 (1%). The infection rate of Entamoeba histolytica was highest in age group (1-13) years old; females was recorded 1 (1%) and males was recorded 4 (4%) and the lowest in age group (26-39) years old; females recorded 1 (1%) while males without infection. Infection with both E. histolytica and H. pylori was recorded the highest in age group (39 and more) years old; females was recorded 3 (3%) and males was recorded 2 (2%) while the group (>1) years do not recorded infections with both E. histolytica and H. pylori.
### Table 2. Distribution of *Entamoeba histolytica* and *H. pylori* infections according to age and gender

<table>
<thead>
<tr>
<th>Age/years</th>
<th>H.pylori + ve %</th>
<th>Direct &amp; Iodine E. histolytica + ve %</th>
<th>H.p &amp; E.h + ve %</th>
<th>Negative %</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male  female</td>
<td>Male  female</td>
<td>Male  female</td>
<td>Male  female</td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>1(1%) 1(1%)</td>
<td>1(1%) 1(1%)</td>
<td>0 0</td>
<td>3(3%) 1(1%)</td>
<td>8(8%)</td>
</tr>
<tr>
<td>1-13</td>
<td>2(2%) 7(7%)</td>
<td>4(4%) 1(1%)</td>
<td>0 2(2%)</td>
<td>9(9%) 5(5%)</td>
<td>30(30%)</td>
</tr>
<tr>
<td>13-26</td>
<td>5(5%) 6(6%)</td>
<td>0 2(2%)</td>
<td>1(1%) 0</td>
<td>3(3%) 6(6%)</td>
<td>23(23%)</td>
</tr>
<tr>
<td>26-39</td>
<td>3(3%) 3(3%)</td>
<td>0 1(1%)</td>
<td>1(1%) 1(1%)</td>
<td>1(1%) 3(3%)</td>
<td>13(13%)</td>
</tr>
<tr>
<td>39 and more</td>
<td>6(6%) 6(6%)</td>
<td>1(1%) 3(3%)</td>
<td>2(2%) 3(3%)</td>
<td>3(3%) 2(2%)</td>
<td>26(26%)</td>
</tr>
<tr>
<td>Total</td>
<td>17(17%) 23(23%)</td>
<td>5(5%) 8(8%)</td>
<td>4(4%) 6(6%)</td>
<td>19(19%) 17(17%)</td>
<td>100(100%)</td>
</tr>
</tbody>
</table>

### Discussion and Conclusion

Intestinal parasites are common in developing countries, poor socioeconomic conditions is major risk factors for acquiring *Entamoeba histolytica* and *Helicobacter pylori*. *Helicobacter pylori* colonizes gastric mucosa and cause gastric ulcer. The spread of intestinal parasitic infection and *H.pylori* associated with contaminated food and water and the extent of personal hygiene. *Entamoeba histolytica* causes amoebic dysentery, infection with *H.pylori* is strongly associated with an increased risk of gastric ulcer. Most persons infected with *H.pylori* will never have gastric ulcer. Other factors that increase the risk of gastric ulcer among persons infected with *H.pylori* need to be detected. Several methods are available for identification of *E.histolytica* oocyst and trophozoites and *H.pylori* including direct smear and lugol’s iodine stain for *E. histolytica* and dipstick for *H.pylori* in stool.

The present study for *Entamoeba histolytica* and *Helicobacter pylori* infection at Wasit province for different ages and gender. A total of 100 samples collected from patients were suffered abdominal pain with solid, semi-solid, or diarrhea in general Hospital of Martyr Fairuz and Saeed Health center at Hay district and Al-Kut Hospital. Stool samples were detected by direct and Lugol’s iodine stain to diagnose *Entamoeba histolytica*, the result showed 14 (14%) positive samples out of 100 samples ; 8(8%) females and 6(6%) males. The highest infection appeared in age group (1-13) years old ; females recorded 1(1%) and males 4(4%), this result agreed with (15). Ten positive samples for both *Entamoeba histolytica* and *Helicobacter pylori*, the highest infection in group age group (39 and more) years old ; females recorded 3(3%) while males recorded 2 (2%). This result disagreed with (16) that showed (0.528) for *E.histolytica* and (0.02) for *H.pylori* out of one hundred and sixty – one patients. *H.pylori* examined by dipstick for each sample, 40 (40%) positive samples of *H.pylori* was detected (17). The highest infection appeared in females 23 (23%) and the lowest infection in males 17 (17%). Our results appeared that age group (39 and more ) years old recorded the highest for *H.pylori* where the females registered 6(6%) and the males recorded 6 (6%), while the age group (>1) years old revealed 1(1%) for females and 1(1%) for males, this results disagreed with (18).The higher infection in males and the lowest infection in females, this result agreed with (19) and disagreed with (20). *Helicobacter pylori* is known to be pathogen in many gastrointestinal disorders, such as...
gastric cancer (21). The infections caused by H. pylori depending on the age, the highest infected patients was in over 30 years old (22,23). The present study showed association between Entamoeba histolytica and H. pylori infections at Wasit province. Females recorded the higher infections with E.histolytica, also the higher infection with both E.histolytica and H.pylori in patients. The highest infection with H.pylori recorded in females 23(23%) while in males 17(17%) out of 100 samples. Microscopic identification of trophozoites and cyst in the stool is the common method for diagnosing Entamoeba histolytica in fresh stool (24).

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Ethical Clearance: Taken from College of Science Committee

Source of Funding: Self

Conflict of Interest: Nil

References


Identification of Cryptosporidium parvum in Stool Specimen Using Different Diagnostic Methods in Wasit Province/Iraq

Abdulsada A. Rahi¹, Salam M. Khlaif²
¹Prof, Post Doctorate, ²Research Scholar, Department of Biology, College of Science, Wasit University, Iraq

Abstract

The present study aimed to investigate the presence and prevalence of Cryptosporidium among immunocompromised patients in Wasit Hospitals and health centers to determine the best method for its diagnosis. The study started from the 1st of August to the end of November 2020. One hundred forty stool samples were collected from immunocompromised patients from both genders who attended the hospital and health centers at Wasit province. Stool samples were inspected by modified acid-fast stain as a standard method, enzyme linked immunosorbent assay (ELISA) and Molecular Nested PCR method. Modified Ziehl-Neelsen technique revealed 46 (33%) positive cases for Cryptosporidium oocysts, of 85 males and 55 females, highly significant relationship was found between the genders and between different age groups of infection with a highly significant difference between rural and urban area. The prevalence of Cryptosporidium was 57.14% using nested PCR and 38.46% for ELISA in comparison with MZN method. It is concluded that cryptosporidiosis found to be endemic in Wasit Province for the first time and the nested PCR was the most reliable technique for its diagnosis.

Keywords: Modified Ziehl Neelson, Nested PCR, Cryptosporidium parvum, Stool, Immunocompromised, ELISA

Introduction

Cryptosporidiosis is an emerging zoonotic disease in humans and animals that contributes to intestinal and extra-intestinal diseases. The major factor controlling the susceptibility and severity of cryptosporidiosis appears to be the immune status of the host [¹]. Those with great risk of infection including the immunocompromised patients [²,³]. They are including AIDS and T.B patients and those with various malignant, cytotoxic drugs receivers, prolonged corticosteroid therapy, the drugs used to prevent organ transplant rejection [⁴]. Those with chronic diseases and persons who have congenital immunodeficiency’s [⁴,⁵]. In such patients, cryptosporidiosis is characterized by painful, persistent, frequently cholera-like diarrhea along with extreme abdominal colic, body weight loss of more than 10%, and dehydration [⁶]. The infection is transmitted through the fecal-oral route and results from the ingestion of Cryptosporidium oocysts through the intake of food, water or direct contact with individuals or animals. It has been documented that infected individuals shed 10⁸-10⁹ oocysts in a single bowel movement and excrete oocysts for up to 50 days after diarrhea cessation [⁷]. The aims of this study were:

1. Detection the infectious rate of cryptosporidiosis in immunocompromised patients at Wasit province.
2. Study of some related variables with cryptosporidiosis infection.
3. Discover the relationship of some immunological factor in cryptosporidiosis infection.
4. Comparison of different technical diagnostic
**Materials and Methods**

**Sample collection**

Samples were collected from patients who attended the Al-kut and Al-zahraa Teaching Hospital in Wasit Province from 1st of August 2020 till the end of November 2020. The samples were classified according to the rural and urban area, gender, age of patients, immune status. All patient samples were freshly collected and placed in a dry, clean, sterile and screw-capped plastic container and each container was labelled with the patient’s number and name. Stool samples were divided into two parts: one section was fixed for the Modified ZN stain, and the other part of the stool sample was retained for the ELISA test and Nested Multiplex PCR as frozen form under (-22°C). The following techniques analyzed each stool sample:

1. **Direct Stool Examination**: A total of 140 stool samples were examined with a microscope as direct identification of *C. parvum* infections by staining using the Modified Ziehl-Neelsen stain technique [8].

2. **Immunoassay Method**: A total of 91 samples of stool were examined by ELISA assay. The ELISA kits were used on the frozen stool specimens. The *Cryptosporidium parvum*-ELISA based fecal antigen detection kit made by (Epitope Diagnostics, Inc. USA) was used according to manufacturer’s instructions to screen 91 randomly selected stool samples for *Cryptosporidium* inclusive of the ones positive by microscopy.

3. **Molecular Diagnosis**: DNA was extracted by using Presto™ Stool DNA Extraction Kit stool (Geneaid, Taiwan) was used according to manufacturer’s instructions to screen 91 randomly selected stool specimens for *Cryptosporidium* inclusive of the ones positive by microscopy then DNA purified and a Nested Multiplex PCR approach was used to amplify a heat shock protein 70 (hsp70) gene were designed in this study using NCBI-Genbank (MT303115.1) and primer 3 plus design fragment of *C. parvum*. The Nested PCR product was analyzed by Agarose gel electrophoresis.

**Statistical Analysis**

For each returned questionnaire, statistical analysis was performed using the SPSS (Statistical Package for Social Sciences version 16.0) software package program for statistical analysis. For both variables, descriptive statistics (numbers and percentages) were measured, as well as empirical statistics were carried out to find the relationships between the variables. The relationship between variables was determined by Chi-square using the necessary statistical tests [9].

**Results**

A total of 140 stool samples were tested, 46 (33 %) of which were positive for *Cryptosporidium* oocysts using MZN stain (Figure 1).

![Figure 1: The rate of Cryptosporidium infection with MZN.](image-url)
Over all, the prevalence of Cryptosporidium infection in male and female was (37.64%) and (25.45%) respectively, with no significant difference in the total rate of infection with Cryptosporidium between males and females (p>0.05), there was a highly significant difference found between the rate of infection among the patient of urban areas (65.51%) and children of rural areas (24.32%) (Table 1).

Patients of age group (48-63) years showed the highest (50%) rate followed by the age group of (32-47) years at a rate of (36.36%), and (1-15 year) with (64-80 year) at rate of (33.33%) then lowest rate of infection (28.94%) was among those of (16-31) years of age, with no significant difference between the rate of infection and the age groups (Table 2).

Table (1): The number of examined samples and the rate of infection with Cryptosporidium according to the gender (n= 140)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Exam. samples</th>
<th>The results of Micro. test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>85</td>
<td>32</td>
<td>37.64</td>
</tr>
<tr>
<td>Female</td>
<td>55</td>
<td>14</td>
<td>25.45</td>
</tr>
<tr>
<td>Residency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>29</td>
<td>19</td>
<td>65.51</td>
</tr>
<tr>
<td>Urban</td>
<td>111</td>
<td>27</td>
<td>24.32</td>
</tr>
</tbody>
</table>

Table (2): The relationship between Cryptosporidium infection and the age of patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Exam. samples</th>
<th>The Result of MZN test</th>
<th>Positive for Cryptosporidium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>1-15</td>
<td>84</td>
<td>28</td>
<td>33.33</td>
</tr>
<tr>
<td>16-31</td>
<td>38</td>
<td>11</td>
<td>28.94</td>
</tr>
<tr>
<td>32-47</td>
<td>11</td>
<td>4</td>
<td>36.36</td>
</tr>
<tr>
<td>48-63</td>
<td>4</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>64-80</td>
<td>3</td>
<td>1</td>
<td>33.33</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.929</td>
<td></td>
</tr>
</tbody>
</table>

From the results of using modified Ziehl-Neelsen stain the oocysts appeared as small as 4μm, spherical in shape, stained with dark pink or red color against green background color(Figure 2).
Figure (2): the Oocyst of *Cryptosporidium* with Modified Ziehl-Neelsen stain in 1000X magnification

Figure (3): Agarose gel electrophoresis image that showed the Nested PCR product analysis of hsp70 gene in *C. parvum* from Human samples. Where, the Lane (M): DNA marker ladder (1500-100bp) and the Lane (1-16) were showed some positive hsp70 gene in *C. parvum* at 650bp PCR product size

The outcome of the use of three techniques in the analysis of stool samples showed that the higher rate of infection was 57.14% by Nested PCR then came ELISA test with prevalence rate of 38.46%, while modified cold Ziehl-Neelsen stain it was 32.85% Table (4).
Table (4): Comparison between efficacy of different methods for diagnosis of Cryptosporidium infection.

<table>
<thead>
<tr>
<th>Test</th>
<th>Total number</th>
<th>Positive No. (%)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified Ziehl- Neelsen</td>
<td>140</td>
<td>46</td>
<td>32.85</td>
</tr>
<tr>
<td>ELISA test</td>
<td>91</td>
<td>35</td>
<td>38.46</td>
</tr>
<tr>
<td>Nested PCR</td>
<td>91</td>
<td>52</td>
<td>57.14</td>
</tr>
<tr>
<td>X2</td>
<td></td>
<td></td>
<td>13.34</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td></td>
<td>0.001(HS)</td>
</tr>
</tbody>
</table>

### Discussion

*Cryptosporidium parvum* accompanied by diarrhea is more frequently seen in bad hygiene, malnutrition cases, among the old and child category, and in immunocompromised patients [10]. Waterborne *Cryptosporidium* outbreak are seen commonly in recent years, and revealed a serious public health problem [11]. The identification of *Cryptosporidium parvum* is important to reveal the epidemiology of *Cryptosporidium* infections in humans [12].

In the present study the percent of Modified acid fast stain technique in patients suffering from diarrhea who attended to Al-kut and Al-zahraa Teaching Hospital in Wasit province was (33%). This finding is disagree with the results obtained in Al-Dewanyia [13] who reported (14.7%), while a previous surveys studies carried out in numerous parts of the world revealed that the prevalence rate of cryptosporidiosis was anywhere from 3-50% [14,15].

In the present study, cryptosporidiosis rate showed a statistically differences between the rural and urban center. *Cryptosporidium parvum* infection was seen in rural population higher than urban area (65.51%). Thus, returns of living in the rural could have been related by such factors as problems of drainage, lack of enough clean water sources, fecal droppings from both animals and humans are found in most places and unselective defecation. The rural population’s also shows that person-to-person transfer through food or water was high and this confirms that there is a high level of contamination with human feces. this result were nearly similar to previous study that carries out the percent rate of Cryptosporidiosis in rural area (55%) [16].

In relation to gender, the present study showed the males were higher percent than females in cryptosporidiosis rate, this is may be due to the male in Iraqi population were more frequenter to coffee shop and restaurant and thus make them more deal with the contaminated instrument , water, vegetable and, this result were nearly similar to previous study in Iraq revealed that 33.74% of samples were positive for *Cryptosporidium*, with higher frequency of cases in male patients [17].

Cryptosporidiosis is accompanying with age and immunity, in this study, the common of the positive cases was frequently seen in older patients (48-63) years. The occurrence of high infection rates in this category may be patients older than 30 years had a higher risk of this infection. Similar age related increases in *Cryptosporidium* infection have previously been reported [18].

Recently, PCR and immunodiagnostic methods have become more popular in diagnosis of cryptosporidiosis to overcome the limitations of microscopy. Compared with microscopy, DNA-based detection methods display several advantages such as an increased sensitivity and specificity, the possibility for molecular typing and an optimized turnaround time [19,20,21]. Given these
trends in diagnosis, PCR was chosen the gold standard in this study. Indeed, it yielded the highest detection rates (57.14%), compared to ELISA (38.46%) and examination of MZN stained slides (32.85%) and this was found to be statistically significant. This result agreed with the results recorded in Beni-Suef, Egypt [22].

Acknowledgement: Authors sincerely wish to acknowledge the members of the Laboratory of the College of Science, Wasit University for their supporting.

Ethical Clearance: Taken from College of Science Committee

Source of Funding: Self

Conflict of Interest: Nil

References


Comparison between Microscopic Identification and Nested PCR for Detection of Cutaneous Leishmaniasis at Wasit Province

Abdulsada A. Rahi¹, Zainab K. Hashim², Ali Mohsin Al-Jamea³
¹Prof, Post Doctorate, ²Research Scholar, Department of Biology, College of Science, Wasit University, Iraq, ³Research Scholar, Dermatologist, Al- Karamah Teaching Hospital, Directorate of Wasit Health, Iraq

Abstract

Cutaneous leishmaniosis in Iraq has 2 forms, zoonotic cutaneous leishmaniasis (ZCL), which is mainly caused by Leishmania major, and anthroponotic cutaneous leishmaniasis (ACL), which is mainly caused by Leishmania tropica. Twenty skin samples were taken from suspected patients with CL and checked for Leishmania amastigote, during the period from October 2019 to February 2020 in Al-Karamah teaching hospital of Kut city, Iraq. The highest infection 100% using Giemsa- smeared and 75% using Nested PCR methods. Totally 20 patients aged from (1- <40) years old were included in current study. The prevalence of CL were in males 9(45) and 11(55) in females and high prevalence in age groups (>20) years old. The current study 12(60%) were brought on Leishmania major and 4(20%) Leishmania tropica using Nested PCR method. In present study the direct smear could be considered a good test for testing the cutaneous Leishmaniasis but Nested PCR assay was more touchy than parasitological technique in diagnosis of Leishmania species in skin lesions. L.major is the main species responsible of cutaneous leishmaniasis in areas of Wasit Province.

Keywords: Cutaneous Leishmaniasis, Nested PCR, Giemsa, Human

Introduction

Leishmaniasis is a disease spread through the bites of a female sandfly and triggered through different varieties of leishmaniasis, which is expressed in three main clinical types: cutaneous, mucous and visceral leishmaniasis (¹–⁴). Cutaneous leishmaniasis exists in at least two shapes; Amastigote elliptical and non-flagellated, 3-5 μm long and promastigote was a cutaneous type contained in the host sand fly (⁵). Cutaneous Leishmaniasis cases were more abundant in winter, with a peak in February, the rate of infection then started to decline from April and reaches its lowest in July and August (⁶).

Cutaneous Leishmaniasis, commonly known as called Baghdad boil, is a very old disease in Iraq, it is a less severe of disease which manifests self-healing ulcers. Leishmania major and Leishmania tropica are causative agents of Cutaneous Leishmaniasis in Iraq (⁷). According to species of parasite and immune response of the patients, the symptoms differ in regions, that beginning as erythematous papule, increase in size producing a nodule, ulcerate and crusts (⁸,⁹). The zoonotic type is caused by Leishmania major and anthroponotic type is caused by Leishmania tropica (¹⁰,¹¹). Other modes of transmission such as parenteral, congenital, sexual, occupational exposures, and person to-person transmission could also theoretically occur(¹²).

Nested PCR approach was applied for the detection and identification of the Leishmania species according to the Noyes et al., (1998) method (¹²). Nested PCR is a one of the best parts of the parasite genome for sequencing.
to identify different *Leishmania* species \(^{(13,14)}\). The current study aimed to compare between microscopic identification and Nested PCR methods for detection of cutaneous leishmaniasis at Wasit province.

### Materials and Methods

#### Population study

This study was carried out during the period from October 2019 to February 2020 in Al-Karamah teaching hospital of Kut city, Iraq. A total of 20 skin samples were taken from suspected patients with CL. All patients were divided into four age groups. Samples were taken from the skin lesion, and kept into two tubes; one stored in freeze at -20°C for Nested-PCR and the second tube for direct smear. After the smears dried completely, they were fixed with 100% methanol, allowed to dry again, and stained with Giemsa stain for microscopic examination for presence of amastigotes \(^{(15)}\).

#### DNA Extraction

Genomic DNA was extracted from skin lesions and aspirates using AccuPrep®Genomic DNA extraction kit (Bioneer, Korea) and done according to company instruction. The extracted genomic DNA was checked using Nanodrop spectrophotometer (Thermo, USA), and measured the purity of DNA through reading the absorbance at (260/280 nm) \(^{(16)}\).

#### Nested-PCR

Nested – PCR was performed as follows: in the first stage two external primers CSB1XR (CGAGTAGCAGAAACTCCCGTTCA) and CSB2XF (ATTTTTCGCGATTTTCGCAGAA CG) and in the second step, two internal specific primers 13Z (ACTGGGGGTT GGTGTAAA ATAG) and LiR (TCGCAGAAGCCCT) was used for amplification of variable minicircles of *Leishmania* kDNA. All primers provided from (Bioneer, Korea) company. Two *Leishmania* species produced the amplified fragments of about 750 bp for *L. tropica* and 560 bp for *L. major*.

Amplification reactions visualized in 1.5 % Agarose Gel Electrophoresis, using a 100 bp DNA ladder \(^{(17)}\).

### Results and Discussion

**Table (1): Prevalence of CL According to the Age Groups and Gender**

<table>
<thead>
<tr>
<th>Age groups / Year</th>
<th>Gender</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (%)</td>
<td>Female (%)</td>
</tr>
<tr>
<td>&gt;20</td>
<td>5(25)</td>
<td>6(30)</td>
</tr>
<tr>
<td>20-40</td>
<td>2(10)</td>
<td>3(15)</td>
</tr>
<tr>
<td>&lt;40</td>
<td>2(10)</td>
<td>2(10)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>9(45)</td>
<td>11(55)</td>
</tr>
</tbody>
</table>

Twenty skin samples of suspected patients were enrolled in our study: 9 (45%) males and 11(55%) females. The high prevalence (55%) in age group (>20) years old and the lowest prevalence (20%) in age group (<40) years old. Which may be due to several factors, such as children’s outdoor activities and sleeping outdoors, which increases exposure to sand fly bites during their active hours. The same result has been reported by some other researchers \(^{(24,25-28)}\). AL-Janabi, (2001) in Najaf province, who found that the age group
10-15 years old was the most affected age group \(^{(31)}\). While the most rate of disease reported by Azizi et al.,(2013) was in age group (10-19) years old assemble for Esfahan region in Iran \(^{(32)}\). The rate of the disease although not significant is more seen in females 11(55%) than males 9(45%) which is do not agreed to previous studies and most likely because of the more exposure to sand fly bites in males than females \(^{(29,30)}\).

| Table (2): Comparison between Giemsa-smeared and Nested-PCR in Diagnosis of CL |
|---------------------------------------------|-----------------|-----------------|
| Test                                        | Positive (%)    | Negative (%)    |
| Giemsa-smeared                             | 20(100)         | 0               |
| Nested-PCR                                 | 15(75)          | 5 (25)          |

In this study, the results showed that the rate of positive cases of CL using Giemsa-smeared were 20 cases which constitute (100%), while Nested PCR recorded 15 positive cases constitute (75%) from total samples. The highest infection (100%) appeared using Giemsa-smeared method while the lowest infection (75%) appeared by Nested-PCR method.

There are many diagnostic tests used to detect the *Leishmania* parasite, microscope examination is the most reliable and conventional method, the parasite is demonstrated in direct smear stained with Giemsa stain or leishman stain to detect the presence of amastigotes \(^{(17)}\).

The diagnosis of CL classically relies on microscopic examination and in vitro cultivation. These classical methods require the presence of a relatively high number of viable or morphologically intact parasites. This may pose a problem particularly in the chronic phase of CL where parasite levels in skin lesions are very low. In contrast, the molecular approach is both sensitive and specific \(^{(18)}\). Several reports have shown the high rates of infection with *Leishmania* species and that agreed with our results, our finding is steady with the discoveries of the study conducted by others \(^{(19)}\). Al Samarai and Al Obaidi, (2009) reported that 73% of the cases were certain to Giemsa stain and 43% were sure in societies for Al Hawija locale of Kirkuk region in Iraq \(^{(9)}\). Rahi, (2015) was recording that the rate of infection was 97.8% by utilizing Giemsa technique for Wasit province in Iraq. Rahi reporting the pervasiveness of positive instances of CL was 94% by utilizing smear strategy for Wasit region in Iraq and microscope examination of smears is quick and simple to use for conclusion of CL \(^{(20)}\). In Tuz- Kirkuk province, suggested the diagnosis of CL by staining the aspirated material with Giemsa stain and culturing on semi-solid media, found that 73% of samples were positive for Giemsa stain, while 27% were negative \(^{(35)}\).

Most regularly utilized strategies for the immediate identification of the parasite (e.g., minute examination of Giemsa-recolored smears and in vitro development) need affectability as a result of the shortage of *Leishmania* parasites in a few examples or the parasites might be inadequate and are generally extracellular in the slide arrangements, or are hampered by the issue of defilement \(^{(21)}\). These techniques have restricted sensitivities since they require coordinate perception of the parasites and the scarcity of parasites inside the injury is a sign of sores with old age. Our result were about like the outcome reported in Iraq \(^{(19)}\). Among these, currently, the most ordinarily utilized strategy is DNA-based systems, utilizing PCR and particular ground works for species and even strains portrayal \(^{(22,23)}\).
Table (3): Distribution of CL cases in relation of Residence

<table>
<thead>
<tr>
<th>Leishmania sp.</th>
<th>No. of patients in Rural areas</th>
<th>No. of patients in Urban areas</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.major</td>
<td>5</td>
<td>7</td>
<td>12(60)</td>
</tr>
<tr>
<td>L.tropica</td>
<td>0</td>
<td>4</td>
<td>4(20)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>5(25)</td>
<td>11(55)</td>
<td>16(80)</td>
</tr>
</tbody>
</table>

In Iraq, cutaneous leishmaniasis (Baghdad boil) caused by two species *L. major* zoonotic disease and *L. tropica* anthroponotic disease (20). The present study revealed that prevalence of *L. major* (60%) were higher than *L. tropica* (20%) in the studied areas. Agreement to the findings were recorded of some other studies (18,33). The present study also agreed with Rahi et al., (2019) revealed that prevalence of *L. major* (63.3%) were higher than *L. tropica* (6.7%) (34).

**Conclusions**

In present study the direct smear could be considered a good test for diagnosis the cutaneous leishmaniasis, the high rate of infection with *Leishmania* species at Wasit province, but Nested PCR assay was more touchy than parasitological technique in recognition of *Leishmania* parasite in skin lesions. *L. major* is the main species responsible of cutaneous leishmaniasis in areas of Wasit Province.

**Acknowledgement:** Authors sincerely wish to acknowledge the members of the Laboratory of the College of Science, Wasit University for their supporting.

**Ethical Clearance:** Taken from College of Science Committee

**Source of Funding:** Self

**Conflict of Interest:** Nil

**References**


20- Rahi AA. Genetic characterization of Leishmania species causing cutaneous Leishmaniasis in Iraq, 2015.


A Comparison of use of Microscopic Examination and PCR for Detection of Entamoeba Histolytica at Wasit Province

Magda A. Ali
Research Scholar, PhD, Department of Biology, College of Science, Wasit University, Iraq

Abstract

A microscopic examination of multiple feces samples of 100 suspected patients with Entamoeba histolytica infection who suffered from diarrhea was attended to Al-Karamah Teaching hospital at Wasit Province from January 2020 to March 2020. The feces samples were collected into two

Parts; first of direct wet film preparation were done for each sample using normal saline 0.9 % for detecting the motility of trophozoites and Lugol’s iodine 5% for demonstrating structures and cyst. The 2nd part was kept in freeze (-20C) until used in PCR method .

The current study recorded that the overall prevalence rate of Entamoeba histolytica 80(80%) by microscopic examination and 88(88%) by PCR methods. The prevalence of E. histolytica was higher among families who live in rural areas 44(55%) than those in urban areas 36(45%) and in males 49(61.3%) more than females 31(38.7%). The highest infection rate 37(46.3) was recorded in age group (21-40) years old. The present study was aimed to compare between the microscopic examination and PCR methods in the diagnosis of E. histolytica in human clinical samples.

We concluded our results that using PCR technique was more accurate and sensitive (88%) than microscopic examination method (80%).

Keywords: Entamoeba histolytica, Lugol’s iodine stain, PCR, Human, Stool

Introduction

The spread of intestinal parasites differ from one region to another and its spread related to many factors such as geographical factors, climate, poorness , malnutrition, personal clean less and other factors (1). One trophozoites of Entamoeba histolytica reach the host intestine can damage the mucosa epithelial layer and prevalence through the sub-mucosa and the lamina propia and other tissues (2).

Entamoeba histolytica infections occur mostly in adults, although they see in children. Since the parasite infects the large intestine, severe infection result in serious loss of blood and also may cause systemic problem such as liver abscesses due to infection of the liver by the parasites, amoebiasis can cause nutrient loss and can lower the levels of circulating proteins, this sometimes leads to under nutrition (3).

The diagnosis of amoebiasis is often difficult and time consuming. Microscopic identification of trophozoites and cysts in the stool is the common method for diagnosing E. histolytica either in fresh stool or stool concentrates (4). The conventional method of diagnosing E. histolytica utilizes microscopy, but its sensitivity is less than 60% (5). To improve the rate and sensitivity of the diagnosis, PCR-based approaches have been used since the early 1990s, and many studies have been conducted to detect target E. histolytica DNA in the human stool (6-9).

The present study aimed to compare between the microscopic examination and PCR methods in the diagnosis of E. histolytica in human clinical samples.
Materials and Methods

Stool Collection

A total of 100 fecal samples were collected from suspected patients who suffering from diarrhea in Alkarama Teaching Hospital at Wasit Province. The fecal sample were collected from January 2020 to March 2020 and it was divided into 2 parts; 1st part it placed in dry plastic container and transported to the laboratory for microscopically examination and the 2nd part kept in freeze (-20 C) until used in PCR method.

Stool DNA Exteraction

Genomic DNA was extracted from stool samples by using (Stool DNA extraction Kit, Bioneer. Korea). The extraction was done according to company instructions by using stool lysis protocol method with Proteinase K (10). After that, the extracted gDNA was checked by Nanodrop spectrophotometer, and then stored at -20C until used in PCR amplification.

Convetion PCR

PCR technique was performed for specific detection *E. histolytica* from human stool samples. This method was carried out according to (10). The primers were provided by (Bioneer company. Korea). PCR reaction solution was prepared by using (AccuPower PCR PreMix Kit) according to company instructions.

Statistical Analysis

Statistical analysis: The statistical analysis was performed using SAS (Statistical Analysis System - version 9.1) (11).

Results

Table 1: Types of diagnostic method for *Entamoeba histolytica*

<table>
<thead>
<tr>
<th>Method</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopic examination</td>
<td>80(80)</td>
<td>20(20)</td>
<td>100(100)</td>
</tr>
<tr>
<td>PCR</td>
<td>88(88)</td>
<td>12(12)</td>
<td>100(100)</td>
</tr>
</tbody>
</table>

Table 2: Distribution of positive cases of *Entamoeba histolytica* in relation to the Age and District

<table>
<thead>
<tr>
<th>Age group (year)</th>
<th>Positive (%)</th>
<th>Rural (%)</th>
<th>Urban (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1 -20</td>
<td>26(32.5)</td>
<td>18(22.5)</td>
<td>8(10)</td>
</tr>
<tr>
<td>21-40</td>
<td>37(46.3)</td>
<td>15(18.75)</td>
<td>22(27.5)</td>
</tr>
<tr>
<td>31 and more</td>
<td>17(21.2)</td>
<td>11(13.75)</td>
<td>6(7.5)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>80(100%)</td>
<td>44(55%)</td>
<td>36(45%)</td>
</tr>
</tbody>
</table>

Table 3: Distribution of positive cases in relation to the Age and Gender

<table>
<thead>
<tr>
<th>Age group (year)</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1 -20</td>
<td>15(18.8)</td>
<td>9(11.2)</td>
<td>24(30)</td>
</tr>
<tr>
<td>21-40</td>
<td>21(26.3)</td>
<td>14(17.5)</td>
<td>35(43.8)</td>
</tr>
<tr>
<td>31 and more</td>
<td>13(16.2)</td>
<td>8(10)</td>
<td>21(26.2)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>49(61.3%)</td>
<td>31(38.7%)</td>
<td>80(100%)</td>
</tr>
</tbody>
</table>
Discussion

In developing countries, poor hygiene and the use of untreated human feces (i.e. fecal sludge) are important factors that contribute to the contamination of food and water. In such countries, prevention of infection with *E. histolytica* can be attained by improved community health education, sanitation, hygiene, and water treatment. To reduce enteric pathogens, including intestinal protozoa that may be present on fresh vegetables and fruits, the produce can be washed by clean tap water and soaked in a solution of acetic acid or vinegar for 10 to 15 minutes before consumption (12).

According to the results of the present study *E. histolytica* had an overall prevalence of 80/100(80%) (table 1). These results in agreement with Rahi and Majeed, (2019) at Wasit Province, Iraq (13) and disagreed with other studies like Ali et al., (2003) that reported 15.6% prevalence of *E. histolytica* from preschool children in Bangladesh (14), Aza et al., (2003) who reported 21% prevalence of intestinal parasites in seven villages of Malaysia (15) and Zahida et al., (2010) who recorded 11% prevalence of *E. histolytica* in children of Delhi, India (16).

The results of current study showed the prevalence of *E. histolytica* in the rural areas 44(55%) was higher than urban areas 36(45%) (table 2). Thus, returns of living in the rural could have been related by such factors as problems of drainage, lack of enough clean water sources, fecal droppings from both animals and humans are found in most places and unselective defecation. The rural population’s also shows that person-to-person transfer through food or water was high and this confirms that there is a high level of contamination with human feces. this result were nearly similar to previous study that carries out of *E. histolytica* infection (17). The present study was appeared the predominance of male 49(61.3%) to female 31(38.7%) as showed in table 3, this result agreed with (18) and it was disagreed with (19).

Acknowledgement: Authors sincerely wish to acknowledge the members of the Laboratory of the College of Science, Wasit University for their supporting.

Ethical Clearance: Taken from College of Science Committee

Source of Funding: Self

Conflict of Interest: Nil

References


Correlation between Interleukin(IL-6) and Procalcitonin (PCT) Level among Diarrheal Children

Nihad Khalawe Tektook¹, Ahmed Salim Hadi Al-Khafaji²

¹Assist Prof Middle Technical University / College of Medical & Health Technology, Medical Laboratory Techniques Dep., ²Pediatrics Specialist, Iraqi Board In Pediatrics, Karbala Pediatric Teaching Hospital/Karbala Health Directorate/Iraq

Abstract

This study was conducted in Baghdad for the period from December 1, 2019 to June 3, 2020 for determination of the level of elevation of interleukin-6 and procalcitonin in children with acute diarrhea. I was included 50 children suffering from diarrhea and 50 children without any other disease, where the ages of children in both groups ranged from one month to five years. The study included collecting information from children in both groups, including gender, age, living situation, number of family members, standard of living and social as well as the number of family members, number of rooms in the house. Amount of 5 ml of blood samples were collected from all the children included in this study, where the blood samples were separated by the centrifuge device and the serum samples were separated from them and kept in the refrigerator until the tests for detecting the level of Interleukin 6 and procalcitonin using ELISA technique. In this study, 76% of diarrheal children were suffered from abdominal pain, 68% with dehydration, 58% of fever, 46% had vomiting. The presented study found that, 78% of families with diarrheal children were lived in crowed houses, 73% didn’t wash vegetables before eat, 69% haven’t wash hands, 66 used tap water for drinking. In this study, IL-16 was observed in elevated mean in diarrheal children (19.66±2.19 ng/ml), when it compared with the control healthy children (4.27±1.46 ng/ml) (P<0.001). In this study, procalcitonin was also observed in elevated mean in diarrheal children (42.17±9.28 ng/ml), when it compared with the control healthy children (28.19±8.27 ng/ml) (P<0.001). The study showed that the maximum means of PCT and IL-6 in diarrheal children were observed in those with fever, followed by abdominal pain. The study revealed a significant positive correlation between IL-6 and PCT level among diarrheal children.

Conclusions:
The study showed a significant relation of PCT and IL-6 with diarrhea.

Keywords: Procalcitonin; diarrhea; IL-6; Abdominal pain

Introduction

Diarrhea is one of the most common diseases among children in various parts of the world, although it is considered one of the common diseases that its severity is almost limited, and this risk is that children are afflicted with what is called the term dehydration, which leads to the loss of salts and water from the body through the digestive system and abroad. The risk of developing dehydration in children increases in children under five years of age (¹).

Corresponding author:
Nihad Khalawe Tektook
Gmail: drnihadkhalawe@gmail.com

Diarrhea is of two types, and they are acute diarrhea, the period of which increases to more than 14 days from the date of injury and chronic diarrhea, and it is not when the patient suffers from diarrhea for a period longer than that (²). It is also classified according to the persistence of stool in children into two types of watery diarrhea, which results from injuries in microorganisms like bacteria, viruses, and parasites, the infection is often in the upper part of the intestine, and the stool is liquid, which may cause dehydration.

Bacteria such as Escherichia coli, salmonella, shigella, and V.cholera, predominantly cause acute diarrhea, in addition to viral infection also could be on of causes of watery diarrhea plus vomiting and
fever in children with gastroenteritis\textsuperscript{(3,4)}. Interleukin-6 is considered one of the most important inflammatory factors among the types of cytokines in the body, which are raised due to infection with bacteria, viruses or parasites in the body.\textsuperscript{(3)} Where the scientific evidence indicates that the high level of interleukin 6 in children with diarrhea may be caused by an acute bacterial or viral infection.

The level of interleukin 6 rises directly with the severity of infection in these children \textsuperscript{(5)}. In addition, the protein factor procalcitonin was found in recent studies to be high in bacterial infections, especially due to gram negative bacteria in diarrhea, urinary tract infection and bacteremia in people with this condition, as its height is directly proportional to the severity of the disease and is also directly proportional to the high level of interleukin.\textsuperscript{(6,7)} This study was conducted for the purpose of determining the level of elevation of interleukin-6 and procalcitonin in children with acute diarrhea who are less than five years old and related to ages, their level of hygiene.

### Patients and Methods

This study was conducted in Baghdad for the period from December 1, 2019 to June 3, 2020, and included 50 children suffering from diarrhea and 50 children without any other disease, where the ages of children in both groups ranged from one month to five years. The study included collecting information from children in both groups, including gender, age, living situation, number of family members, standard of living and social as well as the number of family members, number of rooms in the house. Amount of 5 ml of blood samples were collected from all the children included in this study, where the blood samples were separated by the centrifuge device and the serum samples were separated from them and kept in the refrigerator until the tests for detecting the level of Interleukin 6 and procalcitonin using ELISA technique.

### Results

In this study, 44% of studied children were within the age group \(<1\) year followed by 36% in the age group 1-2 year, 27 of 50 were females and 55% rurals, (Table 1).

<table>
<thead>
<tr>
<th>Age groups (year)</th>
<th>No.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>22 (44%)</td>
</tr>
<tr>
<td>1-2</td>
<td>18 (36%)</td>
</tr>
<tr>
<td>3-5</td>
<td>10 (20%)</td>
</tr>
<tr>
<td>Total</td>
<td>50 (100%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male 23, female 27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residence</td>
<td>55% rural</td>
</tr>
</tbody>
</table>

In this study, 76 % of diarrheal children were suffered from abdominal pain, 68% with dehydration, 58% of fever, 46% had vomiting, Figure 1.
The presented study found that, 78% of families with diarrheal children were lived in crowed houses, 73% didn’t wash vegetables before eat, 69% haven’t wah hands, 66 used tap water for drinking (Figure 2).

In this study, IL-16 was observed in elevated mean in diarrheal children (19.66±2.19ng/ml), when it compared with the control healthy children (4.27±1.46 ng/ml) (P<0.001), Table 2.
Table 2: Level of IL-6 in children groups with and without diarrhea

<table>
<thead>
<tr>
<th>Studied children</th>
<th>Interleukin-6 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>With diarrhea (n:50)</td>
<td>19.66</td>
</tr>
<tr>
<td>Healthy children, (n:50)</td>
<td>4.27</td>
</tr>
</tbody>
</table>

P<0.001

In this study, procalcitonin was also observed in elevated mean in diarrheal children (42.17±9.28 ng/ml), when it compared with the control healthy children (28.19±8.27 ng/ml) (P<0.001), Table 3.

Table 3: Level of PCT in children groups with and without diarrhea

<table>
<thead>
<tr>
<th>Studied children</th>
<th>Procalcitonin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>With diarrhea (n:50)</td>
<td>42.17</td>
</tr>
<tr>
<td>Healthy children, (n:50)</td>
<td>28.19</td>
</tr>
</tbody>
</table>

P<0.001

The study showed that the maximum means of PCT and IL-6 in diarrheal children were observed in those with fever, followed by abdominal pain, Table 4

Table 4: relation of Il-6 and PCT with symptoms of diarrhea

<table>
<thead>
<tr>
<th>Parameter</th>
<th>dehydration</th>
<th>Vomiting</th>
<th>Fever</th>
<th>Abdominal pain</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT</td>
<td>33.4</td>
<td>30.5</td>
<td>46.7</td>
<td>38.5</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-6</td>
<td>17.56</td>
<td>18.5</td>
<td>28.4</td>
<td>25.4</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The study revealed a significant positive correlation between IL-6 and PCT level among diarrheal children, Figure 3.
Discussion

In this study, 44% of studied children were within the age group <1 year followed by 36% in the age group 1-2 year, 27 of 50 were females and 55% rurals,(Table 1).In line with this study that there are many studies that have proven that diarrhea in children often occurs at young ages of less than five years, especially in those who are less than two years of age. A study conducted in previous decades or previous years stated that children who are less than two years old are More vulnerable to acute diarrhea due to bacteria or viruses (6-8).In this study, 76 % of diarrheal children were suffered from abdominal pain, 68% with dehydration, 58% of fever, 46% had vomiting, Figure 1.Dehydration and colic are among the most important symptoms of all ages, especially children, as past studies have shown that most children, and most of those children who are less than five years old and who suffer from severe diarrhea, were implicitly suffering from colic and dehydration, as well as high temperatures, which often accompany anorexia and thinness(9,10).The presented study found that, 78% of families with diarrheal children were lived in crowed houses, 73% didn’t wash vegetables before eat, 69% haven’t wah hands, 66 used tap water for drinking (Figure 2).Past studies show that people or children suffering from diarrhea, the majority of whom are families suffering from limited income and suffer from a disruption of the health system, and a high percentage of children with diarrhea do not wash their hands before eating and use running water for drinking (11-14).In this study, IL-16 was observed in elevated mean in diarrheal children (19.66±2.19 ng/ml), when it compared with the control healthy children (4.27±1.46 ng/ml) (P<0.001), Table 2.In line with this study, the study previously conducted in different countries of the world has shown that Interleukin-6 significantly increases blood serum in people with many diseases, including urinary tract infection, diarrhea, blood market store, and fever of unknown cause(15).In this study, procalcitonin was also observed in elevated mean in diarrheal children (42.17±9.28  ng/ml), when it compared with the control healthy children (28.19±8.27  ng/ml) (P<0.001), Table 3.The study showed that the maximum means of PCT and IL-6 in diarrheal children were observed in those with fever, followed by abdominal pain, Table 4.The study revealed a significant positive correlation between IL-6 and PCT level among diarrheal children, Figure 3.Where the level of interleukin-6 and the level of procalcitonin rises in people with acute diarrhea due to causes that lead to diarrhea such as the bacteria that cause diarrhea (16).Where the researcher(16)mentioned in his study that
the increase in the level of interleukin-6 came with an increase in the level of calcitonin, and the increase was proportional, meaning the higher the first, the higher the level of the second variable was very high, especially in children with diarrhea and those suffering from high temperatures\(^{17}\). The high temperatures in children with diarrhea are often due to the fact that the bacteria that cause diarrhea are of the type gram-negative bacteria, which contain endotoxins that in turn lead to elevated levels of interleukin-6, and the CRP and procalcitonin, which are automatically preserved as a result of infection in infected patients or any diseases, bacteremia, cough and urinary inflammation\(^{18&19}\). Conclusions: The was a significant relation of PCT and IL-6 with diarrhea.

Conflict of Interest: None

Source of Findings: None

Ethical Clearance: None

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Prevalence of Urinary Tract Infections (UTIs) in Children with Inguinal Hermia Repairs

Arkan Kareem Abd1; Ali Ghazi Abbood1; Saud Hussein Mousa Al-jumaily2
1Specialist Surgeon, Fellow Iraqi Council for Medical Specialization General Surgery (F.I.C.M. S), Ministry of Health and Environment / Baghdad Health Directorate- Al Karkh / Al Yarmouk Teaching Hospital; 2Specialist Surgeon M.B.CH. B F.I.C.M.S(uro.) /Al-Salam Teaching Hospital / Ministry of Health and environment / Directorate of Health Nenva/ Iraq

Abstract

This case control study was carried out in Baghdad city from 1st of April 2018 to 1 March 2019 included 40 studied cases (children with inguinal hernia) and 40 healthy children to evaluate the prevalence of UTIs in children with inguinal hernia repairs. It was known that the children are infected or not with urinary tract infection through clinical examination of the signs that indicate urinary tract infection, as well as a laboratory examination of the urine to detect the presence of bacteria that cause urinary tract infection in the laboratory. All children under 15 years of age were included in the study who attended pediatric surgery clinics. Ages over the age of 15 years old, children with complex inguinal hernia such as blocked, irreducible, strangulated hernia and children with recurrent hernia were excluded. All children included in our study included in the study were examined through routine and microscopic examination of urine, urine culture and sensitivity. Children with urinary tract infections were treated according to sensitivity culture reports. This case control study included children with inguinal hernia (90% males and 10% females), their age range was <1 year to 15 year (mean: 9.52±1.3 year), the study also included 40 healthy children (as control group) with the same characteristics of patient. The study revealed that urinary tract infection was observed in 25% of (10 of 40) of children with inguinal hernia compared with 5% of (2 of 40) of the control group (P<0.001). This study showed that most of children with inguinal hernia were suffered from abdominal pain, urge to urinate, polyurea, cloudy urine, fever and pyelonephritis. The study found that, 50% of children with inguinal hernia who suffered from UTI were due E. coli, S. aureus with rate 40% and Klebsiella spp with rate 10%. The study showed that 80% of E. coli isolates were sensitive to ampicillin, 60% to amikacin and 40% to Amoxiclave, while 100% of S. aureus were sensitive to Ceftriaxone and cand 75% to Cefotaxime. It was concluded that, there significant positive correlation between UTIs and inguinal hernia repairs in children.

Keywords: Children; inguinal hernia; UTI; Prevalence

Introduction

Herniorrhaphy operations have become the most important and successful options for treating inguinal hernia in children, as the percentages in these operations reached twenty million successful operations per year(1). The availability of databases for cases who suffer from hernia repair in general, especially in children, may be almost rare. Studies indicate that approximately 26% of men and a small minority of women experience inguinal hernia repair operations worldwide once in their lifetime (2). Some disorders and bacterial infection are associated with the operations of repairing inguinal hernia in children, and among those problems and diseases is urinary tract infection, where urinary tract hernia in children, as the percentages in these operations reached twenty million successful operations per year(1).
infection is one of the most important causes suffered by children under the age of 12 years and the distance between the operations of repairing inguinal hernia and the urinary tract is close\(^{(3-5)}\). Like the bladder and the ureter between, it is expected that they will have urinary tract infections, especially those children who suffer from high temperatures, colic and decreased urination. The infection caused by multidrug resistance (MDR) organisms is more likely to prolong the hospital stay, increase the risk of death, and require treatment with more expensive antibiotics \(^{(6-8)}\). The aim of this study was to evaluate the prevalence of UTIs in children with inguinal hernia repairs.

**Materials and Methods**

This case control study was carried out in Baghdad city from 1\(^{st}\) of April 2018 to 1 March 2019 included 40 studied cases (children with inguinal hernia) and 40 healthy children. It was known that the children are infected or not with urinary tract infection through clinical examination of the signs that indicate urinary tract infection, as well as a laboratory examination of the urine to detect the presence of bacteria that cause urinary tract infection in the laboratory. All children under 15 years of age were included in the study who attended pediatric surgery clinics. Ages over the age of 15 years old, children with complex inguinal hernia such as blocked, irreducible, strangulated hernia and children with recurrent hernia were excluded. All children included in our study included in the study were examined through routine and microscopic examination of urine, urine culture and sensitivity. Children with urinary tract infections were treated according to sensitivity culture reports.

**Results**

This case control study included children with inguinal hernia (90% males and 10% females), their age range was <1 year to 15 year (mean: 9.52±1.3 year), the study also included 40 healthy children (as control group) with the same characteristics of patient. Further features of patients and control were mentioned in Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Children with inguinal hernia</th>
<th>Healthy control children</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Age groups (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>3</td>
<td>7.5</td>
<td>4</td>
</tr>
<tr>
<td>1-4</td>
<td>10</td>
<td>25</td>
<td>11</td>
</tr>
<tr>
<td>5-8</td>
<td>15</td>
<td>37.5</td>
<td>14</td>
</tr>
<tr>
<td>9-15</td>
<td>12</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100</td>
<td>400</td>
</tr>
<tr>
<td>(Mean±SD)</td>
<td>9.52±1.3</td>
<td></td>
<td>8.53±2.2</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>36</td>
<td>90</td>
<td>35</td>
</tr>
<tr>
<td>Females</td>
<td>4</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>20</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>Urban</td>
<td>20</td>
<td>50</td>
<td>18</td>
</tr>
</tbody>
</table>

* P. value >0.05 = non-significant (NS)
The study revealed that urinary tract infection was observed in 25% of (10 of 40) of children with inguinal hernia compared with 5% of (2 of 40) of the control group (P<0.001), Table 2.

**Table 2: Distribution of UTI among children with inguinal hernia compared healthy group.**

<table>
<thead>
<tr>
<th>Rate</th>
<th>Children with inguinal hernia</th>
<th>Healthy control children</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Percentage</td>
<td>25%</td>
<td>5%</td>
</tr>
</tbody>
</table>

P<0.001

This study showed that most of children with inguinal hernia were suffered from abdominal pain, urge to urinate, polyurea, cloudy urine, fever and pyelonephritis, Table 3.

**Table 3: Associated clinical features of UTI in children with inguinal hernia**

<table>
<thead>
<tr>
<th>Associated clinical features</th>
<th>children with inguinal (No.=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>25%</td>
</tr>
<tr>
<td>urge to urinate</td>
<td>27%</td>
</tr>
<tr>
<td>Polyurea</td>
<td>40%</td>
</tr>
<tr>
<td>Cloudy urine</td>
<td>18%</td>
</tr>
<tr>
<td>Fever</td>
<td>19%</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>29%</td>
</tr>
</tbody>
</table>

The study found that, 20 of 40% of children with inguinal hernia who suffered from UTI were due E. coli with rate 50%, S. aureus with rate 40% and Klebsiella spp with rate 10%, Table 4.

**Table 4: Distribution of bacterial isolates from children with inguinal hernia**

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Children with inguinal hernia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>S. aureus</td>
<td>4</td>
</tr>
<tr>
<td>E. coli</td>
<td>5</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
</tr>
</tbody>
</table>

P<0.01
Table 5 shows that 80% of *E. coli* isolates were sensitive to ampicillin, 60% to amikacin and 40% to Amoxiclave, while 100% of *S. aureus* were sensitive to Ceftriaxone and cand 75% to Cefotaxime.

### Table 5: Rate of antibiotics sensitivity toward isolated *E. coli* and *S. aureus*

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>% of <em>E. coli</em> (No.=5)</th>
<th>% of <em>S. aureus</em> (No.=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>Amikacin</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td>Amoxiclave</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>20</td>
<td>75</td>
</tr>
</tbody>
</table>

**Discussion**

Numerous studies have shown that the results that we have reached in our study are limited by the fact that the most vulnerable children to me hernia are males, especially those over the age of five years (5,7). This is due to the fact that males are more susceptible to movement and activity than females and that ages more than five years are among the factors (8).

The task and influencing the movement of the child, where he has knowledge and love in movement. Going outside the house, which leads to exposure to a hernia in the lower abdomen (9). In view of the prevalence of UTIs in children with inguinal hernia repairs. Different studies also showed that there is a strong relationship between urinary tract infection and people at risk of inguinal hernia (11-13). Several studied also found similar finding as *S. aureus* and *E. coli* were the predominate bacterial isolates of UTI in children (14,15). Other studies indicated that most of *E. faecalis* were resistant to Amikacin (16,17), as another study found that most of *E. faecalis* isolates were resistant to vancomycin and Ceftriaxone with rate reach to 80% (18). The reasons for the high resistance of these bacteria to many antibiotics are due to the fact that they are present and in abundance the medical body as well as the community hospital environment (19). And that the excessive and wrong use of antibiotics in the community has a negative impact on these bacteria and made them resistant to antibiotics, which are used frequently and in excess in all pathological conditions such as urinary tract infection, diarrhea and coughing. To the other by many means, which makes it more harmful than other types that are resistant to antibiotics (20,21). On the related level, there are many studies that prove that most people, especially children who suffer from urinary tract infection, actually suffer from a high temperature, the desire to urinate, in which women who suffer from diabetes do not suffer from frequent urination and fatigue (22,23).

**Conclusions:** It was concluded that, there significant positive correlation between UTIs and inguinal hernia repairs in children.

**Ethical Clearance:** None

**Source of Funding:** None
Conflict of Interest: None

References


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Risk factors of Prostate Enlargement among Patients with History of Lower Urinary Tract Symptoms

Mazin Anwer Yadgar Al-Obaidi¹; Abid Ahmad Salman Al-Mahmood²; Suha Karam Jameel³; Azzawi M Hadi⁴

¹ Assist Prof, Urosurgery, College of Medicine, Tikrit, Iraq; ² Prof Community Medicine, College of Medicine, Tikrit University, Tikrit, Iraq; ³ Specialist, Tikrit Health Center Office; ⁴ Prof College of medicine, Ninevah University, Department of Surgery

Abstract

Background: Prostate enlargement is defined as an increase in size of the prostate gland. The disease may be presented clinically as lower urinary tract symptoms (LUTS). The disease is increasing with age. There are many risk factors as age, genetic, geographical, obesity, physical activity, diabetes mellitus, hypertension.

Subjects and Method: A descriptive study was conducted on patients with lower urinary tract symptoms who were attending Tikrit teaching hospital outpatient’s clinic during the period from 1st July - 1st October 2018. The demographic characteristics of patients were obtained according to a questionnaire and the patients were examined clinically to determine if there was enlargement of prostate or not. The diagnoses was confirmed by PR and ultrasound examination.

Results: The frequency of among sample study was (83%). The cases were more prevalent among the followings: age group 60 years and more (94.3%), positive family history (89.7%), low physical activity (93.4%), hypertensives (87.4%) and with those with erectile dysfunction (94.9%).

Keywords: Prostate enlargement; Risk factors; lower urinary tract symptoms (LUTS)

Introduction

Prostate enlargement means there is an increase in size of the prostate gland. The clinical features include frequent urination, weak stream, loss of bladder control, inability to urinate and difficulty in starting urination (1). This disease may lead to many complications such as bladder stone, infection and chronic renal diseases (2).

The causes are unclear (1). About 105 million men are affected in the world (3). The disease begins after age 40 (1) and half of males age 50 and over are affected (2). and the age after 80 years about 90% of males are affected (1) and reach in 2010 (6%) of population (4, 5, 6).

Prostate enlargement rises markedly with increased age (5-7) and may reach in male freely asymptomatic with age 46 years, the risk of developing the disease in the next 30 years, may reach 45% (8). The older age will be at risk to be affected with clinical features of the disease (9-11).

There are other risk factors other than aging which are positive family history (12-14), obesity (15, 16), diabetes mellitus type 2, hypertension (17), not enough exercise (18, 19) erectile dysfunction (1). Eating red meat, fat, dairy product and starch increase risk while eating vegetables, fruits decrease the risk (19, 20).

Some drugs like calcium channel blockers, anticholinergics and pseudoephedrine may worsen symptoms (2).

Diagnosis is depend on clinical bases which based on lower urinary tract symptoms, digital rectal examination then Ultrasound (2).

Patients and Methods

A descriptive study was conducted on adults attending urology outpatient clinic in Tikrit general hospital. The study started from 1st July - 1st October 2018. The patients were diagnosed clinically by the specialist and sent for
further investigation as ultrasound. The sample study individuals demographic information was obtained according to structured-designed questionnaire and by direct interview. Body weight, height, were measured in addition to obtain from patients other investigation which help in relation with common risk factors.

**Statistical Analysis:** Frequencies, per cent and Chi-square test was used to assess association. Statistical analysis at p-value < 0.05 was considered significant.

**Results**

It has been revealed that the frequency of prostate enlargement among study sample was about (83%) (Fig. 1).

![Fig. 1: Distribution of sample study according to presence of prostate enlargement](image)

The frequency of prostate enlargement was progressively increased with age group, the highest frequency was among age group 60 years and more (94.3%) followed by age group 50-60 years (87.1%) and the less frequency among age group less than 50 years (44.4%) with significant difference.

Regarding the residence factor, there was no statically difference between urban and rural area patients even there was slightly high frequency among rural area (84.7%). There frequency of prostate enlargement cases was more frequent among the patient with a positive family history (89.7%) with a significant difference. Table (1)
Table 1: Distribution of cases according to certain demographic characteristics.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Normal size prostate</th>
<th>Enlarged prostate</th>
<th>Total</th>
<th>Chi square test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 50 years</td>
<td>10 (55.6%)</td>
<td>8 (44.4%)</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>50-59 years</td>
<td>8 (12.9%)</td>
<td>54 (87.1%)</td>
<td>62</td>
<td><strong>P-value is 0.000013.</strong> Significant at <strong>P &lt; 0.05</strong></td>
</tr>
<tr>
<td>60 years and more</td>
<td>2 (5.7%)</td>
<td>33 (94.3%)</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20 (37%)</td>
<td>95 (83%)</td>
<td>115</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Residence</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>11 (19.6%)</td>
<td>45 (80.4%)</td>
<td>56</td>
<td><strong>P-value is 0.534855.</strong> Not Significant at <strong>P &lt; 0.05</strong></td>
</tr>
<tr>
<td>Rural</td>
<td>9 (15.3%)</td>
<td>50 (84.7%)</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20 (37%)</td>
<td>95 (83%)</td>
<td>115</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Family history</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>9 (10.3%)</td>
<td>78 (89.7%)</td>
<td>87</td>
<td><strong>P-value is 0.00044.</strong> Significant at <strong>P &lt; 0.05</strong></td>
</tr>
<tr>
<td>Negative</td>
<td>11 (39.3%)</td>
<td>17 (60.7%)</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20 (37%)</td>
<td>95 (83%)</td>
<td>115</td>
<td></td>
</tr>
</tbody>
</table>

The frequency of cases with prostate enlargement was higher among patients with low physical activity (93.4%) than those with high physical activity (62.5%), the difference was highly significant.

Regarding the presence of obesity, prostate enlargement cases were higher among obese than nonobese patient (83.3%, 82.4% respectively), but there was no significant difference.

Hypertensive patients had more frequent cases than normotensive (87.4%, 60% respectively) and there was a significant difference.

Hypertensive patients had more frequent cases than normotensive (87.4%, 60% respectively) and there was a significant difference.

Regarding presence of diabetes mellitus, prostate enlargement was more frequent among diabetics (83.3%, 82.4% respectively) with no significant difference.

It has been reported that prostate enlargement cases were more frequent among smokers than nonsmokers’ cases (85.2%, 79.6% respectively) but there was no significant difference.

There was a significant relation between erectile dysfunction and presence of prostate enlargement. The frequency of prostate enlargement cases was higher among patients with erectile dysfunction than those without erectile dysfunction (94.9%, 69.6% respectively). Table (2).
Table (2) Distribution of cases according to certain risk factors.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Normal prostate</th>
<th>Enlarged prostate</th>
<th>Total</th>
<th>Chi square test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal prostate</td>
<td>Enlarged prostate</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>Low</td>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 (6.7%)</td>
<td>15 (37.5%)</td>
<td>20 (37%)</td>
<td>P-value is 0.000033. Significant at P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>70 (93.4%)</td>
<td>25 (62.5%)</td>
<td>95 (83%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75 (100%)</td>
<td>40 (100%)</td>
<td>115 (100%)</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>Normal</td>
<td>Obese</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 (17.6%)</td>
<td>4 (16.7%)</td>
<td>20 (37%)</td>
<td>P-value is 0.916148. Not significant at P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>75 (82.4%)</td>
<td>20 (83.3%)</td>
<td>95 (83%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>91 (100%)</td>
<td>24 (100%)</td>
<td>115 (100%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 (12.6%)</td>
<td>8 (40%)</td>
<td>20 (37%)</td>
<td>P-value is 0.003336. Significant at P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>83 (87.4%)</td>
<td>12 (60%)</td>
<td>95 (83%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>95 (100%)</td>
<td>20 (100%)</td>
<td>115 (100%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 (16.7%)</td>
<td>13 (17.8%)</td>
<td>20 (37%)</td>
<td>P-value is 0.87642. Not significant at P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>35 (83.3%)</td>
<td>60 (82.2%)</td>
<td>95 (83%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>42 (100%)</td>
<td>73 (100%)</td>
<td>115 (100%)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 (14.8%)</td>
<td>11 (20.4%)</td>
<td>20 (37%)</td>
<td>P-value is 0.427768. Not significant at P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>52 (85.2%)</td>
<td>43 (79.6%)</td>
<td>95 (83%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>61 (100%)</td>
<td>54 (100%)</td>
<td>115 (100%)</td>
<td></td>
</tr>
<tr>
<td>Erectile dysfunction</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (5.1%)</td>
<td>17 (30.4%)</td>
<td>20 (37%)</td>
<td>P-value is 0.000352. Significant at P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>56 (94.9%)</td>
<td>39 (69.6%)</td>
<td>95 (83%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>59 (100%)</td>
<td>56 (100%)</td>
<td>115 (100%)</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The frequency of prostate enlargement in the current study was about (83%). This frequency is higher to that reported in general population , the prevalence of 8%, 50%, and 80% in the 4th, 6th, and 9th decades of life, respectively(9-11). This result may be attributed to that our study result was obtained from the patients with lower urinary tract symptoms which is considered as a risk factor of prostate enlargements(21-24).

The prostate enlargement cases in this study was increasing with increasing age and it was with high frequency among age group (60 years and more) (94.3%) followed by age (50-59) years (87.1%) with significant difference. This results were similar to studies report that prostate enlargement increasing positively with increased age(25). Many researchers revealed that older age is a risk factor of prostate enlargement(9-11,26-29).

According to residence there were slightly high frequency among rural (84.7%) than urban (80.4%) residence but without significant difference.

There was highly significant difference in the current study between those with positive family history of prostate enlargement and those without for occurrence of the disease. The disease was high frequent among those with positive family history (89.7%) than those without(60.7% ). This result was similar to that documented by others.4,12,14,30,31

Prostate enlargement cases in our study were more frequent among patients with low physical activity (93.4%) than those with high physical activity (62.5%) with significant difference. This results were similar to that reported by other in which they revealed that moderate to vigorous physical activity was a protective factors from the disease19,18

It has been found in the current study that there were no significant difference between obese and non-obese and between diabetics and non-diabetics even that obesity and diabetes mellites are considered as a risk factors of prostate enlargements15,16.

Regarding hypertension disease it was documented that prostate enlargement cases were more prevalent among hypertensive patients (87.4%) than non-hypertensive(60%) with significant difference. This result is going to results which consider hypertension is a risk factor of the disease17.

Regarding smoking habit ,the disease was more frequent among smoker than nonsmoker with no significant difference. This result is with agreement of other studies32-Zheeno found that most of patients with benign prostatic enlargement were nonsmokers.

Regarding erectile dysfunction , it has been reported in the current study that prostate enlargement cases were more frequent among patients with erectile dysfunction (94.9%) than those with normal erectile function (69.6%) with significant difference. This evidence support the relation between erectile dysfunction and prostate enlargements21-24.

Conclusions The current study revealed that there are a significant association between hyperchloremia and hypertension, cardiac diseases and diabetes mellites.

Acknowledgement: The authors are thankful to College of Medicine/Tikrit University for helping to carry this research to a fruitful outcome.

Ethical Clearance: Protocol approval and the Ethical Committee Approval were achieved from the College of Medicine/Tikrit University for the protocol of the study.

Conflict of Interest: The authors declare that there are no conflicts of interest.

Source of Funding: Self-funding.

References


Prevalence of Neonatal Septicemia in Karbala Pediatric Teaching Hospital and Al-alwiyah Pediatric Teaching Hospital, Iraq

Ahmed Salim Hadi Al-Khafaji1; Mohammed Ahmed Jassim Alogaidi2; Anfal Akram Hasan3; Yasir Ayad Khallawi4


Abstract

Background: Neonatal sepsis is a major cause of morbidity and mortality worldwide and especially in developing countries. The incidence of neonatal septicemia varies widely between the developed world and developing countries. Methods: The data was collected from the records of the Department during period (February 2019- December 2020), incubated blood culture at 37°C for 7 days. Subcultures were done onto blood agar and MacConkey agar plates. Results: Clinical feature of neonatal septicemia as Fever 31(67%); Feeding difficulty 22 (48%); both Jaundice and Lethargy as 9(19.5%); also, Diarrhea 7(15%); Skin rash 6(13%) and Meningitis 3(6.5%).so current results showed male with positive bacterial culture (69.5%) when compare with Female (30.5%), Among a total of 46 bacterial isolates recovered, 34 (74%) were Gram-negative isolates more than Gram-positive isolates 12 (26%) , so among a total of 46 bacterial isolates recovered, E.coli were recovered from the cases as (24%) followed by both Klebsiella pneumoniae and Pseudomonas as (13.5%), So Citrobacter species and Proteus mirabilis as (8.5%) , and Strep pneumonia and Staphylococcus aureus were recovered from a single case , Staphylococcus epidermedis (7 cases - 15%). Conclusions: Most clinical feature of neonatal septicemia as Fever; Feeding difficulty; and Jaundice and Lethargy, so the male with positive bacterial culture more than Female as well as gram negative bacteria is more common septicemia children with predominant of E.coli .

Keywords: neonatal septicemia; Karbala Pediatric Teaching Hospital; Karbala.

Introduction

Neonatal sepsicaemia (NNS) may be defined as systemic bacterial infection in a neonate documented by positive blood culture within the first twenty eight days after birth1, or define as the clinical syndrome of bacteraemia with signs and symptoms of infection in the first twenty eight days after birth2.

Neonatal sepsis accounted for 1.4 million neonatal deaths or around 40% of total lives lost, annually 3. About 99% of neonatal deaths occur in low and middle-income countries (LMIC) and approximately 62% occurred during the first 3 days of life 4.

There are two types of neonatal sepsis, early- and late-onset. There is little consensus about applicable age limits in literature5. Usually, the age limit defined for early-onset sepsis varies from 3 to 7 days6. Some clinicians and researchers use 7 days as the limit7. Late-onset sepsis is usually caused by organisms acquired after delivery and considered as nosocomial community-acquired infection8.

Breast feeding is another effective strategy in term and preterm infants that improves cognitive and behavior skills, and decreases rates of infection9. An increase in sepsis caused by Gram-negative organisms has been
reported in recent years\(^{(10)}\). Neonatal sepsis caused by Gram-negative microorganisms is responsible for 18%–78% of all neonatal sepsis\(^{(11)}\). Early-onset neonatal sepsis is caused by microorganisms acquired from the mother before or during birth (vertically transmitted and perinatally acquired); thus, microorganisms from the maternal genital tract may play an important role in early infection\(^{(12)}\).

In the developing world, \textit{Escherichia coli} (\textit{E. coli}), \textit{Klebsiella} species, and \textit{Staphylococcus aureus} (\textit{S. aureus}) are the most common pathogens of EOS, whereas \textit{S. aureus}, \textit{Streptococcus pneumoniae}, and \textit{Streptococcus pyogenes} are the most commonly reported organisms in LOS\(^{(13)}\).

**Patients and methods:**

This retrospective observational study was conducted in the Department of Microbiology in Karbala Pediatric Teaching Hospital and Al-alwiyah Pediatric Teaching Hospital. The data was collected from the records of the Department during period (February 2019- December 2020). 2ml blood drawn under aseptic precautions and inoculated into 20 ml blood culture bottles, these blood culture bottles were incubated at 37°C under aerobic conditions in the incubator for 7 days. The first subculture was done after 24 hours of incubation, the second on the third day and a final on the seventh day. Subcultures were done onto blood agar and MacConkey agar plates. The inoculated plates were incubated aerobically at 37°C for 24 hours, and the plates were observed for growth. The growth was identified by colonial characteristics, gram’s stain and standard biochemical tests as well as used Vitek system for identified bacterial isolates.

**Results**

Table (1): Common Clinical features of Neonatal Septicemia (46 case)

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>31</td>
<td>67</td>
</tr>
<tr>
<td>Feeding difficulty</td>
<td>22</td>
<td>48</td>
</tr>
<tr>
<td>Jaundice</td>
<td>9</td>
<td>19.5</td>
</tr>
<tr>
<td>Lethargy</td>
<td>9</td>
<td>19.5</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Skin rash</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Meningitis</td>
<td>3</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Results in table 2 showed clinical feature of neonatal septicemia as Fever 31(67%); Feeding difficulty 22 (48 %); both Jaundice and Lethargy as 9(19.5%); also, Diarrhea 7(15%); Skin rash 6(13%) and Meningitis 3(6.5%).
Table (2): Bacterial culture according to gender patients of Neonatal Septicemia

<table>
<thead>
<tr>
<th>Gender</th>
<th>Positive bacterial culture</th>
<th>Negative bacterial culture</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>Male</td>
<td>32</td>
<td>69.5</td>
<td>18</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>30.5</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>100</td>
<td>25</td>
</tr>
</tbody>
</table>

This table (2) showed male with positive bacterial culture (69.5%) when compare with Female (30.5%).

Table (3): Types of bacterial isolate from patients of Neonatal Septicemia

<table>
<thead>
<tr>
<th>Number</th>
<th>Type of bacteria</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>Gram negative bacteria</td>
<td>74</td>
</tr>
<tr>
<td>12</td>
<td>Gram positive Bacteria</td>
<td>26</td>
</tr>
<tr>
<td>46</td>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

Among a total of 46 bacterial isolates recovered, 34 (74%) were Gram-negative isolates more than Gram-positive isolates 12 (26%) were (table-3).

Table (4): Distribution of isolated bacteria according to gram stain

<table>
<thead>
<tr>
<th>Name of bacterial isolate</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram negative bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>11</td>
<td>24</td>
</tr>
<tr>
<td>Klebsella pneumonia</td>
<td>6</td>
<td>13.5</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>6</td>
<td>13.5</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>4</td>
<td>8.5</td>
</tr>
<tr>
<td>Citrobacter species</td>
<td>4</td>
<td>8.5</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>3</td>
<td>6.5</td>
</tr>
<tr>
<td>Total Gram negative bacteria</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Gram positive bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strep pneumonia</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus hemolyticus</td>
<td>3</td>
<td>6.5</td>
</tr>
<tr>
<td>Staphylococcus epidermedis</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Total Gram positive bacteria</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>100</td>
</tr>
</tbody>
</table>
Among a total of 46 bacterial isolates recovered, *E. coli* were recovered from the cases as (24%) followed by both *Klebsiella pneumoniae* and *Pseudomonas* as (13.5%), So *Citrobacter species* and *Proteus mirabilis* as (8.5%), and *Strep. pneumonia* and *Staphylococcus aureus* were recovered from a single case, *Staphylococcus epidermedis* (7 cases - 15%) (Table-4).

**Discussion**

Data from developing countries shows variable prevalence of Gram negative and gram-positive organisms in neonatal septicemia. Some have predominant gram-negative isolates (14), others show Gram positive isolate predominance (15&16). During lysis, Gram-positive bacteria release peptidoglycans whilst the Gram-negatives release lipopolysaccharides-A (LPS-A) or endotoxins. These substances initiate a cascade of events that lead to the sepsis syndrome, septic shock, multiple organ failure and death. Bacterial fragments, endotoxins and/or exotoxins stimulate monocytes and neutrophils to produce inflammatory mediators (17).

The reason for male preponderance is unknown, but this could be due to sex-dependent factors (18). The synthesis of gamma globulins is probably regulated by X–linked immuno regulatory genes and as males are having one X chromosome, they are more prone for neonatal septicemia than females (19). Previous study as Aletayeb et al., Celicia et al., Rabia et al., and Ahmad et al., have reported higher number of male neonatal septicaemia than female neonatal septicaemia (20 ;21;22).

A study by Ojukwu reported Gram-positive organisms as the predominant with Staphylococcus aureus accounting for 45% while for Gram-negative, *E.coli* accounted for 18.2% (23). Although Gram-positive organisms are the most common causes of nosocomial blood stream infections, Gram-negative bacteremia carries higher risks of severe sepsis, septic shock, and death. Of these, one-third were caused by gram negative bacilli and 70 (18.6%) were multidrug resistant (24). Some of the most frequently isolated bacteria in sepsis are *Staphylococcus aureus* (*S. aureus*), *Streptococcus pyogenes* (*S. pyogenes*), *Klebsiella spp.*, *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*P. aureginosa*) (25). Also Hornik et al., showed most commonly bacterial isolate as GBS, Escherichia coli, CONS, *Haemophilus influenzae* and Listeria monocytogenes (26).

Sundaram et al., (27) records *S. aureus* was (22%), *Klebsiella spp.* (18%) and NFGO were (17%). Other organisms in decreasing frequencies were Enterobacter spp. (11%), *E. coli* (9%), *Acinetobacter spp.* (7%), CONS (6%), and *Pseudomonas spp.* (3%). So Kaith et al., 2010 (28) found that CONS were 41%, NFGO were 27% and Klebsiella were 18% of the total isolates, So Al-Shamahy et al., from Yemen had 97% Gram negative isolates. Klebsiella spp. (36%) and *Pseudomonas spp.* (30%) were the two commonest isolates (29).

**Conclusions**

1) Most clinical feature of neonatal septicemia as Fever; Feeding difficulty; and Jaundice and Lethargy.

2) Male with positive bacterial culture more than Female.

3) Gram negative bacteria is more common septicemia children with predominant of *E. coli*.

**Ethical Clearance:** None

**Source of Funding:** Self.

**Conflict of Interest:** None

**References**


8) Vohr , BR ; Poindexter, BB. Dusick; AM et al., Persistent beneficial effects of breast milk ingested in the neonatal intensive care unit on outcomes of extremely low birth weight infants at 30 months of age Pediatrics.2007;120: e953-9.


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Seroprevalence of BKV-Antibodies among Cancer Patients and Healthy Individuals

Vian Ibraheem Husain¹, Salah Mahdi Hassen², Amal Aziz Kareem²
¹Msc Student, Health and Medical Technical College, Kirkuk. Northern Technical University, ²Assistance Professor, College of Health and Medical Technology
Baghdad. Middle Technical University

Abstract

BKPyV, a small DNA virus classified into the polyomaviridae family, is holoendemic with global distribution among human population. The objective of this study is to determine the prevalence of anti-BK polyomavirus IgG antibodies among cancer patients and healthy controls. In the present study, A total of 85 serum samples were collected (65 cancer patients and 20 healthy controls) with age range between 15-75 years, the female-to-male ratio was 3:1. All obtained sera samples were tested using ELISA method for the detection of anti-BK polyomavirus IgG antibodies. BKV-IgG seroprevalence show high rate of exposure to the virus 48.23% (41 of 85 samples) revealed positive result (50.77% in cancer patient and 40% in healthy control). Seroprevalence was not different between female and male. The frequency of IgG antibodies decreased from 66.67% in the age group <40 years to 46.94% among age group 40-60 years, and drop again to 38.1% in >60 years old.

Key words: BK polyomavirus, seroprevalence, cancer, ELISA, Kirkuk.

Introduction

Human polyomaviruses (HPyVs) are recognized as a source of benign initial infection during early age. These viruses are highly prevalent in the population with frequent incidences of reactivation and pathologic consequences in elderly and immunosuppressed patients [1]. HPyVs are a growing challenge in those patients in view of the increasing number of currently 12 HPyV species and their various diseases potential [2].

BKPyV represent one of the first two HPyVs that have been identified. It was isolated from the urine and ureteral epithelial cells of a Sudanese renal transplant patient with renal failure and ureteral stenosis [3]. BKV is a small, non-enveloped DNA tumor virus with icosahedral capsid and small, circular and double-stranded DNA [4, 5, 6]. Primary infection with BKPyV usually occurs during early childhood, after waning of the maternal antibodies. In children under the age of 10 years, the seroprevalence rises to 91% and the overall frequency of seropositivity for the virus is 81% [7].

After BKPyV primary infection, the virus establishes a lifelong infection in the renourinary tract [8, 9, 10]. The infection is completely asymptomatic in most immunocompetent individuals, but frequent episodes of viral reactivation can occur and the virus shed into the urine [11, 12]. In seropositive individuals with altered immunity and immunocompromised patients, viral reactivation is followed by high-level of replication and this can result in severe diseases such as polyomavirus-associated nephropathy and hemorrhagic cystitis [12, 13].

Several early studies on the seroprevalence of BKPyV were done using indirect immunofluorescence (IFA), complement fixation assay (CFA), or immunolectroosmophoresis (IEOP). Though, the hemagglutination inhibition assay (HIA), has been used for most of the seroprevalence studies with human polyomaviruses [14]. HIA have been now mostly replaced by ELISA techniques for the detection of antibodies against BKPyV capsid protein using recombinant proteins as antigens. Virus-like particles are produced by expressing the VP1 protein in eukaryotic expression
systems or expressed in *E. coli* as a glutathione-S-transferase (GST) fusion protein \[^{[15]}\].

**Methodology**

**Specimen Collection:** This study was carried out in Kirkuk Specialist Center for Oncology and Hematology, during the period February 2020 to September 2020 and included 85 individuals (65 cancer patients and 20 controls). Single serum specimen were collected and stored at – 20 °C until processed.

**Methods:** To detect the presence of BKV-IgG in the serum, Human BK Virus IgG (BK-IgG) ELISA Kit (Abbkine\China, **item no.** KTE63708) was used according to manufactures instructions with a cutoff concentration 3.43 pg/ml.

**Results**

Table (1) shows the result of IgG ELISA test among the two groups and demonstrates that 33 (50.77%) of cancer patient and 8 (40%) of healthy control revealed positive results while 32(49.23%), 12 (60%) of cancer patients and healthy control, respectively showed negative results. The results show no significant difference at (P>0.05).

<table>
<thead>
<tr>
<th>IgG ELISA results</th>
<th>Study Groups</th>
<th>Cancer patients</th>
<th>Healthy controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>33</td>
<td>8</td>
<td>41</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td>50.77%</td>
<td>40%</td>
<td>48.24%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>12</td>
<td>44</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td>49.23%</td>
<td>60%</td>
<td>51.76%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65</td>
<td>20</td>
<td>85</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

P-value : 0.39 > 0.05 (NS) *

*BKV IgG antibodies were found in the serum samples of 41 (48.24) of samples tested, 12 (57.14%) of males and 29 (45.31%) of females (Table 2) with no significant difference at P.value> 0.05.

Table 2: Sex dependent prevalence of BKV antibodies.

<table>
<thead>
<tr>
<th>IgG ELISA results</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>12</td>
<td>29</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>57.14%</td>
<td>45.31%</td>
<td>48.24%</td>
</tr>
<tr>
<td>Negative</td>
<td>9</td>
<td>35</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>42.86%</td>
<td>54.69%</td>
<td>51.76%</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>64</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

P-value = 0.3 >0.05 (NS) *
The age-distribution of BKV-IgG is shown in Table (3). The frequency of IgG antibodies was 23 (46.94%) among age group 40-60 years, 10 (66.67%) in the age group <40 years and 8 (38.1%) in >60 years old. The results show no significant difference at (P>0.05).

### Table 3: Age dependent prevalence of BKV antibodies

<table>
<thead>
<tr>
<th>IgG ELISA results</th>
<th>Age</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;40</td>
<td>40-60</td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>66.67%</td>
<td>46.94%</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>33.33%</td>
<td>53.06%</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

P.value = 0.2 >0.05 (NS) *

### Discussion

BK polyomavirus (BKPyV), a member of the family Polyomaviridae, comprises dsDNA viruses [16]. The virus infect about 90% of human populations at early life and remains latent in the epithelium cells of the proximal tubule of the kidney and the urothelial cells of the bladder, ureters, renal pelvis and other tissue types [17]. The viral reactivation and replication occur when individuals become immunosuppressed or immunocompromised due to cancer, diabetes, pregnancy, HIV-infection, organ or bone marrow transplantion[18].

BKPyV seroprevalence is the lowest at the age of 6 months after losing maternal antibodies. Serological studies reported 50% seropositive rate in children aged 3–4 years old, suggesting that 50% of children had been infected with BKPyV. The seropositive rate rises from 50% to about 90% in adult population. In healthy population, 5% individuals show low-level BKPyV replication with asymptomatic viruria. During adulthood, a gradual drop in antibody prevalence was reported in some studies. This was possibly due to the decrease in antibody titer which was undetectable using hemagglutination inhibition assay or enzyme link immunosorbent assay. This phenomenon revealed a lack of BKPyV antigenic stimulation in older immunocompetent individuals [19].

Reports show that positive serology for BKPyV infections in adults ranged between 60% and 100% [18, 20, 21].

Antibody seroprevalence has been reported by Stolt et al, 2003 in 290 Swedish children aged (1-13 years), grouped by age in 2 year intervals, revealed that seropositivity increased with increasing children’s age, reaching 98 % at (7-9 years) of age, followed by a minor decline in seropositivity [22].

In a study by Knowles et al, 2003 from individuals aged 1–69 years. 81% of sera tested for antibody to BKV revealed positive result and the prevalence varied significantly with age (P < 0.001). 64% of children in the age group of (1–4 years) showed positive results, with rise in the figure to 91% at (5–9 years). This seroprevalence was sustained until around 40 years of age with subsequent decline in the frequency of detectable antibody to 68% in the age group (60–69 years)[7].

In a study by Egli, et al., 2009 included the analysis of serum samples from 400 healthy blood doners. IgG
seroprevalence was 82% for BKV. The seroprevalence decreased from 87% in the youngest group (aged 20 –29 years) to 71% in the oldest group (aged 50 –59 years) (P <0 .006). No significant difference was present between female and male (83% vs. 82%; P > 0.795) [13]. Another study by Kean et al, 2009, found that 82% of 1501 healthy blood donors over the age of 21 revealed antibodies to BKV and 73% of 721 study subjects under the age of 21 was positive [23].

Ethical Clearance: None

Source of Funding: Self.

Conflict of Interest: None

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Evaluation the Role of Malondialdehyde in Myocardial Infarction Patients

Dina A. Ibrahim¹, Sami A. Zbbar², Muhib A. Salih³

¹MSC Student, Kirkuk Health Directorate, Kirkuk City, Iraq; ²Assist Prof. Department of Biochemistry, College of Medicine, Tikrit University, Iraq; ³Prof. Department of Internal, College of Medicine, Tikrit University, Iraq

Abstract

Case control study was carried out in Kirkuk city in the intensive care unit in Kirkuk general hospital from Feb 2020 to May 2020. The study included 90 individuals including sixty myocardial infraction patients and thirty healthy individuals to evaluate the level of Malondialdehyde (MDA) in myocardial infraction patients and healthy controls. All patients were aged between (40-80) years, with a negative family history of heart attack. Blood samples were collected from each patients and controls to evaluate the levels of malondialdehyde by using immunofluorescence technique. The study showed no significant differences between myocardial infraction patients and control groups regarding their age, although 33.33% of MI patients belonged to the age group 60-69 year flowed by 28.33% in the age group 50-59 year. The study displayed that the highest mean of MDA was recorded in MI (104.7±13.0 ng/ml) as compared with the control group (66.7± 22.7 ng/ml), The result was significant (P: < 0.05). The study concluded that, the MDA levels were elevated significantly in patients group as compared with the control group and MDA is may be an important cardiovascular parameter, which may be followed before and during the disease.

Keywords: Malondialdehyde; Myocardial infraction; Oxidative stress.

Introduction

Myocardial infarction (MI) is heart muscle damage that results from interruption of tissue blood flow caused by occlusion of coronary arteries due to thrombus. It potentially causes permanent damage (myocardial tissue death) unless there is prompt intervention to open up the coronary artery either by percutaneous or surgical methods¹. According to the World Health Organization (WHO), 17.9 million people die each year from cardiovascular diseases (CVDs), an estimated 31% of all deaths worldwide. This implies that CVDs are the first cause of death globally with heart attacks and strokes producing 85% of all CVD deaths². The most popular form of coronary heart disease is myocardial infarction and is responsible for over 15% of mortality each year³. Unlike the other type of acute coronary syndrome, unstable angina, a myocardial infarction occurs when there is cell death, diagnosis of MI is dependent on the sensitivity and specificity of the clinical criteria, electrocardiographic findings, imaging studies and biomarkers (such as cardiac troponins)⁴. Oxidative stress plays a crucial role in the development of endothelial dysfunction and is a potent modulator of the inflammatory response, cell growth and differentiation, apoptosis and changes in vascular tone⁵. Studies have demonstrated that the excessive activation of lipid peroxidation has a key role in the development of many diseases such as angina and MI. This is because the lipid peroxidation is a chain of reactions providing a continuous supply of free radicals that increase further peroxidation⁶,⁷. Malondialdehyde (MDA) is currently considered to the most widely used representative of oxidative lipid damage⁸. The best evidence of lipid peroxidation is the increased formation of malondialdehyde (MDA) which is one of the principal breakdown products by the action of endoperoxidase, and hence the determination of MDA has been widely used in human studies to prove the involvement of lipid
peroxidation in various diseases. A number of studies reported the elevated level of serum MDA in heart diseases, indicating a link between oxidative stress and AMI\(^9,10\). The study aim of this work was to evaluate the level of Malondialdehyde (MDA) in Myocardial infarction patients and healthy controls.

**Patients and methods**

Case control study was carried out in Kirkuk city, in the intensive care unit in Kirkuk general hospital from Feb 2020 to May 2020. The study included 90 individuals including sixty myocardial infarction patients and thirty healthy individuals to evaluate the level of Malondialdehyde (MDA) in myocardial infarction patients and healthy controls. The information about patients in this study was retrieved from patient’s itself. The myocardial infarction patients were diagnosed by Clinical features, Electrocardiographic (ECG) findings, Elevated values of biochemical markers such as (Cardiac troponin). All patients were aged between (40-80) years. The criteria of exclusion include negative family history of heart attack. The results of the patients groups were compared with healthy individuals nearly comparable age and. About five milliliters of blood were collected from the antecubital vein of patients and controls in plain tubes without any anticoagulant at room temperature for 10-15 minutes and allowed to clot. The tube then were centrifuged (3000 rpm) for 15min. The clear serum was pipetted into clear dry Eppendorf’s and stored at (-20°C) until used for the various investigations. The level of malondialdehyde, was measured by using immunofluorescence technique.

**Results**

The study showed no significant differences between AMI patients and control groups regarding their age, although 33.33% of AMI patients belonged to the age group 60-69 year flowed by 28.33% in the age group 50-59 year, Table 1.

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>MI patients (n:60)</th>
<th>Control group (n:30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>40-49</td>
<td>12</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>50-59</td>
<td>17</td>
<td>28.33</td>
<td>11</td>
</tr>
<tr>
<td>60-69</td>
<td>20</td>
<td>33.33</td>
<td>5</td>
</tr>
<tr>
<td>70-80</td>
<td>11</td>
<td>18.34</td>
<td>6</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>58.4±10.8</td>
<td>55.4±10.9</td>
<td>0.22</td>
</tr>
</tbody>
</table>

**Serum Malondialdehyde (MDA) Level Comparison Between Patients Group and Control Group.**

The study displayed that the highest mean of MDA was recorded in MI (104.7±13.0 ng/ml) as compared with the control group (66.7± 22.7 ng/ml). The result was significant (P: < 0.05), as shown in the Table 2. and Figure 1.
### Table 2.: Serum Malondialdehyde Level Comparison Between Patients Group and Control Group.

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (ng/ml)</th>
<th>Mean</th>
<th>SD</th>
<th>Max</th>
<th>Min</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI Patients Group</td>
<td>104.7</td>
<td>13.0</td>
<td>143.79</td>
<td>83.145</td>
<td></td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Control Group</td>
<td>66.7</td>
<td>22.7</td>
<td>100.918</td>
<td>18.87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** : Serum Malondialdehyde Level Comparison Between Patients Group and Control Group in (ng/ml).

**Discussion**

The study showed no significant differences between AMI patients and control groups regarding their age, although 33.33% of AMI patients belonged to the age group 60-69 year flowed by 28.33% in the age group 50-59 year. In agreement with these finding, previous research has found that older age is associated with a higher prevalence of AMI presentation\(^\text{11}\). The elderly with acute myocardial infarction (AMI) have been reported to present with more atypical symptoms\(^\text{12,13}\). AMI is associated with significantly higher mortality in the elderly compared with the young, yet the elderly are treated less aggressively than the young\(^\text{14}\). Another study found that, elderly patients, those 65 years of age and older, represent 13% of them yet account for half of hospital admissions for acute myocardial infarction (AMI) and 80% of AMI deaths\(^\text{15}\). Despite the greater risk of AMI among older patients and the increasing size of this population, the relationship between age, clinical presentation and outcome of AMI in elderly patients is incompletely understood. Many prior observational studies and clinical trials have classified elderly patients as a single population, not specifically evaluating differences in older subgroups, particularly those 75 years of age and older\(^\text{16-17}\). In the elderly, numerous disorders often coexist. Ischemic heart disease, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, chronic renal failure, digestive system disorders, as well as, joint and bone disorders occur more often in this group of patients. The coexistence of several diseases may cause the clinical picture of acute coronary syndrome to be uncharacteristic\(^\text{18,19}\). The study displayed that the highest mean of MDA was recorded in MI (104.7±13.0 ng/ml).
ng/ml) as compared with the control group (66.7 ± 22.7 ng/ml). The result was significant (P: < 0.05), as shown in the Table (2.) and Figure (1.). In agreement with this finding, Yin et al. (11) found that, circulation MDA was significantly increased in patients with acute myocardial infarction as compared with healthy individuals. Majid (12) showed that, patients with acute myocardial infarction have a significant increase in serum MDA comparing with healthy persons. Additionally, Lopes-Virella et al. (13) stated that, levels of circulated MDA showed a significant increase in MI patients when compared with normal persons. Several studies also indicated significant increase in MDA levels in AMI patients as compared to controls (14-16). Another study on 22 AMI patients and 15 controls has found serum MDA levels significantly elevated (20). While in AMI patients a significant increase in serum MDA was observed in the days following the acute event, reaching a maximum 6-8 days later, when 90% of the patients had values higher than the upper normal limit of the control group (17). Another study showed that, the levels of thiobarbituric acid reactive substances (TBARS, predictor of MDA) were significantly increased and total antioxidant status was significantly decreased in AMI (18). Moreover, Bagatini et al. (19) have demonstrated a significant increase in MDA levels and a decrease in nonenzymatic antioxidants such as vitamin C and vitamin E levels in 40 AMI patients when compared with the same number of normal subjects. The significantly higher level of MDA in patients with unstable angina and myocardial infarction than in the control group were attributed to significantly higher level of serum cholesterol, high blood pressure, smoking and increased BMI in patients (21). Oxidative stress has been regarded as one of the most important contributors to the progression of atherosclerosis (13). MDA levels increased following by oxygen free radicals accumulation, which lead to lipid peroxidation in cell membranes, acute cardiac injury, mitochondrial function impairment and reduced myocardial systolic function, and eventually induced AMI (12,19).

Conclusions

The study displayed that the MDA levels were elevated significantly in patients group as compared with the control group and MDA is may be an important cardiovascular parameter, which may be followed before and during the disease.

Ethical Clearance: None

Source of Funding: Self.

Conflict of Interest: None

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Assessment of the Coverage Rate of Expanded Program of Immunization among Five Years Children in Tikrit City – Iraq

Hala Mohammad Yosif1, Nisreen Mohammed Ibraheem2
1Physician, 2Prof. Community Medicine Department, Medical of College, Tikrit University, Salahaddin, Iraq

Abstract

Background: Childhood immunization is the initiation of immunity through the application of vaccines. It is considered important for improving child survival. Immunization is a primary health care preventive measure and remain the most cost-effective public health intervention to reduce child morbidity and mortality attributed to infectious diseases. Subjects and Method: Cross sectional study was conducted on five year children who attended (8) kindergarten in Tikrit city and their information from PHCC sector. Children were selected by cluster sample then simple random from each cluster. The study was conducted in the period from November 2019 to August 2020. Results: The study revealed a significant inverse relation was observed between household crowding and socioeconomic status children’s parents. The percentage of full immunized children (40.90%) these children were considered as having had complete immunization, but more than half of the children had one or more than one missed doses and were considered as is having had partial immunization (56.25%). The number of unvaccinated children were five children (2.84%). Complete immunization of children were found among male (54.77%), while complete immunization among children female were (28.26%) and there strong association statistically. The partial immunization among male children were (39.28%) and female were (71.74%) the coverage rate of vaccination among immunized children the coverage rate was (97.2%). The highest for immunized children was for the vaccines Bacillus Calmette Guerin (BCG*)+OP90+first dose of Hepatitis B Virus vaccine (HBV1) (92.04%) while the lowest rate (6.0%) was for the vaccines OPV2booster+MMR+TETRA (DTaP +IVP ). Conclusion: this study concluded that the highly percentage of partially immunized children in kindergarten (56.25%).

Key Word. Bacillus Calmette Guerin (BCG), Oral polio vaccine (OPV), Hepatitis B Virus vaccine (HBV1), Measles Mumps Rubella (MMR), Expanded Programm Of Immunization (EPI).

Introduction

Vaccination is that the administration of agent-specific, however comparatively harmless, substance elements that in immunized people will induce protecting immunity against the corresponding infective agent. Clinically, the terms “vaccination” and “immunization” area unit usually used interchangeably(1). Vaccination could be a extremely effective technique of preventing bound infectious diseases. Vaccines area unit usually safe, and heavy adverse reactions area unit uncommon(2,3). Routine protection programs shield most of the world’s children from variety of infectious diseases that antecedently caused millions deaths annually(4). Several years after wars have ended, children living in war and conflict settings are at risk of developing immunogenic preventable and infectious diseases. Except in times of war, immunogenic campaigns are common(5), but the environment remains conducive to the spread of communicable disease epidemics. Vaccine shortages, problems with the cold chain for immunogenic fidelity, and the inability to locate and inoculate children all result from the war (especially those not in their original families or who are wounded)(6).

Subject and Methods

Study sampling.

Cross sectional study was conducted on five year children who attended (8) kindergarten in Tikrit city and...
their information from PHCC sector. The study included 176 children at five years old who attended kindergarten and their parent and caretaker agreed to take part in the study. Children selected by cluster sample then simple random from each cluster.

**Data Collection and Analysis.**

A suitable questionnaire designed to collect the related information to immunization coverage particularly EPI. Questions included opened and closed questions, demographic information for the child as (age, weight, residence, birth order, place of delivery number of living children in a household, medical history of the child) and Demographic data of the parents like (educational level of the father/mother, occupation of father/mother, socioeconomic status for the family, source of the information about immunization).

**Result**

Regarding to the coverage rate of vaccination among immunized children the coverage rate were 171 (97.2%) while non-immunized children were 5 (8.3%) (figure 1). The highest for immunized children was for the vaccines Bacillus Calmette Guerin (BCG)+OP0+first dose of Hepatitis B Virus vaccine (HBV1) 162 (92%) while the lowest rate (6.0%) was for the vaccines OPV2booster+MMR+TETRA (DTaP +IVP)

![Figure (1) coverage rate of vaccination among children](image)

However the coverage rate decrease for the vaccine OPV2 + Hexa2+ Rota2+ Pneumococcal2 139 (79%), OPV3 + Hexa3+ Pneumococcal3 116 (66%), then increase for the Measles vaccine and MMR (154 (87.5%), 22 (83%) respectively, coverage rate decrease for the vaccine OPV2booster+MMR+TETRA (DTaP +IVP) 72 (41%). Table (1)

Figure (2) show the immunization status for the children. Seventy two of the children were immunized with all vaccination doses; 23 (41%) these children were considered as having had complete immunization, but more than half of the children had one or more than one missed doses and were considered as is having had partial immunization 44 (56%). The number of unvaccinated children were 5 (3%).
Table (1) Distribution of vaccination among 176 immunized children

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
</tr>
<tr>
<td>BCG+OPV0+HBV1</td>
<td>162 (92%)</td>
<td>14 (8%)</td>
</tr>
<tr>
<td>OPV1 + Hexa + Rota1 + Pneumococcal1</td>
<td>144 (81.8%)</td>
<td>32 (18.2%)</td>
</tr>
<tr>
<td>OPV2 + Hexa2 + Rota2 + Pneumococcal2</td>
<td>139 (79%)</td>
<td>37 (21%)</td>
</tr>
<tr>
<td>OPV3 + Hexa3 + Pneumococcal3</td>
<td>116 (66%)</td>
<td>60 (34%)</td>
</tr>
<tr>
<td>Measles</td>
<td>154 (87.5%)</td>
<td>22 (12.5%)</td>
</tr>
<tr>
<td>MMR</td>
<td>146 (83%)</td>
<td>30 (17%)</td>
</tr>
<tr>
<td>OPV 1st booster + TETRA 2nd booster</td>
<td>77 (43.8%)</td>
<td>99 (56.2%)</td>
</tr>
<tr>
<td>OPV2 booster+MMR+TETRA (DTaP+IVP)</td>
<td>72 (41%)</td>
<td>104 (59%)</td>
</tr>
</tbody>
</table>

Figure (2) Immunization status among 5 years children in Tikrit city
A higher percentage of complete immunization of children were found among male 46(54.8%). The partial immunization among female children were 66(71.7%) this finding was very strong association statistically (significant) (P=<0.05) Table (2).

Table(2) The relation between vaccination state and gender of children

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number (%)</th>
<th>Vaccination status</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Complete</td>
<td>Partial</td>
<td>Not vaccinated</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>84 (47.7%)</td>
<td>46(54.8%)</td>
<td>33(39.3%)</td>
<td>5(5.9%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>92 (52.3%)</td>
<td>26(28.3%)</td>
<td>66(71.7%)</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>176(100%)</td>
<td>72(100%)</td>
<td>99(100%)</td>
<td>5(100%)</td>
<td></td>
</tr>
</tbody>
</table>

Chi=21.2 d.f=2 correlation =0.3 p -value <0.05

The result showed that most of children had have this card 159(90%) but the percentage of children without this card 17(10%).

The study found that 166 (94.3%) of fully immunized children were from urban area and 10(6.7%) from rural area. The finding was statistically not significant at P value <0.05.

Table(3) shows the relationship between immunization status and the Socioeconomic level of the families according to the level of the education, occupation of the parents and the household crowding index for these families. The percentage of the complete immunization among high Socioeconomic level 40% and 35% among low Socioeconomic level. This finding was insignificant statistically (P=<0.05).

Table 3 Distribution of immunization status according to the Socioeconomic level

<table>
<thead>
<tr>
<th>Socioeconomic level</th>
<th>Immunization status</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete immunization</td>
<td>Partial immunization</td>
<td>No immunization</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>32(40%)</td>
<td>48(60%)</td>
<td>00</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>27(45.7%)</td>
<td>29(49.2%)</td>
<td>3(5.1%)</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>13(35.1%)</td>
<td>22(59.4%)</td>
<td>2(5.5%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>72(40.9%)</td>
<td>99(56.3%)</td>
<td>5(2.8%)</td>
<td></td>
</tr>
</tbody>
</table>

Chi=5.6 d.f.=4 P value=0.05 correlation =0.2 not significant association.
The reason behind delay or incomplete immunization 25(23.9%) because lack of time for vaccination by the parents or less frequent reasons were absence of vaccination or unavailability of vaccines 6(5.9%) for reach (Table-(4))

<table>
<thead>
<tr>
<th>Causes</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of time for vaccination</td>
<td>25 (23.9%)</td>
</tr>
<tr>
<td>Causes not given</td>
<td>22(20.9%)</td>
</tr>
<tr>
<td>Afraid from Side effect of vaccine</td>
<td>16(15.4%)</td>
</tr>
<tr>
<td>Ignorance</td>
<td>11(10.9%)</td>
</tr>
<tr>
<td>Ill child</td>
<td>9(8.5%)</td>
</tr>
<tr>
<td>Mother travel with her child</td>
<td>9(8.5%)</td>
</tr>
<tr>
<td>Absence of vaccination card</td>
<td>6(5.9%)</td>
</tr>
<tr>
<td>Unavailability of some vaccines</td>
<td>6(5.9%)</td>
</tr>
</tbody>
</table>

**Discussion**

The highest coverage rate for children in this study was for vaccines BCG+OPV0+HBV1. This is may be attributed to the fact that more than three fourth of the children were born in hospitals where these vaccine doses were given soon after birth. This is in agreement with Maheshwari et al in India\(^7\), study in 2014 which had the highest coverage rate for the same vaccine doses, also agree with study by Al-Kazrajy and et al, which conducted on the displaced Iraqi children\(^8\).

Coverage rate for poliovirus vaccine was ≥65% for ≥3 doses of vaccine, this percentage are lower than the National Vaccination Coverage in the America which reach to (92.7%) for more than three doses\(^9\), but similar to this finding with other study was conducted in Africa\(^10\).

Measles and rubella elimination remain a top WHO priority\(^94\). The coverage rate for measles vaccine in current study reach to 87.5%. This finding are agree with Al-Kazrajy and et al study\(^8\) and many study conducted in the many countries\(^11\). The explanation for this finding return to some studies which proved that one-dose vaccines have high coverage of vaccination compared to more than one dose vaccines, which may be due to shortages and delays in completing immunizations on time.

This finding are similar to many studies in the world like the study by Rahman M, Obaid from Bangladesh which revealed the male children were more likely to be fully immunized than female\(^12\). But disagree with the another studies from Iraq by Omer Qutaiba B Al- lemon and et al\(^13\), which mention that many studies in different countries, such as Nigeria and Bangladesh, have found no significant connection between gender and immunization completeness\(^13,14\).

When children are not vaccinated according to the prescribed schedule, they not only miss out on timely protection from preventable diseases at a time when they are most vulnerable, but they also increase their chances...
of never finishing the course. Any of these results jeopardize the effectiveness of a childhood vaccination program\textsuperscript{(15)}.

It’s likely that partial immunization is attributable to a lack of vaccination awareness among parents or providers. Inadequate vaccination information can result in inconvenient timing or missing immunizations, resulting in less disease safety, more side effects, and higher costs\textsuperscript{(16)}.

Furthermore, partial immunization may have occurred as a result of the immunization card or health reports failing to provide a consistent and accurate record of immunization. The immunization card is essential for the immunization provider to know which vaccines are due at the time of the child’s visit. Furthermore, the immunization card is mandatory for both father and mother to be capable to assess or verify their child’s immunization status\textsuperscript{(16)}.

Forty percent of children with high socioeconomic status were fully immunized. This finding was agree with study by Noh J-W, and et al from Pakistan\textsuperscript{(17)} Level of Socioeconomic status for the families in this study were determined by the level of education , occupation of parents and household crowding index. Previous research has found a link between living in wealthier households and having complete full immunization coverage\textsuperscript{(18,19,20)}, which is consistent with a Bangladeshi study that found a positive impact of high socioeconomic status on full immunization coverage\textsuperscript{(12)}. Perhaps one explanation for this result is that income no longer has as much of an impact on full immunization coverage as it once did since immunization offered by the EPI program is free, and public efforts to reach poor mothers and infants are still ongoing.

So the effort made by Iraqi Ministry of Health through vaccination campaign have contributed to the delivery of vaccines to as many children as possible. Incomplete immunization for children with high socioeconomic status may be return to the lack of time due to employed parents and education.

**Ethical clearance:** None

**Source of funding:** Self.

**Conflict of Interest:** None

**References**


Factors Affecting the Severity of RH Incompatibility Newborn

Nfal Akram Hasan1, Mohammed Ahmed Jassim Alogaidi2, Areej Ali Abbood3

1Pediatrics Specialist, CABP Pediatrics Al-alwiyah Pediatric Teaching Hospital, Baghdad-Alrusafa Health Directorate, 2Pediatrics Specialist F.I.B.M.S pediatrics, Al-alwiyah Pediatric Teaching Hospital, Baghdad-Alrusafa Health Directorate/ Ministry of Health and Environment; 3Gynecologist Specialist FICOG CABOG, Al-elwiyah Maternity Hospital, Baghdad- Alrusafa Health Directorate/ Ministry of Health and Environment / Baghdad / Iraq

Abstract

Rh incompatibility is a not common pediatric problem, that cause morbidity and mortality in children, and it is an important cause of hemolysis, anemia and jaundice in newborn. To study Rhesus hemolytic disease, its severity, its complications; to evaluate if ABO incompatibility is protective or not, so to find out the efficacy of anti-D globulin; to evaluate the efficacy of phototherapy and exchange transfusion as a treatment.

This study was done on neonates with jaundice, seventy five patients (1-10 days old) who had Rh incompatibility were studied during period from the first of January 2008 to the 30th of June 2008.

History was taken about age, gender and gestational age of the patients, determination of gestational age and hepatosplenomegally as a cause of extramedullary hematopoiesis. Investigations done for patients were hemoglobin, total serum bilirubin, reticulocyte count, blood group and Rh, and direct coombs test. From 75 patients studied, 55 patients (73%) required treatment for jaundice; 25 of them (46%) required only phototherapy due to mild degree of hemolysis, and 30 of them (54%) required exchange transfusion with phototherapy due to severe degree of hemolytic. The remaining 20 patients (27%) required observation alone.

Family history of previous hemolysis was positive in 44 patients and it was a risk factor for having hemolysis in present pregnancy. Early evaluation of patients for jaundice was useful in early recovery. ABO incompatibility in association with Rh incompatibility was not necessarily protective against hemolysis. The administration of anti-D globulin to the mother within first 72 hours after delivery was protective against sensitization.

Early and proper management of Rh incompatibility may reduce need for exchange transfusion. ABO incompatibility was not necessarily protective against hemolysis. Anti-D globulin administered to mothers within 3 days after delivery was protective against sensitization, History of hemolytic in previous siblings is considered as a risk factor for present hemolytic in neonates with Rh incompatibility.

Keywords: RH Incompatibility; newborn; hemolytic disease

Introduction

Hemolytic disease of newborn due to Rh-incompatibility is an isoimmune hemolytic disease results from transplacental passage of RH(-ve) maternal blood containing antibodies active against RH(+ve) red blood cells antigens of the infant and is characterized by an increased rate of RBC destruction1, When Rh-positive blood is infused into an Rh-negatives woman through error or when small quantities(usually more than 1ml) of Rh-positive fetal blood containing D antigen inherited from an RH-positive father enter the maternal circulation during pregnancy, with spontaneous or induced abortion or at delivery, antibody formation against D antigen may be induced in the unsensitized RH-negative recipient mother.1
Hemolytic disease rarely occurs during first pregnancy because transfusion of RH-positive fetal blood into an RH-negative mother occurs near the time of delivery, too late for the mother to become sensitized and transmit antibodies to her infant before delivery.\(^1\)

When the mother and fetus are also incompatible with respect to group A or B, the mother is partially protected against sensitization by the rapid removal of RH-positive cells from her circulation by her preexisting anti-A and anti-B, which are IgM antibodies and not cross the placenta.\(^2\) In the fetus, anemia and heart failure are associated with hyperdynamic Circulation in both arterial and venous vessels.\(^3\) Fetal anemia in RH isoimmunization is the reduced life span of erythrocytes coated with antibodies, presumably from phagocytosis by reticuloendothelial cells.\(^4\) In severe cases of RH isoimmunization (erythroblastosis fetalis), hydrops and heart failure related to severe anemia in the fetus occur.\(^5\) Hydrops is often resulting in fetal or neonatal death without appropriate antenatal intervention.\(^5\)

Laboratory evaluation include blood typing, coombs test, complete blood picture with blood film.\(^5\) Reduced hemoglobin levels, reticulocytosis and blood film characterized by polychromasia and anisocytosis are expected with isoimmune hemolysis.\(^7\)

In Rh-negative women, a history of previous transfusion, abortion or pregnancy should suggest the possibility of sensitization.\(^1\)

Parents blood types should be tested, and maternal titer of IgG antibodies to D antigen should be assayed at 12-16, 28-32, and 36wk; the presence of elevated antibody titers at beginning of pregnancy, or rapid rise in titer, or titer of 1:64 or greater suggests significant hemolytic disease\(^1\). Immediately after birth of any infant to Rh negative woman, blood from the umbilical cord or from infant should be examined for blood group, Rh type, hematocrit and hemoglobin, and reaction of the direct coombs test; if the coombs test is positive, baseline serum bilirubin should be measured being done not only to establish the diagnosis but also to ensure the selection of the most compatible blood for exchange transfusion.\(^1\)

**Aim of current study:** To determine the severity of Rh hemolytic disease and its subsequent complications, and determine the protective effect of ABO incompatibility, so to find out the efficacy of anti-D globulin and effect of treatment of Rh incompatibility with phototherapy and exchange transfusion.

**Patients and methods:**

About 75 neonates with jaundice and Rh incompatibility admitted to Central Child Teaching Hospital and Al-Yarmook Teaching Hospital were studied during the period from the first of January 2008 to the 30th of June 2008. Forty six patients were males and twenty nine were females and their age range from 1-10 days.

**Clinical information collected** include: gestational age, gender, parity of the mothers, the presence of previous hemolysis or previous abortions, administration of anti-D antibody to the mothers, previous blood transfusion to the mother, presence of jaundice and its time of onset, and presence of pallor. So all the neonates were examined thoroughly for the presence of jaundice determination of gestational age, hepatosplenomegaly and ascitis. the pallor, in addition to investigations were done: for neonates = hemoglobin, reticulocyte count, blood group and Rh, total serum bilirubin, and direct coombs test (the method of direct coombs test in hospitals mentioned above is by putting on drop of whole blood and wash it 4 times with normal saline then remove supernatant and add 2 drops of antihuman globulin then test for agglutination by naked eye); *for mothers* = blood group and Rh. With regards to treatment, we found that -20 patients need no specific treatment. -25 patients need treatment with phototherapy alone (Group A). 30 patients need treatment with phototherapy and exchange transfusion (Group B).

**Results**

The frequency of severe Rhesus hemolytic disease and the need for exchange transfusion were more in those with multiparous mothers, as shown in table (1):
Table (1): Relationship between hemolysis in neonates and parity of their mothers.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Gravida 5≤</th>
<th>Gravida 5&lt;</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>63</td>
<td>11</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>48</td>
<td>13</td>
</tr>
</tbody>
</table>

P value=0.05 (significant).

Results showed in table (2) blood group in mothers and their neonates is shown in table (2), There were No.= 58(78%) and No.= 27(36%) for both mothers and neonates patients with type O , whilst about No.= 4(5%) and No.= 25(33%) for both mothers and neonates patients with type A.

Table (2): The distribution of different blood groups in the mothers and babies with Rh incompatibility

<table>
<thead>
<tr>
<th>Blood groups</th>
<th>mothers with Rh incompatibility</th>
<th>babies with Rh incompatibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.(%)</td>
<td>No.(%)</td>
</tr>
<tr>
<td>A</td>
<td>4(5)</td>
<td>25 (33)</td>
</tr>
<tr>
<td>B</td>
<td>10(13)</td>
<td>12 (16)</td>
</tr>
<tr>
<td>AB</td>
<td>3 (4)</td>
<td>11(15)</td>
</tr>
<tr>
<td>O</td>
<td>58(78)</td>
<td>27 (36)</td>
</tr>
</tbody>
</table>

History of previous hemolysis was present in 44 babies who need treatment and no one in neonates who not need treatment as shown in table(3):

Table (3): The incidence of previous hemolysis in Rh incompatibility neonates

<table>
<thead>
<tr>
<th>History of previous hemolysis in neonates siblings</th>
<th>Patients who need treatment</th>
<th>Patients who not need treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Not present</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Present</td>
<td>44</td>
<td>80</td>
</tr>
<tr>
<td>Total no.</td>
<td>55</td>
<td>100</td>
</tr>
</tbody>
</table>

P-value =0.0001 (very significant).
History of previous hemolysis was present in 17 of 25 neonates treated with phototherapy and in 27 of 30 neonates treated with exchange transfusion, as shown in table (4), so only five neonates (9%) of those who need treatment (55 neonates), their mothers had history of regular administration of anti-D following every pregnancy.

Table (4): The frequency of previous hemolysis in phototherapy and exchange transfusion groups.

<table>
<thead>
<tr>
<th>History of previous hemolysis in babies siblings</th>
<th>Phototherapy alone Group(A)</th>
<th>Exchange transfusion Group(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Not present</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>Present</td>
<td>17</td>
<td>68</td>
</tr>
<tr>
<td>Total no.</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

P-value = 0.002 (significant).

Table (5) showed about fifteen neonates (75%) of those did not need treatment (20 patients), their mothers had history of regular administration of anti-D following every pregnancy, which means that administration of anti-D was protective against hemolysis.

Table (5): The frequency of anti-D administration among treated and untreated neonates with Rh incompatibility.

<table>
<thead>
<tr>
<th>Anti D administration</th>
<th>Hemolysis</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Not given</td>
<td>50</td>
<td>91</td>
<td>5</td>
</tr>
<tr>
<td>Given</td>
<td>5</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Total no.</td>
<td>55 neonates need treatment</td>
<td>20 neonates need no treatment</td>
<td></td>
</tr>
</tbody>
</table>

P-value = 0.0001 (very significant).

From data collected, ABO incompatibility presence with Rh incompatibility was not protective, and the incidence of ABO incompatibility in treated and untreated neonates is shown in table (6).
### Table 6: The incidence of ABO incompatibility in patients groups

<table>
<thead>
<tr>
<th>ABO incompatibility</th>
<th>Patients who need treatment</th>
<th>Patients who not need treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Not present</td>
<td>14</td>
<td>25.5</td>
</tr>
<tr>
<td>Present</td>
<td>41</td>
<td>74.5</td>
</tr>
<tr>
<td>Total no.</td>
<td>55</td>
<td></td>
</tr>
</tbody>
</table>

P-value = 0.34 (not significant).

### Discussion

In this study we found that when the parity increases, the severity of hemolysis will be more and the need for exchange transfusion will also increase as shown that exchange transfusion was needed more in those with multiparous mothers, this result was also proved by Swinhoe & Gilmore which found that Rh hemolytic disease increased with subsequent pregnancies\(^{(29)}\) and also Joseph & Kramer who found the same results\(^{(28)}\); and this is because fetomaternal transfusion occur with each pregnancy and the illness will be more worse with successive pregnancies\(^{(1)}\).

We found that the majority of those who required exchange transfusion had a history of previous hemolysis in their siblings (27 from 30 neonates) (90%) and 17 neonates of 25 neonates (68%) who required phototherapy had history of previous hemolysis siblings and this means that the history of previous hemolysis increases the the possibility that subsequent pregnancies and babies need active treatment; while no one in the untreated group had history of previous hemolysis; and this goes with the fact that consider previous kernicterus or severe erythro-blastosis fetalis in a sibling as a further factor to decide treatment and not only to base the decision for treatment on the degree of anemia and/or hyperbilirubinemia\(^{(1)}\).

Fifteen of 20 neonates (75%) who did not require treatment, their mothers received dose of anti-D immunoglobulin and it was effective in preventing hemolysis in the successive pregnancies and that is why their neonates did not need treatment. Only 5 of 55 mothers (9%) whom their babies required treatment, received dose of anti-D. This result is similar to what was found by Swinhoe & Gilmore \(^{(29)}\), and also by LAD & Jane \(^{(30)}\); while Hundric found that prevention of Rh immunization by anti-D immunoglobulin does not comprise all the Rh negative mothers especially inadequate after abortions and multiple pregnancies\(^{(34)}\).

So, we found that the presence of ABO incompatibility between neonates and their mothers was not effective in protection against hemolysis; this result is similar to that found in Vox who found that ABO incompatibility has no effect on ameliorating the severity of erythroblastosis after Rh-incompatibility has developed \(^{(32)}\), while VOS in his study found that Rh immunization in pregnancy is significantly less in ABO incompatible matings than in ABO compatible matings\(^{(31)}\). The fact that ABO incompatibility is protective against Rhesus hemolysis by the rapid removal of the fetal red blood cells by the mother’s natural IgM anti-A and anti-B antibodies which do not cross the placenta\(^{(1)}\).

Clinical features of hemolysis like jaundice and pallor were evident in 89% and 40% of the neonates respectively, this is because that Rhesus incompatibility is a cause of hemolysis; and this evidence of hemolysis was more clear in the group who required exchange transfusion than those patients treated with phototherapy alone.
We found in this study regarding the hematological findings that the difference in the hemoglobin and reticulocyte count between neonates who required exchange transfusion and those treated with phototherapy alone was highly significant (p-value less than 0.001), which means that there is lower hemoglobin level and higher reticulocyte count in the exchange transfusion group than in phototherapy group, also the TSB levels were significantly higher in the group who required exchange transfusion than those treated with phototherapy alone.

All the above findings indicate that hemolysis was more severe in the exchange group than in the phototherapy group, and this was approved by Hayde & Widness in their study when they found that among infants with severe Rh isoimmunization, high total serum bilirubin levels and low hemoglobin levels indicate continuing severe hemolysis. Direct coombs test was positive in 27 from 30 neonates (90%) who required exchange transfusion due to the presence of high titer of maternal anti-bodies against the babies Rhesus positive red blood cells and it was positive in 15 from 25 neonates(60%) treated with phototherapy alone.

**Conclusion**

1) Early and proper management of the Rh-incompatibility may reduce the need for exchange transfusion. So, ABO incompatibility if occur with Rh incompatibility is not necessarily protective. also Anti-D immunoglobulin was protective against Rhesus hemolytic disease.

2) History of previous hemolysis in previous siblings is considered as risk factor for present hemolysis in neonate with Rh-incompatibility.

**Ethical Clearance:** Hospital and patient approvals were taken

**Source of Funding:** None

**Conflict of Interest:** None

References


A Comparative Study of Efficacy of Esmolol and Fentanyl For Blood Pressure and Heart Rate Attenuation During Laryngoscopy and Endotracheal Intubation

Bashar Naser Hussein1; Mohammad Kheiri Mahmod1, Ammar Hamid Hanoosh1
1Anesthetist, MBchB; DA Diploma of Anesthesia, AL-Yarmook Teaching Hospital, Ministry of Health and Environment, Baghdad, Iraq

Abstract

Background: Blood pressure and heart rate elevation during laryngoscopy and tracheal intubation may cause serious medical problems ex: patients with cardiovascular disease as well as patients with increased intracranial pressure. Aim of study: Comparism between Fentanyl and Esmolol in attenuating blood pressure and heart rate during laryngoscope and tracheal intubation. Patients and Method: 60 adult patients of both sex, ages between (20-33), labeled as ASA I and ASA II will undergo elective surgery, divided into two groups, each group contain 30 patients: Esmolol Group (E) takes 0.5 mg/kg esmolol IV. And Fentanyl Group (F) takes 1 mic/kg fentanyl IV. Blood pressure and heart rate was recorded before drugs administration as baseline reading and another reading was taken after drug administration as well as after tracheal intubation. Result: blood pressure and heart rate readings of both groups, when compared to baseline reading shows that Regarding heart rate, there are significance differences between the two groups, in that, esmolol reduce heart rate in all stages when compared to fentanyl. Regarding systolic blood pressure, both drugs reduce systolic blood pressure and there are significances in all stages, favored esmolol except in T3 due to rise in sbp when using fentanyl. Regarding diastolic blood pressure, both drugs also reduce diastolic blood pressure but there are no significances in these reading except in T3 due to esmolol reduce dpb much more than fentanyl does. Conclusions: esmolol in a dose of 0.5mg/kg is more effective than fentanyl in a dose of 1mic/kg in attenuation of hemodynamic response after endotracheal intubation.

Key words: Esmolol, fentanyl, hemodynamic changes, endotracheal intubation

Introduction

Due to sympathoadrenal discharge resulting from direct stimulation of epipharyngeal, laryngopharyngeal and par-pharyngeal area, together with increase plasma level of nor epinephrine [2]. The afferent sensory pathway is comprised of the glosopharyngeal nerve, which innervates the pharyngeal, structures superior to the anterior surface of the epiglottis, and the vagus, which innervates the posterior epiglottis distally into the trachea. The efferent responses to laryngoscopy and endotracheal intubation are mediated by both the parasympathetic and sympathetic nervous systems. The parasympathetic response, which is mediated by the vagus, can produce sinus bradycardia or arrest (occur mainly in children), but the main response in adult is sympathetic stimulation[3].

These response start with the first 5 second from laryngoscopic manipulation and intubation, and reach a plateau level after 45-60 seconds from intubation and return to normal level in 5 minutes[4].

Esmolol is cardioselective beta1 receptor blocker with rapid onset, a very short duration of action, and no significant intrinsic sympathomimetic or membrane stabilizing activity at therapeutic dosage. Esmolol reduce heart rate and, to a lesser extent, blood pressure. It has been successfully used to prevent tachycardia and hypertension in response to perioperative stimuli, such as intubation, surgical stimulation and emergence[13].
Although fentanyl has no significant effect on the cardiovascular system, it depends on the central Vigus stimulation \[14\]. Opioids neutralize the hemodynamic responses to intubation and surgical stresses, but are associated with nausea, vomiting, and hypoventilation, there are many studies into the reduction of hemodynamic responses to laryngoscopy and intubation, and many medications have been used, but there is still no specific medication for it \[14&15\].

**Patients and method:**

After approval obtaining from Arabic board council committees, and attaining a written consents from patients a double blind study was done to 60 patients.

**Inclusion criteria :**

* classified as ASA I , ASA II .
* scheduled for elective surgery ( lower abdominal surgery ) under general anesthesia and muscle relaxation with endotracheal intubation

the study was done in Al-yarmouk teaching hospital , in November the 1\textsuperscript{st} 2014 to December 1\textsuperscript{st} 2015 .

Exclusion criteria include:

* Pregnant patients .
* Patients refusing .
* Patients with ENT surgery .
* baseline pulse rate <60 bpm .
* Patient with suspected difficult intubation.
* patient on drug effecting autonomic nervous system .
* patients on antihypertensive drugs.
* Patient’s allergy to the corresponding drugs of the study.

Demographic data including:

1. Age (20-33) years.
2. Sex , male (36 ) , female (24 ).

3. Body weight (70 -90 kg ).

All patients were fasting for at least 6-8 hours. Basic monitoring which includes (Bp - HR - PR - SpO2) was attained by using non-invasive devices.

Patients divided into two groups as Group F take 1 mic / Kg fentanyl IV and Group E take 0.5 mg / Kg esmolol IV

**DATA collection:**

* At 0 time To (time just before the drug of the study was injected), blood pressure and pulse rate were taken as baseline measure , and the drug of the study were injected slowly over one minute .

* T1 after 3 minutes from drug administration the reading of blood pressure and heart rate were taken.

* T2 after general anesthesia was given; a reading was taken just before performing endotracheal intubation. (two minutes after T1 )

* T3 another reading was was taken just after performing endotracheal intubation.

* T4 final reading was recorded after 5 minutes from performing endotracheal intubation. Intubation was fascillating with a sleeping dose of propofol (1.5 - 2.5) was injected slowly and 0.1 mg / kg of vecuronium bromide.

At the end of the operation, Halothane was stopped and muscles reversal was done with neostigmine (0.01 mg / kg) together with Atropine (0.01 mg / kg) were given.

All patients were awake extubated in left lateral position and safely discharged from operating room.

**Statistical Analysis**

Analysis of data was carried out using the available statistical package of SPSS-20 (Statistical Packages for Social Sciences- version 20).
Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values).

The significance of difference of different means (quantitative data) was tested using analysis of variance (ANOVA) for more than two groups, using independent student-t-test for difference between two independent means, and paired t-test for difference between two dependent means (paired observations). Statistical significance was considered whenever the P value was equal or less than 0.05.

Results

First of all there are no difference between the two groups according to body weight, gender and age.

Table (1): Distribution of study group according to age, weight and Male / female ratio

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27.8</td>
<td>25.8</td>
</tr>
<tr>
<td>Weight Weight</td>
<td>73.2</td>
<td>79.1</td>
</tr>
<tr>
<td>Male / female ratio</td>
<td>17/13</td>
<td>19/11</td>
</tr>
</tbody>
</table>

P value for age = 0.491
P value for Weight = 0.104

There were no significance differences between baseline readings of systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate.

Table (2): corelated between baseline readings of systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate in patients groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic Baseline</td>
<td>E</td>
<td>30</td>
<td>132.50</td>
<td>8.854</td>
<td>.314</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>135.33</td>
<td>12.433</td>
<td></td>
</tr>
<tr>
<td>diastolic Baseline</td>
<td>E</td>
<td>30</td>
<td>83.03</td>
<td>6.965</td>
<td>.462</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>81.47</td>
<td>9.273</td>
<td></td>
</tr>
<tr>
<td>MAP Baseline</td>
<td>E</td>
<td>30</td>
<td>99.6</td>
<td>6.911</td>
<td>.949</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>99.47</td>
<td>8.966</td>
<td></td>
</tr>
<tr>
<td>Heart rate Baseline</td>
<td>E</td>
<td>30</td>
<td>90.73</td>
<td>8.65</td>
<td>.581</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>88.90</td>
<td>15.86</td>
<td></td>
</tr>
</tbody>
</table>

T1: Systolic blood pressure was decrease in both groups, from the baseline reading, the reduction in sbp was so obvious with esmolol and it was a significant change when compared with fentanyl. P value <0.05.
Diastolic blood pressure was decreased in both groups, from baseline reading, although dbp was reduced, when using esmolol, more than when using fentanyl but it was not significant. P value > 0.05

Mean blood pressure was decreased in both groups, although esmolol reduce mbp more than fentanyl, but the changes were not significant. P value > 0.05

Heart rates were decreased in esmolol group only and fentanyl group show slight increase in heart rate, but the overall changes was a significant value which favored esmolol. P < 0.05.

Table (3): comparison between values of systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate in esmolol group and fentanyl group after 3 minutes.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic after 3 minutes</td>
<td>E</td>
<td>30</td>
<td>125.53</td>
<td>8.982</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>133.23</td>
<td>13.836</td>
<td></td>
</tr>
<tr>
<td>Diastolic after 3 minutes</td>
<td>E</td>
<td>30</td>
<td>76.70</td>
<td>6.824</td>
<td>0.543</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>78.27</td>
<td>12.259</td>
<td></td>
</tr>
<tr>
<td>MAP after 3 minutes</td>
<td>E</td>
<td>30</td>
<td>92.93</td>
<td>6.94</td>
<td>0.145</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>96.70</td>
<td>12.112</td>
<td></td>
</tr>
<tr>
<td>Heart rate after 3 minutes</td>
<td>E</td>
<td>30</td>
<td>83.43</td>
<td>7.99</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>92.07</td>
<td>17.475</td>
<td></td>
</tr>
</tbody>
</table>

Systolic blood pressure decrease was a minimal with esmolol when compared to fentanyl because fentanyl augments the hypotensive effect of the induction agent and the reduction of dbp was significant, which favored fentanyl. P < 0.05

Diastolic blood pressure decrease in both groups in equal manner and the changes were not significant. P value > 0.05

Mean blood pressure decrease was in both groups, but fentanyl reduce mbp more than esmolol but not to a significant value. P value > 0.05

Heart rates were decreased in esmolol group only to a significant value because the rate was not change in fentanyl group. P value < 0.05.
Table (3): comparison between values of systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate in esmolol group and fentanyl group before intubation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic before intubation</td>
<td>E</td>
<td>30</td>
<td>124.7333</td>
<td>8.250</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>113.3333</td>
<td>19.106</td>
<td></td>
</tr>
<tr>
<td>diastolic before intubation</td>
<td>E</td>
<td>30</td>
<td>68.63</td>
<td>5.986</td>
<td>0.974</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>68.73</td>
<td>15.682</td>
<td></td>
</tr>
<tr>
<td>MP before intubation</td>
<td>E</td>
<td>30</td>
<td>87.33</td>
<td>6.076</td>
<td>0.259</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>83.73</td>
<td>16.188</td>
<td></td>
</tr>
<tr>
<td>Heart rate before intubation</td>
<td>E</td>
<td>30</td>
<td>78.00</td>
<td>7.456</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>92.97</td>
<td>16.923</td>
<td></td>
</tr>
</tbody>
</table>

Systolic blood pressures were decreased in esmolol group also in period of intubation and sbp were going up in case of fentanyl and the changes were not significant. P value > 0.05

Diastolic blood pressure was slightly decrease in esmolol group, but increased to a significant value in case of fentanyl. P value < 0.05

Mean blood pressure were decreased slightly in esmolol group but mbp were jumped in fentanyl group but not to a significant value. P value > 0.05

Heart rate were increased slightly in esmolol group compared to fentanyl where heart rate was increased to significant change which favored esmolol. P value < 0.05.

Table (4): comparison between values of systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate in esmolol group and fentanyl group after intubation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic after intubation</td>
<td>E</td>
<td>30</td>
<td>119.66</td>
<td>9.140</td>
<td>0.689</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>121.533</td>
<td>23.739</td>
<td></td>
</tr>
<tr>
<td>diastolic after intubation</td>
<td>E</td>
<td>30</td>
<td>67.93</td>
<td>5.994</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>77.90</td>
<td>19.787</td>
<td></td>
</tr>
<tr>
<td>MP after intubation</td>
<td>E</td>
<td>30</td>
<td>85.20</td>
<td>6.509</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>92.43</td>
<td>20.051</td>
<td></td>
</tr>
<tr>
<td>Heart rate after intubation</td>
<td>E</td>
<td>30</td>
<td>80.43</td>
<td>7.682</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>99.90</td>
<td>16.283</td>
<td></td>
</tr>
</tbody>
</table>
Systolic blood pressure was increased slightly in esmolol group and sbp remain constant in fentanyl group and the changes were not significant. p value < 0.05

Diastolic blood pressure was slightly increased in esmolol group and slightly decreased in fentanyl group but the changes were not significant. p value > 0.05

and Mean blood pressure were slightly increased in esmolol group and slightly decreased in fentanyl group and the changes were not significant. p value > 0.05 so, Heart rate were slightly increased in esmolol group and slightly decreased in fentanyl group and the changes were significant. p value < 0.05.

Table (4): Comparison between values of systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate in esmolol group and fentanyl group after 5 minutes intubation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic after 5 minutes intubation</td>
<td>E</td>
<td>30</td>
<td>125.33</td>
<td>8.727</td>
<td>0.350</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>121.63</td>
<td>19.658</td>
<td></td>
</tr>
<tr>
<td>Diastolic after 5 minutes intubation</td>
<td>E</td>
<td>30</td>
<td>70.40</td>
<td>6.420</td>
<td>0.256</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>74.30</td>
<td>17.477</td>
<td></td>
</tr>
<tr>
<td>MP after 5 minutes intubation</td>
<td>E</td>
<td>30</td>
<td>88.67</td>
<td>6.535</td>
<td>0.671</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>90.10</td>
<td>17.161</td>
<td></td>
</tr>
<tr>
<td>Heart rate after 5 minutes intubation</td>
<td>E</td>
<td>30</td>
<td>82.83</td>
<td>7.808</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>30</td>
<td>97.27</td>
<td>18.486</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

In the present study we compare between esmolol in a dose of 0.5/kg and fentanyl in a dose of 1mic/kg in order to attenuate the hemodynamic response that occur during endotracheal intubation. and we found that esmolol is more effective than fentanyl in that respect [16]. Esmolol provide a smothe and gradual control of the blood pressure .and prevent the rise of blood pressure during intubation.

Esmolol also prevent the rise of the heart rate during intubation [17]. Increasing the dose of the study drugs may cause unplanned hypotension and bradycardia [18], especially fentanyl which may augment the hypotensive effect of the propofol.

We need to increase the dose of fentanyl in order to control the heart rate [19], as it shows in our study fentanyl failed in attenuating the rise of heart rate during intubation.

The following two studies below were performed by using same dose [or even less] of esmolol that has been used in our study and proved that esmolol in a dose of 0.5 mg/kg and 0.4 mg/kg was effective in attenuating the rise of blood pressure and heart rate during endotracheal intubation as Jazaer state that esmolol in a dose of 0.5mg/kg is enough for attenuating hemodynamic response during intubation [20], whilst Kasey found that esmolol in a dose of 0.4mg/kg is enough for attenuating hemodynamic response that occur during intubation [21].
Many previous studies favored esmolol in attenuation the rise of blood pressure and heart rate during endotracheal intubation, when compared to fentanyl, although the doses of the study drugs were higher from the doses in current study: Shobhana and Purvi found that both fentanyl in a dose of 2mic/kg and esmolol in a dose of 2 mg/kg, can attenuate the hemodynamic response. But only esmolol provided consistent and reliable protection against increases in both heart rate and systolic blood pressure accompanying laryngoscopy and endotracheal intubation [22], so Feng et al., found that esmolol in a dose of 2 mg/kg could reliably offer protection against increase heart rate and blood pressure, while fentanyl in a dose of 3mic/kg only protect against hypertension not tachycardia [23] as well as Gogus et al., found esmolol in a dose of 2 mg/kg is better than fentanyl in a dose of 2 mic/kg in attenuation of hemodynamic response [24], whilst Sampangiramaiah and Jodumut found that esmolol in a dose of 1.5 mg/kg is better than fentanyl in a dose of 2 mic/kg, in attenuating hemodynamic response, and fentanyl in such dose may cause severe hypotension [25]. So Habib Bostan, Ahmet Eroglu found that both esmolol in a dose of 1 mg/kg and fentanyl in a dose of 1 mic/kg, can attenuate the hemodynamic response, but esmolol is more effective [26].

**Ethical Clearance:** Hospital and patient approvals were taken

**Source of Funding:** None

**Conflict of Interest:** None

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Study of Possible Predisposing Factors that May Result in Congenital Abnormalities among Newborn Infants a Hospital-based Study

Munaf Authman Hreeth¹, Ahmed Nadher Kareem², Sabah Noori Rashid²


Abstract

The term congenital abnormalities signifies that there has been disruption in the normal process of organogenesis occurring before birth. The earlier the insult, the more gross the abnormality.

This study aimed to study some predisposing factors that may result in congenital abnormalities among newborn infants in neonatal care unit (NCU) of Al-Kadhymia teaching hospital-Baghdad/Iraq. This prospective study was carried out during the period from 1st February to 1st August 2011. A total of 2700 neonates were admitted to NCU, and (100) newborn infants were proven to have congenital abnormalities by physical examination alone in the nursery care unit.

The results showed that of the total (100) affected neonates, 63 (63%) were full term as compared to only 37 (37%) preterm baby (<37 weeks gestation) with a significant difference (P <0.05). Of the total 2700 neonates, 1440 (53%) were females and the remaining 1260 (47%) were males.

Out of the (100) neonates who were proven to have congenital anomalies, 55 (55%) were males and 45 (45%) females. The percentage of newborns with congenital abnormalities was 3.7% with respect to total number of newborns (2700); 2.1% were males and 1.6% were females.

Our results showed that (55%) had neurological abnormalities followed by (12%) cleft lip and palate then (11%) chromosomal abnormalities (most of them Down syndrome and only 3 cases had Edward syndrome and 1 case had Patau syndrome). In this study, it was shown that the highest incidence of congenital abnormalities (55) occurred between (20-30) years.

It can be concluded that most the affected newborns were full terms, with a slight male predominance. Incidence of neurological abnormalities was higher than other types of congenital abnormalities, the highest incidence of congenital abnormalities occurred between 20-30 years, and in multigravida mothers, and most of parents were reported to be consanguineous or relatives.

Key words: Congenital abnormalities, newborn infants, predisposing factors.

Introduction

There are two types of congenital abnormalities, the major congenital anomaly, which is a structural abnormality present at birth, and has a significant effect on function or social acceptability, e.g. cleft lip, while the minor congenital anomaly is a structural abnormality present at birth, which has minimal effect on clinical functions, but may have a cosmetic impact, e.g. pre auricular pit [1].
Congenital malformations or birth defects are common among all races, cultures and socioeconomic strata. Birth defects can be isolated abnormalities or part of a syndrome and continue to be an important cause of neonatal and infant morbidity. Based on a World Health organization (WHO) report, about 3 million fetuses and infants are born each year with major congenital malformations; congenital malformations accounted for an estimated 495,000 deaths worldwide in 2005 [2].

Regarding etiology, congenital abnormalities can be the result of monogenic, chromosomal, maternal infections, maternal illness, twinning, environmental agents, medications, nutritional and unknown etiologies [3].

Congenital anomalies can be classified either based on timing of insult, underlying histological changes, or based on its medical and social consequences.

Congenital anomalies based on insult can be placed into following three categories: malformation, disruption and deformation. Classification based on underlying histological changes includes: aplasia, hypoplasia, hyperplasia and dysplasia [4].

Regarding management of congenital abnormalities, newborns with one or more malformations should receive ongoing care and may require multidisciplinary care and case management. Some clinical problems or physical findings may evolve over time and become more apparent with age [5,6].

Patients and Methods

This prospective study was carried out in Al-Kadhymia teaching hospital (Neonatal care unit) during the period from 1st February to 1st August 2011.

A total of 2700 neonates were admitted to NCU. One hundred newborn infants were proven to have congenital abnormalities by physical examination alone in the nursery care unit. The questionnaire for neonatal evaluation included: gestational age, sex, body weight and type of congenital anomaly. A detailed maternal history including age, parity, antenatal care, any history of abortion, previous baby with congenital abnormality, still births, medical illness, or drug intake during pregnancy were also obtained.

Moreover, the residency of the family and consanguinity between father and mother were recorded.

Statistical Analysis

The Statistics Package for Social Science (SPSS) version 17 was used for data analysis. The results are expressed in form of numbers, percentages and Chi-square Pearson correlation which was statistically significant at P.value less than 0.05 and statistically not significant at P.value more than 0.05.

Results

Of the total (100) affected neonates, 63 (63%) were full term when compared with 37 (37%) preterm babies (<37 weeks gestation), as shown in table (1), with significant difference (P<0.05).

<table>
<thead>
<tr>
<th>Gestational age</th>
<th>Number</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm babies</td>
<td>37</td>
<td>37%</td>
</tr>
<tr>
<td>Full term babies</td>
<td>63</td>
<td>63%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (2) showed that out of the (100) neonates who were proven to be have congenital anomalies, 55 (55%) were males and 45 (45%) were females, with no significant difference (P>0.05). The male to female ratio was 1.3:1.

**Table (2): Gender distribution of neonates with congenital abnormalities**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>55</td>
<td>55%</td>
</tr>
<tr>
<td>Females</td>
<td>45</td>
<td>45%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

Results showed that 55% were diagnosed to have neurological abnormalities followed by (12%) cleft lip and palate then (11%) chromosomal abnormalities (most of them were Down syndrome and only 3 cases were Edward syndrome, while 1 case Patau syndrome) as shown in table (3), with significant differences (P <0.05).

**Table (3): Distribution of newborns according to the type of congenital anomalies**

<table>
<thead>
<tr>
<th>Types of anomalies</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological (mainly neural tube defects)</td>
<td>55</td>
</tr>
<tr>
<td>Cleft lip &amp; palate</td>
<td>12</td>
</tr>
<tr>
<td>Chromosomal</td>
<td>11</td>
</tr>
<tr>
<td>Cardiovascular system (C.V. S)</td>
<td>6</td>
</tr>
<tr>
<td>Musculoskeletal system</td>
<td>5</td>
</tr>
<tr>
<td>Alimentary system</td>
<td>5</td>
</tr>
<tr>
<td>Genitourinary system (GUS)</td>
<td>5</td>
</tr>
<tr>
<td>Respiratory system</td>
<td>1</td>
</tr>
</tbody>
</table>

Results in table (4) showed the highest incidence (55%) of congenital abnormalities occurred within the age group (20-30) years, and (38%) within the age group (30-40) years, while (5%) occurred within (> 40) years and only (2%) within <20 years. The table demonstrated that the occurrence of congenital abnormalities in multigravida mothers was the highest (81%) when compared with primigravida mothers (19%). Out of 100 cases, (34%) had regular antenatal care and folic acid supplement during pregnancy, while (20%) were with maternal illness during pregnancy, which included (11% D.M), (8%) with hypertension and (1%) with epilepsy. Table (4) also showed that (13%) had a history of congenital abnormalities in their families, which was mainly neurological (10%), cleft lip and palate (2%) and (1%) chromosomal disorder (Down syndrome). Out of
100 cases, (56%) were living in urban areas and (44%) in rural areas. In regard to consanguinity between father and mother, (70%) were reported to be consanguineous, and only (30%) were negative (strange). Finally, (6%) of mothers were reported to be giving birth to a baby with congenital abnormalities and (2%) with stillbirth.

Table (4): Relationship between congenital abnormalities and predisposing factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of mothers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 years</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>20-30 years</td>
<td>55</td>
<td>55%</td>
</tr>
<tr>
<td>30-40 years</td>
<td>38</td>
<td>38%</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>5</td>
<td>5%</td>
</tr>
<tr>
<td>Residency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>55</td>
<td>55%</td>
</tr>
<tr>
<td>Rural</td>
<td>45</td>
<td>45%</td>
</tr>
<tr>
<td>Parity of mother</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multigravida</td>
<td>81</td>
<td>81%</td>
</tr>
<tr>
<td>Primigravida</td>
<td>19</td>
<td>19%</td>
</tr>
<tr>
<td>Antenatal history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had regular care &amp; folate supplement during pregnancy</td>
<td>34</td>
<td>34%</td>
</tr>
<tr>
<td>Maternal illness</td>
<td>20</td>
<td>20%</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of congenital abnormalities</td>
<td>13</td>
<td>13%</td>
</tr>
<tr>
<td>History of previous birth with congenital abnormality</td>
<td>6</td>
<td>6%</td>
</tr>
<tr>
<td>Still birth</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>Consanguinity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>consanguineous parents (relatives)</td>
<td>70</td>
<td>70%</td>
</tr>
<tr>
<td>Strange</td>
<td>30</td>
<td>30%</td>
</tr>
</tbody>
</table>
Discussion

Our results indicate the prevalence of congenital malformation disorders at Al-Kadhymia teaching hospital in NCU was 3.7% (100/2700), where males were higher than females, and male to female ratio was 1.3/1. This finding is in agreement with the Iranian study [7].

In a recent study done in Iran in June 2008, there was 2.9% of live births have major congenital abnormalities [7], while another study in Kuwait documented 1.25% of children with congenital abnormalities [8]. Considerable variation in frequency in different populations has been reported, from as low as 1.07% in Japan [9] to as high as 4.3% in Taiwan [10]. This wide variability could be due to the difference in population’s genetics.

Also, in this study, out of 100 newborn infants with congenital abnormalities, (63%) were full term with body weight (2.5-3.5 Kg), and only (37%) preterm babies with body weight (1–2.5 Kg), whereas a slight increment in incidence had been found in preterm and small for gestational age infants in a studies done in U.K [11] and India [12]. Such results could be attributed to the absence of facilities regarding antenatal diagnosis of such anomalies in our country leading to delivery of a full term babies with such anomalies.

Moreover, this study showed that the commonest anomalies were neurological (55%), followed by cleft lip and/or palate (12%), then chromosomal abnormalities (11%); among the neurological anomalies, the neural tube defects was the commonest one.

Different observations were recorded in other studies. A study in India [13,14] revealed an increase in frequency of musculoskeletal anomalies (30%), neurological (20.5%), then cleft lip and palate (18.5%); Other studies in Iran and Tunis [15,16] showed higher incidence of cleft lip and palate, while a study in Saudi Arabia [17] reported that the major anomalies are genitourinary (25%), cardiovascular (15%) then neurological (10%).

This partly implies a poor compliance of pregnant women regarding the intake of folic acid, in addition to poor antenatal care in regard to screening for such anomalies.

The neurological anomalies in this study were 55%, while in Wales (1%) [18], and in Germany (2%) [19]; this could be related to inadequate education of our people in regard to supplementation of folic acid during pregnancy and poor antenatal care, while in Wales and Germany, there are facilities for prenatal diagnosis and interruption of affected pregnancy.

The chromosomal abnormalities in this study was 11% which is similar to the study of United Kingdom, but higher than a study in in Norway 0.1% [20].

The chromosomal abnormalities in this study was 11% which is similar to the study of United Kingdom [11], whereas 0.1% in Norway [20].

On the other hand, this study showed that the percentage of occurrence of congenital abnormalities was (70%) among newborns delivered to consanguineous parents, which is similar to the figure in India and Iran and Saudi Arabia studies [15,17,21]. Despite the high prevalence of consanguineous marriages, the overall incidence of congenital abnormalities was not higher than developed countries [22].

The percentage of congenital abnormalities was very high among mothers aged (20-30) years (55%). A study done in England concluded that the increasing age of the mother may increase the risk of congenital abnormalities especially chromosomal defects [23], which may be attributed to the fact that this age is a common age of child bearing and higher fertility rate that is why most congenital abnormalities can be diagnosed in this age group.

Also, this study revealed that the residency of the families was from urban areas (56%) and this could be related to physical and environmental exposures (hot climate, air pollution, chemicals like lead exposure [24] and ionizing radiation [25,26]. In addition to the proximity of Al-Kadhymia teaching hospital to these areas, more anomalies can be diagnosed and documented. This result was in disagreement with a study done in India by Datta V Chaturvedi [14] who revealed higher incidence
of congenital malformations (57%) among people living in rural areas.

With respect to parity of mothers, this study revealed that most anomalies occur in multigravida mother (81%). Similar observations were recorded in other studies \[27,28\], which could be related to presence of a history of previous abortion, still births or delivery of affected baby with major anomaly, making such mothers attend hospital for their delivery of subsequent babies, whereas the least occurrence of anomalies in primigravida mother (19%). Chaturvedi et al \[21\] recorded increase in frequency of congenital anomalies in primi mothers.

Our study showed that there is significant family history of congenital abnormalities, as there were thirteen (13%) cases reported to have such a history which could be explained by the fact that most anomalies would be the result of genetic inheritance or mutation of certain gene in the family. This had been approved by a study done in Tokyo by Otake et al., \[27\] who revealed high frequency of congenital anomalies (10%) out of 1000 families with history of congenital anomalies).

Finally, this study showed that the diseases during pregnancy with drugs taken during such period were relatively insignificant regarding the occurrence of congenital abnormalities in newborns, as only (20%) mothers who had taken drugs during pregnancy. This could be due to that either most pregnant ladies in our society have no regular antenatal care follow up so most diseases passed undiagnosed, or the pregnant women who had medical disease during their pregnancy are well controlled by medication that had no impact on the growing fetus. Other studies done in Pakistan by Mishra and Baweja \[29\] and Garner \[30\] revealed increased incidence of congenital abnormalities with maternal illness.

**Ethical Clearance:** Hospital and patient approvals were taken

**Source of Funding:** None

**Conflict of Interest:** None

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Risk Factors of Rebound Hyperbilirubinemia in Post Phototherapy Hyperbilirubinemia Infants

Alexander Leonard Caesar Josediputra¹, Martono Tri Utomo², Risa Etika³

¹Researcher, Pediatric Resident, ²Associate Professor, ³Scientist, Head of Neonatology Division, Faculty of Medicine, Airlangga University, Dr. Soetomo General Hospital, Surabaya, Indonesia

Abstract

Background: Post phototherapy rebound hyperbilirubinemia is a cause for readmission in some infants. However, this phenomenon data of rebound hyperbilirubinemia is lacking from Indonesia. Our study aims to describe the risk factor of post phototherapy rebound hyperbilirubinemia in the infant.

Method: Cross-sectional study of all infants with indirect hyperbilirubinemia who were treated phototherapy according to standard guidelines in neonate intermediate unit Dr. Soetomo hospital for 6 months from June 2017 until December 2017. Bilirubin was measured 24 hours after phototherapy. Bilirubin rebound is considered as increasing total serum bilirubin that needs reinstitution of phototherapy.

Result: A total of 53 (44.9%) infants developed rebound hyperbilirubinemia. We revealed the following risk factor for rebound hyperbilirubinemia was the onset of jaundice on < 3 days, (10 babies, p <0.05). Other results are 30 (56.6%) female infants, 39 (73%) birth weight < 2500 g, 36 (67%) infants with a history of cesarean section, and 38 (71%) preterm infants but there are not statistically significant.

Conclusion: Post phototherapy rebound hyperbilirubinemia should be considered in the onset of jaundice < 3 days.

Keywords: Phototherapy, Neonatal hyperbilirubinemia, Rebound hyperbilirubinemia

Introduction

Hyperbilirubinemia in neonates is a common condition. Approximately 60-70% of term neonates and 80% of preterm neonates develop jaundice in the first week of life. Most of the hyperbilirubinemia is physiological and does not require special therapy, but there are some conditions where after phototherapy the hyperbilirubinemia recurs. Rebound hyperbilirubinemia after stopping phototherapy is a rare phenomenon.¹,² Some authors define it as a post-phototherapy bilirubin level that requires reinstitution of phototherapy according to AAP guidelines.³ The detection of rebound hyperbilirubinemia after stopping phototherapy is a common practice that prolongs hospital stay and increases laboratory costs.

In the previous study, recurrent hyperbilirubinemia occurred in 24.9% of the neonates who were the study subjects, the majority causes are hemolytic disease (71%) (ABO incompatibility 54.8%, Rhesus 4.8%, other etiologies unknown 16.9%). From previous studies, it was found that low birth weight increased...
the risk of recurrent hyperbilirubinemia by 3.5x and the male gender increased the risk by 1.6x. Other risk factors include gestational age, mode of delivery, birth weight based on gestational age, pregnancy more than once, and the onset of jaundice. Phototherapy is widely recognized as a relatively safe and effective method for the treatment of neonatal hyperbilirubinemia.

Bilirubin levels often increase after discontinuation of phototherapy. The factors causing the elevated bilirubin levels after stopping phototherapy have been investigated in several previous studies with different risk factors. This research has not been carried out in Dr. Soetomo hospital so that this study is the beginning to find risk factors for recurrent hyperbilirubinemia babies so that it can find prevention and prognosis for babies with the disease.

Material and Methods

Study Population

A case-control study was conducted on hyperbilirubinemia infants (age 0-28 days) in Dr. Soetomo General Hospital, Surabaya. Subjects were chosen using the total sampling technique and were included in the study if they met the following inclusion criteria: hyperbilirubinemia which had phototherapy. Exclusion criteria in this study include multiple congenital anomalies, sepsis, and direct hyperbilirubinemia. The control of this study is an infant with hyperbilirubinemia but does not have rebound hyperbilirubinemia.

Phototherapy is using blue light (minimum intensity of phototherapy light 10-12mW/cm²/nm) with an effective surface area of 60x30 cm and a distance of the light from the baby of 40 cm for 1-2 x 24 hours.

Hyperbilirubinemia is defined as a condition in which bilirubin levels increase with normal values depending on gestational age or birth weight and postnatal age (in hours) and clinically requires phototherapy or exchange transfusion.

Rebound hyperbilirubinemia is defined as post-phototherapy bilirubin level requiring reinstitution of phototherapy according to AAP (American Academy of Pediatrics) guidelines (Bansal et al., 2010). Data of sex, the onset of jaundice, birth weight, gestational age, and mode of delivery from all infants that met the criteria of rebound hyperbilirubinemia were recorded from the medical records. Birth weight is grouped by ³ 2500 gram < 2500 gram; onset of jaundice is grouped by and ³ 3 days and < 3 days old; mode of delivery is grouped by vaginal delivery and caesarean section; gestational age is grouped by term and preterm infant.

Statistical Analysis

The data were analyzed using Microsoft Excel 2019 and using IBM SPSS Statistic Version 21.0. A descriptive analysis was used to present the correlation. A Chi-square test was used to seek the bivariate correlation of rebound hyperbilirubinemia and risk factors.

Variables that have correlations were analyzed further by analysis of variance to define any influences between variables. A value of $p < 0.05$ was considered to be significant.

Ethics

This study was conducted after obtaining ethical approval from the Health Research Ethics Committee of Dr. Soetomo General Hospital, Surabaya. Before conducting the research, the procedure was fully explained to the parents. The study was conducted only after informed consent was signed by the parents/guardians. The confidentiality of the research subjects was maintained in this study.

Result and Discussion

A total of 118 infants in neonatal inpatient installation of Dr. Soetomo hospital with hyperbilirubinemia requiring phototherapy were involved in this study. The base characteristics of this study are described in table 1. Correlation of sex, onset of jaundice, mode of delivery, birth weight, and gestational age with rebound hyperbilirubinemia described in table 2
Table 1. Characteristics of subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rebound hyperbilirubinemia</strong></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>53(44.90)</td>
</tr>
<tr>
<td>no</td>
<td>65(55.10)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>54(45.80)</td>
</tr>
<tr>
<td>female</td>
<td>64(54.20)</td>
</tr>
<tr>
<td><strong>Birth weight</strong></td>
<td></td>
</tr>
<tr>
<td>&gt; 2500 gram</td>
<td>39(33.10)</td>
</tr>
<tr>
<td>&lt;2500 gram</td>
<td>79(66.90)</td>
</tr>
<tr>
<td><strong>Onset of Jaundice</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 3 days</td>
<td>10(8.40)</td>
</tr>
<tr>
<td>&gt; 3 days</td>
<td>108(91.60)</td>
</tr>
<tr>
<td><strong>Mode of delivery</strong></td>
<td></td>
</tr>
<tr>
<td>vaginal delivery</td>
<td>32(27.10)</td>
</tr>
<tr>
<td>caesarean section</td>
<td>86(72.90)</td>
</tr>
<tr>
<td><strong>Gestational age</strong></td>
<td></td>
</tr>
<tr>
<td>term</td>
<td>42(35.60)</td>
</tr>
<tr>
<td>preterm</td>
<td>76(64.40)</td>
</tr>
</tbody>
</table>
Table 2. Correlation of sex, onset of jaundice, mode of delivery, birth weight, and gestational age with rebound hyperbilirubinemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rebound hyperbilirubinemia</th>
<th>p</th>
<th>OR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yes</td>
<td>no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>0.71</td>
<td>0.84</td>
<td>0.40-1.74</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset of jaundice</td>
<td></td>
<td>&lt;0.05*</td>
<td>2.51</td>
<td>1.99-3.16</td>
</tr>
<tr>
<td>&lt; 3 days</td>
<td>10</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 3 days</td>
<td>43</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td>0.21</td>
<td>1.72</td>
<td>0.75-3.93</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>17</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caesarean section</td>
<td>36</td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight</td>
<td></td>
<td>0.08</td>
<td>0.08</td>
<td>0.92-4.55</td>
</tr>
<tr>
<td>&gt; 2500g</td>
<td>14</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2500g</td>
<td>39</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td></td>
<td>0.17</td>
<td>0.21</td>
<td>0.75-3.93</td>
</tr>
<tr>
<td>Term</td>
<td>15</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-term</td>
<td>38</td>
<td>38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p-value statistically significant

In this study, 53 infants (44.9%) were found with rebound hyperbilirubinemia. Female babies are more than male babies. Hyperbilirubinemia was more found in cases of infants with birth weight <2500g. The onset of hyperbilirubinemia occurs mostly at <3 days of life. Most cases of hyperbilirubinemia occurred in infants caesarean section delivery. In addition, the most cases of hyperbilirubinemia were also found in infants with pre-term infant.
Female infants with rebound hyperbilirubinemia are more than male infants. The results of this study are different from previous studies with 9% of female babies compared to 12.9% of male infants who experience recurrent hyperbilirubinemia. The cause of the differences in this study is that it does not exclude the possibility of hemolytic diseases such as ABO incompatibility, Rhesus, Coomb test, G6PD, sepsis, type of nutrition, and other risk factors so that there is a possibility that female patients are included in the study sample that will affect the study results.

Onset of jaundice in infants with rebound hyperbilirubinemia was found to be significantly different in infants with yellow onset <3 days with a risk of 2.5 times compared to ³ 3 days. The highest incidence of jaundice was observed on the second to fourth day after birth. Among all infants with the onset of jaundice within 72 hours after birth, 13.8% showed hyperbilirubinemia after cessation of phototherapy. Contributing factors to the development of physiological hyperbilirubinemia in the neonate include increased bilirubin load due to relative polycythemia, shortened erythrocyte life span (80 days compared to 120 days of adults), immature hepatic absorption and conjugation processes, and increased enterohepatic circulation. This study does not record the nutrition of the baby so that the result can be biased.

Mode of delivery by caesarean section also showed more results in infants with rebound hyperbilirubinemia (30.50%) compared to normal delivery (14.40%), but in this study, it was not statistically significant. In another study after discontinuation of phototherapy, rebound hyperbilirubinemia was found in 13.3% of neonates born via cesarean section, but no statistically significant association was found (p = 0.520). In this study, the data on infants with cesarean delivery were not recorded with the indication.

Low birth weight babies and preterm infants are not statistically significant in this study. In another previous study, birth weight <2000gram was found to be a significant risk for recurrent hyperbilirubinemia (p <0.001). Similar results were also found by Kaplan M et al. The difference in this result can be caused by the other risk factor like ABO, Rhesus, G6PD were not excluded in this study. Premature babies have immature hormonal and enzyme responses, and hyperbilirubinemia is caused by the liver maturity factor, where the baby’s liver function is not fully mature to process erythrocytes so that indirect bilirubin conjugation is not perfect either.

Jaundice can be aggravated by polycythemia, hemolysis, and infection because hyperbilirubinemia can cause a jaundiced kern, so the baby’s skin color must be monitored frequently. The three best predictors for recurrent hyperbilirubinemia were gestational age of the infant, age at initiation of phototherapy, and total serum bilirubin.

**Conclusion**

Jaundice in infants with rebound hyperbilirubinemia was higher in subjects with onset of jaundice < 3 days. There was no correlation of sex, mode of delivery, birth weight, and gestational age with rebound hyperbilirubinemia in infants in this study. Factors affecting rebound hyperbilirubinemia such as ABO, Rhesus, G6PD, and Coomb’s test should be investigated at the time of sample selection.

**Acknowledgment:** The author thanks to Dr. Soetomo general hospital-Universitas Airlangga, Surabaya, Indonesia for the medical record collection.

**Ethical Clearance:** We obtained an approval of whole project from Ethical Committee Review Board of Dr. Soetomo General Hospital Surabaya.

**Source of Funding** - Self

**Conflict of Interest** - Nil

**References**


Abuse of Topical Glucocorticoids among Patients Visiting Community Pharmacy in Basrah-Iraq

Alhassan Shaker Abed¹, Jubran K. Hassan²
¹Researcher, ²Assistant Professor, Department of Clinical Pharmacy, College of Pharmacy, University of Basrah, Basrah-Iraq

Abstract

Introduction: Topical glucocorticoids have been the mainstay for the treatment of many skin conditions since their introduction due to their anti-inflammatory, anti-proliferative, vasoconstrictive, and immunosuppressive properties. Availability of topical glucocorticoids in different potencies as over the counter medication in Iraq encourages their use for non-labeled indications, thus increasing the side effect of such drugs.

Objectives: The aim of this study was to evaluate the use, knowledge of the patients and patient education of topical glucocorticoids.

Methodology: A cross sectional community pharmacy based survey was conducted throughout Basrah city. Inclusion criteria were customers visiting the community pharmacies asking for topical corticosteroids without prescription, aging 15 years and older and willing to participate in the study. 600 questionnaire forms for topical corticosteroids were distributed in the center and the districts of Basrah city. 254 forms out of 600 were returned out of which 212 were completed. Incomplete forms were excluded.

Result: 106 (50%) participants out of 212 misused topical glucocorticoids. As a single product, Betamethasone valerate and Clobetasol propionate was majorly used by the participants which are potent and ultra-high potent topical steroid respectively. The major source of topical steroid prescription was relatives. 49.8% participants didn’t recognize any side effect of topical glucocorticoids while hypopigmentation was the most side effect identified by the participants (21.8%). Only 35% of the participants, whose topical steroid prescription source by physicians and pharmacists, was educated about the use of topical glucocorticoids.

Conclusion: Topical glucocorticoids are commonly abused in Basrah city and probably other cities of Iraq. Looking fair and improving face appearance is a common cultural believe in Iraq that push people to seek topical glucocorticoids from community pharmacies for skin lightening in addition to other unapproved indications without prescription.

Key words: topical corticosteroids, abuse of topical corticoids, misuse of topical corticoids.

Introduction

Topical glucocorticoids possess various properties which make them very effective to be used for different non-infectious dermatological conditions¹. These properties are anti-inflammatory, anti-proliferative, vasoconstrictive, and immunosuppressive properties². Hydrocortisone was the first introduced then with various structural modifications others, such as betamethasone, mometasone, clobetasol, dexamethasone and triamcinolone, was produced³. Due to their usefulness and effectiveness, they have been used widely for treating inflammatory dermatologic diseases which
led to their abuse and emergence of their side effects such as hypopigmentation, telangiectasia, acne, hypertrichosis, suppression of hypothalamic–pituitary–adrenal (HPA) and rosacea(4)(5). Topical glucocorticoids are classified according to their potency into 7 classes by WHO, Class 1 (most potent) to Class 7 (least potent). The individual topical glucocorticoids may exist in different classes depending on formulation (cream or ointment) and concentration.

For example, betamethasone valerate ointment 0.05% is in Class II (potent), while betamethasone valerate cream 0.05% is in Class III (upper mid-strength(6).

Abuse of the drug is using drug for non-medical purposes while misuse is the wrong use of the drug for medical purposes(7). In Iraq and other developing countries topical glucocorticoids are purchased as over the counter drug without medical supervision, from some community pharmacies, for different medical purposes which is not accepted legally. That may be due to different reasons such as low patient income and insufficient regulatory enforcement level(7). Usually Iraqi patient keeps the prescription after being dispensed to refill it again with or without medical supervision which may encourage drug abuse or misuse(8). Low level of patients counseling and education or insufficient knowledge of healthcare providers decrease patients adherence and awareness about the side effect of glucocorticoids(9)(10).

Topical glucocorticoids misuse occurs at different levels starting from pharmaceutical companies, prescription, sales ending with the patients and layperson. Pharmaceutical companies make irrational combinations such adding moderate potent steroid with other combination for treatment of melasma(3). Prescription misuse of Topical glucocorticoids occurs when prescription requirements are not taken into consideration that ranges from incomplete prescription (not writing the strength and vehicle of TC, dosing, site of application and length of treatment on the prescription) to erratic prescription of topical glucocorticoids for the treatment of skin disorders with undetermined diagnoses(2).

Sales misuse can occur from over-the-counter (OTC) availability of all strengths and formulations of Topical glucocorticoids. This does not allow for proper supervision of patients’ using topical steroid resulting in indiscriminate purchase for self, family and friends. Salespersons play a critical role, as they often dispense these agents(2).

Topical glucocorticoids are used for incorrect indications tinea, nonspecific pruritus and acne, etc. by general practitioners, homemade doctors (parents, siblings, neighbors) and chemists. Use of combination of antibacterial or antifungal with topical glucocorticoids for the treatment of bacterial or fungal infection randomly has led to emergence of resistant organism(11).

Brown and dark races with no skin disease abuse topical glucocorticoids, to look fair and hide their blemishes, due to their vasoconstrictive and hypopigmentation effect. Some of the, reasons indicates why topical glucocorticoids are abused as cosmetic cream, are shown below(11):

- inexpensive.
- Easily available.
- careless about adverse effects.
- incomplete counseling by prescribers when prescribing topical glucocorticoids for appropriate indication.
- Social attitudes to skin color

Topical glucocorticoids have been used for the treatment of melasma either alone or in combination with tretinoin and hydroquinone, this triple combination is known as Kligman’s formula. Majority of cases treated with this combination subjected to relapse once stopped the treatment forcing them to use the treatment again for months without medical advice leading to adverse effects on their face such as telangiectasia, hypertrichosis, acne, skin atrophy(12).
Topical steroids are being misused by parents for diaper rash in their children where excess use of which lead to Cushing’s syndrome\(^{(13)}\).

**Methodology**

This study was a community pharmacy-based survey. It was carried out in Basrah city only as other cities of Iraq where inaccessible because of COVID19 Lock down.

This forma was allocated for costumers visiting the community pharmacies asking for topical or oral corticosteroids without prescription, aging 15 years and older and willing to participate in the study.

The questionnaires form comprised of six parts where the first part was about the sociodemographic information such as age, sex, place of residence and chronic diseases. The second question was to investigate the most topical corticoid asked by the patients while the third part identified the source of topical corticosteroids prescription. The fourth part was to investigate if the patients was educated properly about the use of corticosteroids and the fifth part was to identify the reason for using topical steroids. The last part to evaluate the awareness of the patients about the side effect of both topical corticosteroids.

Community pharmacists was, after providing the topical corticosteroids, asking the patients for their agreement verbally about participation in the study. After their agreement, the community pharmacist introduces the questionnaire paper (who was previously trained on) of the topical corticosteroids and report the answers of the patients. The survey was completely voluntary and required about 10 minutes to complete.

In this study we define abuse of the drug is using drug for non-medical purposes without prescription\(^{(7)}\)

**Sample size**

600 forma of questionnaire for topical corticosteroids were distributed in the center and the districts of Basrah city. 254 forma out of 600 were returned out of which 212 were completed. Incomplete questionnaires were excluded from the study.

**Statistical Analysis**

Data has been tested using specialized software for data analysis and Microsoft Excel 2019. The data of this study were analyzed using descriptive statistic and chi-square test. The level of significant was considered for p less than 0.05.

**Result**

Table 1 shows characteristics of participants answered the questionnaire related to topical glucocorticoids where the total number of participants where 212. 52% of them are female and 48% are male, p=0.4922.

There was non-significant (p<0.05) difference among the ages of the patients that participated in the study where 33.1% had an age of (15-20), 31.1 had an age of (25-35) and 35.8 had an age of (>40), p=0.6)

There was significant (p<0.05) difference in the residency of participants where 37% in the center of the city versus 63% outskirt, p=0.0001.
Table 1 Characteristics of participants in Questionnaire about topical glucocorticoids, N=212

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>111</td>
<td>52</td>
<td>0.4922</td>
</tr>
<tr>
<td>Female</td>
<td>101</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Age Distribution (years)</td>
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<td>0.6987</td>
</tr>
<tr>
<td>15-25</td>
<td>70</td>
<td>33.1</td>
<td></td>
</tr>
<tr>
<td>25-35</td>
<td>66</td>
<td>31.1</td>
<td></td>
</tr>
<tr>
<td>&gt;35</td>
<td>76</td>
<td>35.8</td>
<td></td>
</tr>
<tr>
<td>Scientific degree</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>39</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>51</td>
<td>24.1</td>
<td>0.1011</td>
</tr>
<tr>
<td>High school</td>
<td>60</td>
<td>28.3</td>
<td></td>
</tr>
<tr>
<td>Graduated</td>
<td>62</td>
<td>29.2</td>
<td></td>
</tr>
<tr>
<td>Residency of participants</td>
<td></td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td>center</td>
<td>78</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>outskirt</td>
<td>134</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Disease history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>36</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>19</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>seizure</td>
<td>3</td>
<td>1.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Others</td>
<td>35</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td>Non</td>
<td>119</td>
<td>56.1</td>
<td></td>
</tr>
</tbody>
</table>

P value <0.05 considered significant

A Comparison the frequency of prescriptions sources for common types of topical glucocorticoids used by the participants

As shown in table 2, there was a significant (p < 0.05) difference in sources of topical steroid prescription (physicians 22.5%, 24.3% from pharmacist, 7.3% from nurses, 30.3% from relatives, 7.3% from internet and 8.3 % from other sources).

Table 2 also represents the frequency of dispensed topical steroids; there was a significant difference (p<0.05) among the dispensed steroids (betamethasone valerate 20.2%, Clobetasol propionate 15.1%, mometasone furoate 7.1%, hydrocortisone acetate 4.7% and others or combinations 52.8%, p <0.0001).
### Table 2: A Comparison the frequency of prescriptions sources for common types of topical glucocorticoids used by the participants in the questionnaire

<table>
<thead>
<tr>
<th>Source of prescription</th>
<th>Beta-methasone valerate</th>
<th>Clobetasol propionate</th>
<th>Mometasone furoate</th>
<th>Hydrocortisone acetate</th>
<th>Others*</th>
<th>Total N(%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>physicians</td>
<td>7</td>
<td>4</td>
<td>10</td>
<td>5</td>
<td>23</td>
<td>49(22.5)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>pharmacists</td>
<td>9</td>
<td>9</td>
<td>2</td>
<td>4</td>
<td>27</td>
<td>51(24.3)</td>
<td></td>
</tr>
<tr>
<td>nurses</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>16(7.3)</td>
<td></td>
</tr>
<tr>
<td>relatives</td>
<td>20</td>
<td>14</td>
<td>3</td>
<td>0</td>
<td>28</td>
<td>65(30.3)</td>
<td></td>
</tr>
<tr>
<td>internet</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>10</td>
<td>13(7.3)</td>
<td></td>
</tr>
<tr>
<td>others</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>18(8.3)</td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>43(20.2)</td>
<td>32(15.1)</td>
<td>15(7.1)</td>
<td>10(4.7)</td>
<td>112(52.8)</td>
<td>212(100)</td>
<td></td>
</tr>
</tbody>
</table>

*P value <0.05 considered significant
*others are products containing topical steroids with other products

### A Comparison the frequency of reasons led to use topical glucocorticoids by participants

Table 3 shows the reasons for using topical glucocorticoids by the participants; where there was a significant difference (p<0.05) for the causes of using topical glucocorticoids among the patients. Eczema 7.5%, Allergic dermatitis 38.7%, Psoriasis 1.9%, Melasma 16.5%, Vaginal infection 3.3%, Acne 2.4%, Skin lighting 16%, Diaper rash 4.3%, Skin ulcer 0.5%, Wound healing 0.9%, Skin fungal infection 6.1%, p<0.0001
Table 3 Frequency of Reasons for using topical glucocorticoids mentioned by participants in the questionnaire. Data expressed as N(%)  

<table>
<thead>
<tr>
<th>Reason of use</th>
<th>N(%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema</td>
<td>16(7.5)</td>
<td></td>
</tr>
<tr>
<td>Dermatitis</td>
<td>86(38.7)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>4(1.9)</td>
<td></td>
</tr>
<tr>
<td>Melasma</td>
<td>35(16.5)</td>
<td></td>
</tr>
<tr>
<td>Vaginal infection</td>
<td>7(3.3)</td>
<td></td>
</tr>
<tr>
<td>Acne</td>
<td>5(2.4)</td>
<td></td>
</tr>
<tr>
<td>Skin lighting</td>
<td>34(16)</td>
<td></td>
</tr>
<tr>
<td>Diaper rash</td>
<td>9(4.3)</td>
<td></td>
</tr>
<tr>
<td>Skin ulcer</td>
<td>1(0.5)</td>
<td></td>
</tr>
<tr>
<td>Wound healing</td>
<td>2(0.9)</td>
<td></td>
</tr>
<tr>
<td>Skin fungal infection</td>
<td>13(6.1)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Total</td>
<td>212(100)</td>
<td></td>
</tr>
</tbody>
</table>

P value <0.05 considered significant  
Bold is irrational use (14)(15).  

A Comparison the frequency of known adverse effects of topical glucocorticoids mentioned by the participants in the questionnaire  

Table 4 shows a significant (p<0.05) difference in the knowledge of the patients about the side effects of steroids, (Delayed wound healing 6.3%, Striae 11.7%, Hypo/hyperpigmentation 21.8, Hirsutism 4.1, and the patients who didn’t know were 49.8%, p<0.0001).  

Table 4 Frequency of adverse effects of topical glucocorticoids described or known by the participants. Data expressed as N(%)  

<table>
<thead>
<tr>
<th>Side effects of glucocorticoids(16)</th>
<th>N(%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delayed wound healing</td>
<td>15(6.3)</td>
<td></td>
</tr>
<tr>
<td>Striae</td>
<td>28(11.7)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Acne</td>
<td>15(6.3)</td>
<td></td>
</tr>
<tr>
<td>hypopigmentation</td>
<td>52(21.8)</td>
<td></td>
</tr>
<tr>
<td>Hirsutism</td>
<td>10(4.1)</td>
<td></td>
</tr>
<tr>
<td>Don’t know</td>
<td>126(49.8)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>246(100)</td>
<td></td>
</tr>
</tbody>
</table>

P value <0.05 considered significant  
Some participants indicated > 1 side effect
A comparison the frequency of education of participants by pharmacists and physicians about the use and adverse effects of topical glucocorticoids

In Table 5 there is significant (p<0.05) difference between number of the patients that have been educated by physician and pharmacists and those who have not been (65% versus 35%, p=0.0027).

Table 5: A comparison between the source (physicians a pharmacists) of prescription and if the patient has been educated about the topical glucocorticoids or not. data expressed as N(%) 

<table>
<thead>
<tr>
<th>Source of prescription</th>
<th>Physicians</th>
<th>Pharmacists</th>
<th>Total N(%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients who have been educated</td>
<td>20</td>
<td>15</td>
<td>35(35)</td>
<td></td>
</tr>
<tr>
<td>Patients who haven’t been educated</td>
<td>29</td>
<td>36</td>
<td>65(65)</td>
<td>0.0027</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>51</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

P value <0.05 considered significant

Discussion

Table 2 demonstrates the frequency of prescriptions sources for common types of topical glucocorticoids used by the participants in the questionnaire. This study found that relative personnel are considered the main source of topical glucocorticoid prescription (p value < 0.0001) followed by pharmacists and physicians respectively. Meena S et al. found that pharmacists were the main source of prescription(17).

That is may be due to most participants where from outskirt (63% versus 78%) or due low level of education (about 30% graduated versus 70% non-graduated). Other reasons are patients may have trust in their relative personnel that tried same product for same indication without getting advice from healthcare provider, this due to low economic conditions made the patients seek free of charge advice from the pharmacists rather than visiting the physicians.

A firm regulations about dispensing topical glucocorticoids by prescriptions from qualified healthcare advisers should be made.

In contrast to this study, Hon et al 2006 in china found that physicians are the main source of topical glucocorticoids prescriptions followed by newspaper(18). Also a different results were found by Lee et al 2015 in Korea where internet was the main source of prescriptions followed by media or TV(19).

As a single product betamethasone was the most prescribed topical glucocorticoids followed by clobetasol. Deena et al. 2014 in Mosul-Iraq found the same result while different results was found by Al-Dhalim et al 2006 in Najaf-Iraq where clobetasol was the most prescribed followed bybetamethasone(20)(21) In Meena S et al. Clobetasol was the most prescribed topical glucocorticoids(17).

These are highly potent topical glucocorticoids and only physicians have the right to prescribe them while other sources are considered unofficial sources which includes pharmacists also as these products are non-OTC products. In this study relatives of the patients are more
familiar with betamethasone and clobetasol than other single products, therefore, they are the most prescribed and misused because relatives are unofficial source of prescription.

Majority of mometasone prescriptions has been prescribed by its official source (physicians) because relatives of the patients, main source of prescription in this study, are not familiar with mometasone or due to its high cost. Hydrocortisone is the other topical products prescribed by its official sources as relatives may not be familiar with it.

In Charman et al 2000 in UK; hydrocortisone was the most prescribed topical glucocorticoids followed by betamethasone which is not matched with this study(22). Also different results were found in.

Table 3 expresses what topical glucocorticoids was used for by the patients. 50% of the participants used topical glucocorticoids for unapproved indications where skin lightening and melasma were the most reason of use. Also same result were found by Deena et al. 2014 in Mosul-Iraq(20). In Al-Dhalim et al 2006 in ALNAJAF-IRAQ skin lightening and acne were the most reason of use(21). Fungal infection and acne were the most reasons of use while skin lightening was the third most reason of use in Meena S et al.(17).

Table 4 expresses the knowledge of the patients about the side effect of topical glucocorticoids. About 50% of the patients didn’t know any side effects of topical glucocorticoids. Hypopigmentation is the most side effect known by the patients and this clarifies the use topical steroids for skin fairness.

Table 5 demonstrate the level of education for patients whose source of prescription was physicians and pharmacists only as its their duty to ensure safety and a suitable use of the medication(23). A significantly higher percent (p value 0.0027) of patients was not educated by either physicians and pharmacists indicating inadequate level of patient’s education practiced by them.

A similar result were found by Wing Man Lau et al. where pharmacists were less likely to advice the patients about topical glucocorticoids(24). Ammar Abdulrahman Jairoun et al. found that deficiency in curriculum of pharmacy courses also affects the practice and advising process regarding topical glucocorticoids(25).

Low level of patients counseling may be due to lack of information or time, the patients feel negatively about topical glucocorticoids or/and the physicians feel negatively about the counseling for topical glucocorticoids by the pharmacists. Min Jung Kang et al. found that bad feeling and physicians attitude was the most barrier for counseling(9).

This study finds that topical glucocorticoids are abused commonly in Basra city and probably in other cities of Iraq also where Al-Dhalim et al 2006 in Najaf (another city in Iraq) and Deena et al. 2014 in Mosul(another city in Iraq) found the same results. In this study the major responsibility of misuse was put on the relatives of the patients. Pharmacists also bear a part of the responsibility as they were dispensing a potent topical glucocorticoids as OTC, or with prescription but with inadequate level of education. On other hand potent topical glucocorticoids relieve the symptoms of the patients and after stopping the medication the symptoms recur leading the patients to reuse the potent topical glucocorticoids again without seeking medical advice.

Physicians and pharmacists should develop an intense relationship, reduce the inter-professional practice gap and improve communication lead to delivering a unified clear massage about drug use and safety for the patients(26)(9).

Conclusion

In Basrah-Iraq, topical glucocorticoids are used without prescription for unapproved indication such as skin lightening and melasma addition to other unapproved indication. Having culturally accepted skin color represents the dominant incentive that urges people to abuse topical glucocorticoids. Betamethasone valerate is the predominant abused or misused topical glucocorticoids as a single product. Half of the participants who abuse topical glucocorticoids are
unaware of their adverse effect.

Community pharmacists can enhance people knowledge about the adverse effects of topical glucocorticoids through counseling before dispensing. Finally, Iraqi health officials need to promote people awareness about the risks of misusing topical glucocorticoids without medical supervision through education programs and advertisements in public health settings, media, and social networks.

**Ethical Clearance:** Ethical permission was taken from ethics review committee of college of pharmacy/ university of Basrah

**Conflict of Interest:** The authors declare no conflict of interest

**Source of Funding:** Self

**References**

1. Eswaramurthi Balasubramanian MD, DVL MRMD. A Study on Topical Steroid Abuse and Its Consequences in Dermatology.


Factors Affecting Professional Integrity in Nurses: A Qualitative Content Analysis

Ali Asghar Jesmi, Khadijeh Yazdi, Zahra Sabzi, Hadi Ahmadi Chenari, Abdolmotalleb Hasani

1Assistant Professor, Faculty of Nursing and Midwifery, Sabzevar University of Medical Sciences, Sabzevar, Iran,
2Assistant Professor, Faculty of Nursing and Midwifery, Golestan University of Medical Sciences, Gorgan, Iran,
3Assistant Professor, Ferdows School of Paramedical and Health, Birjand University of Medical Sciences, Birjand, Iran,
4Ph.D. Candidate of Nursing, Faculty of Nursing and Midwifery, Golestan University of Medical Sciences, Gorgan, Iran

Abstract

Background: Integrity is one of the professional values mentioned in the ethical codes of the Professional Nursing Association. It is defined as a commitment to the five fundamental values of integrity, trust, equality, respect, and accountability. This study aims to explore factors affecting professional integrity in nurses.

Methods: A descriptive content analysis was carried out by 17 interviews with Iranian nurses who were chosen through purposive sampling. The data collection instruments were semi-structured interviews and observation. The interviews were recorded and transcribed word by word; then, they were coded and analyzed by Graneheim and Lundman qualitative content analysis method. For the trustworthiness of the study, Guba and Lincoln’s criteria were used.

Result: After analysis, the latent meanings formulated into one theme,” keeping up confident beliefs,” which consists of “religious beliefs,” “inner call,” “philanthropic sense,” “empathetic care” and “divine rewards” categories.

Conclusion: Many factors are heading to internalizing professional integrity in nurses in Iran. Authorities, managers, and faculty members should pay special attention to the internalization of professional integrity and ethical values, such as altruism, conscience, empathy, and the presence of God in life to decrease the mistakes and promote quality of care.

Keywords: Nurse, Integrity, Spirituality, Qualitative research

Introduction

Professional values are standards for action accepted by the professional groups and experts, which provide a framework for evaluating values and beliefs that affect behavior. These values are the basis of nursing practice and guides nurses’ interactions with patients, colleagues, other professionals, and the public. Values provide a framework for commitment to patient well-being and guide them to ethical behaviors in the provision of humanitarian and safety care.

The American Nursing Association has identified a set of ethical values and behaviors specific to the nurses. Nurses are needed to internalize these values in order to maintain and develop their profession. These values include respect for human dignity, accountability, compassion, trust, and professional integrity.
Professional integrity, as a profound individual phenomenon and as a part of professionalization, provides a connection between actions on the one hand, and beliefs and principles, on the other hand. It is defined as a commitment to the five fundamental values of integrity, trust, equality, respect, and accountability, besides referring to the acceptance of principles plus ethical standards and practices based on ethical codes. The value systems of a person form the basis of professional integrity.

A struggle for maintaining professional integrity in a care challenging environment can lead to moral distress and consequently leaving the profession. Moral distress with deleterious effects on mental health is followed by anxiety and failure in nursing care. In the case of vulnerable, the negative effects are expressed as anger, worthlessness, depression, and discomfort. Determine factors affecting professional integrity in nurses are very important. However, the factors affecting professional integrity in nurses are unknown and no study has been conducted in this field to reveal these factors. Few studies in this field have been mostly quantitative and have considered only one aspect of professional integrity. For example in a study in Iran, which has been done in a cross-sectional descriptive method, has only examined the level of professional honesty in nurses and in the recommendations section of this study, it has been suggested a study should done for identifying factors can reduce or increase the rate of professional integrity. On the other hand, quantitative study cannot answer complex and less known issues, with a qualitative study can be discovered, deep description and explanation of unknown or less known phenomena. Qualitative studies in the field of professional integrity have also focused on other aspects of this phenomenon that are different from the purpose of the present study besides these research were done in other countries which are different in the context and culture. Such as a study in USA aimed to explore local experiences of professional integrity in pre-registration nurse education by grounded theory that objective of this study, research method and also participants of this study are different with our study. Therefore, this study aims to investigate factors affecting professional integrity in Iranian nurses.

**Materials and Methods**

This study is a qualitative content analysis driven from a Ph.D. thesis carried out from April to October in 2019. The research environment was the educational hospitals affiliated to Golestan University of Medical Science (North of Iran). By a purposive sampling approach, 17 participants were enrolled. Participants were nurse practitioners, head nurses and faculty members. Data collection instruments were semi-structured interviews and observation. The interview lasted from 25 to 90 minutes, depending on the participants. Interviews were held in a calm room according to the nurse’s suggestion. Two interviews were held in the faculty of nursing and midwifery and the rest in their wards. All interviews were conducted face-to-face by one researcher (first author) and audio-taped. The interview began with tow general questions: “Can you please describe professional integrity in nursing?” and based on your opinion, what factors are affecting to professional integrity in nurses. Participants were also asked to expand on their responses. Then, more questions were asked depending on the answers of the participants. Each interview transcript verbatim in the first 24 hours, coded within 48 hours by one researcher (Ph.D. candidate), and checked by the rest of the authors. In addition to interviews, the researcher wrote 14 field notes from his observations in teaching hospitals. Observations were purposive and based on interviews data analysis. All observations which were in 19 shifts (eight in the morning, seven in the afternoon and four in the night shift) were written. MAXQDA10 software was used for managing the data.

**Data Analysis**

Data analysis was performed by the Graneheim and Lundman qualitative content analysis method. The researcher transcribed the interviews and observations
verbatim and read them all several times to obtain an immerging sense of the data. Paragraphs, sentences, or words were taken as a meaning unit; they were coded according to their underlying concepts. In the second step, these codes were compared based on their similarities and differences; then, they were assigned specific labels and came to specific subcategory. After deep reflection on subcategories content, subcategories were merged into a category based on their similarity. Finally, similar categories with the same meaning merged the main theme. In addition of interviews several observations has done in various shifts to enrich the data analysis. Observation notes were coded and these codes merged with interview code index. In analyzing observations, the researcher was reviewing what was witnessed and recorded. Then researcher arranged them by converting all the data into a text format. The next step was organizing observations by going back to research objectives and then organizing the data based on the questions of research. After organizing they coded by the first author. In coding stage categorizing and assigning properties and patterns to the collected data was done. Ultimately, codes were used in data analysis process.

Trustworthiness

For the validity and reliability of the study, Guba and Lincoln’s criteria were used. It has four criteria including credibility, dependability, conformability, and transferability. The researchers tried to fulfill the credibility by engaging with the participants and the data collection process for a long period and checking the transcripts with the participants. Dependability was established by performing the process of step-wise, as well as auditing reviews by supervisors (second and third authors), consultants (third and fourth authors), and other experts. Conformability was improved over reviewing the study by checking the analyses process by supervisors. To achieve the transferability of this study, the researchers tried to provide an accurate report of the participants’ statements for the feasibility of findings in other contexts.

Ethical Consideration

The study was approved by the Research Deputy and Research Ethics Committee of Golestan University of Medical Sciences (Project number: 970405058, Ethics Code: ir.goums.rec.1397.032). Information included aim of the study, the voluntary nature of the participation, their right of confidentiality as well as right to withdraw from the study at any time were explained to the participants. Additionally, permission for voice recording was taken from the participants. The participants then signed an informed consent form.

Results

17 participants were interviewed, 17 interviews were overall held, seven of whom were male and the rest female. From the job position, three participants were head nurses (HN), two of them were a faculty member (FM) and the rest were clinical nurses (NP). Age ranges of participants were 23 to 53 years with a work experience of 6 months to 30 years.

Theme and categories

After deep reflection on the data transcripts (interviews, observation notes), 1583 initial codes were extracted; by the merging of similar codes, 686 primaries were extracted, these codes were categorized in 19 subcategories and consequently merged to the five categories according to their similarities and differences. After analysis, the latent meanings were formulated into one main theme,” keeping up confident beliefs,” which consists of “religious beliefs,” “inner call,” “philanthropic sense,” “empathetic care,” and “divine rewards” categories (Table 1).
Table 1. Theme, categories and subcategories emerged from data analysis.

<table>
<thead>
<tr>
<th>Theme</th>
<th>Categories</th>
<th>Subcategories</th>
</tr>
</thead>
<tbody>
<tr>
<td>keeping up</td>
<td>Religious beliefs</td>
<td>God’s observation on acts”, “considering God in care affairs”, “acquiring Halal income”, “trust in God” and “acquiring God’s satisfaction”.</td>
</tr>
<tr>
<td>confident beliefs</td>
<td>Inner call</td>
<td>“Inner persuasion to do the best for patients”, “job conscience “and” inner call for having integrity in care”.</td>
</tr>
<tr>
<td></td>
<td>Philanthropic sense</td>
<td>“Being interested in serving the people”, “cherishing the existence of humans”, “Feeling the need to help people” and “loving the profession”.</td>
</tr>
<tr>
<td></td>
<td>Empathetic care</td>
<td>“Consider myself as a patient”, “Consider relatives as a patient” and “Empathy with patients”.</td>
</tr>
<tr>
<td></td>
<td>Divine rewards</td>
<td>“God returns the result of honest care, “having God’s blessings,” “low worthiness of profession financial aspect,” and “tranquility in life”</td>
</tr>
</tbody>
</table>

1 (Religious Beliefs)

These categories were labeled on “God’s observation on acts,” “considering God in care affairs,” “acquiring Halal income,” “trust in God,” and “acquiring God’s satisfaction” subcategories. Almost all participants disclosed the role of God in internalization of professional integrity. One of the most important factors which influenced their care quality was their beliefs about God. They were believed that God is always observing their acts, record them and return the result of their acts to them.

1.1. God’s observation on acts: participant 2 said: “What I see, God is watching everything; we do our care, may nobody see what I am doing, but I’m sure that God is always watching our activities” [P2].

1.2. Acquiring God’s satisfaction: one of the participants said: “we have to acquire God satisfaction by doing our best for patient’s wellness [P12].

1.3. Considering God in care affairs: Another one emphasized that” we do what asked by the doctor; besides these, God asks us to do the right thing for patients. Situations in which nobody is present, you are yourself and God. May patient does not know what you are doing exactly. It is integrity based on which, for example, a medication that should be injected is injected properly “[P5].

1.4. Trust in God: The third participant said: “Every day, I start my work keeping trust in God. Before going to work and after prays, I say to God: please help me to have a chance helping patients and not being shameful in front of them.”[P3].

1.5. Acquiring Halal income: Two participants said that our income has to be earned in a Halal way (the right way); so, we must consider integrity in our professional affairs [P1, P5].

2) Inner call

The inner call is assumed as an appropriate title for “inner persuasion to do the best for patients,” “job conscience,” and “inner call for having integrity in care” subcategories. Some participants had a sense of hearing a voice that pushes them to do their care accurately. Most of them call this inner voice as “conscience,” and some call it “inner force feeling.” They know it as a strong force, which is not ignorable. They revealed that if they ignore it or make a mistake in care affairs due to clinical challenges, they get disturbed; in some cases,
they also involved with bad feelings, such as “a doom of conscience” or “sorrow” after work at home.

2.1 Inner persuasion to do the best for patients: Another one said: “I’m a responsible person. If someone asks something to do, I have to do my best; if I do not, a doom of conscience comes to me. I have to complete my responsibility. I try not to harm anybody; if a bad event is going to happen, I prefer it to happen to me, not to others”[P7].

2.2 Job Conscience; Participants 6 said I always remind myself that as a head nurse I have some responsibilities which is important for improving the quality of care delivering by nurses who work in the ward [P6].

2.3 Inner call for having integrity in care: One of the participants disclosed: “Conscience is with me everywhere. Whatever I do, my conscience is with me. Yesterday evening it happened, I said to myself that I do not check the temperature of patients today. It was in the morning; I checked their symptoms in the morning. I checked the temperature. Patients were not febrile; I decided to only check the blood pressure in the afternoon. I feel I can’t do it”[P8].

3) Philanthropic Sense

The philanthropic sense was labeled on “being interested in serving the people,” “cherishing the existence of humans,” “feeling the need to help people,” and “loving the profession” subcategories. All study participants except the last one (contrary case) adored their profession and helping others. It gave them a great energy for overcoming many challenges that exist in clinical setting.

3.1 Being interested in serving the people: participant 5 said: “Interest is very important. I terribly love my job. If I go backward, I will choose this job again. I love working with sick persons. When I do something for a patient, and he/she says God bless you or God forgives/bless your parents, it’s very valuable for me”. [P5].

3.2 cherishing the existence of humans: Participants I said: “All humans are valuable in my opinion. We are gathered to help each other and I think helping others is the philosophy of human existence” [P1].

3.3 feeling the need to help people: participant 10 stated that: “I chose to work as a nurse here to meet the patient’s needs I feel happy when the patients’ condition improves. as a matter of fact, I have a need to help others to feel more happiness in my life.”[P8]

3.4 Loving the profession: Another participant disclosed: “I love nursing. I like to continue my education. If I have a choice for a job again, I will choose to be a nurse practitioner. Even if I continue studying for the Master or Doctoral level, I will never leave the clinical practice” [P14].

4) Empathetic care

Empathy was one of the prominent characteristics of all study participants. The item subcategories consist of “consider myself as a patient,” “consider relatives as a patient,” and “empathy with patients.” All participants emphasized that when they are taking care of patients, they consider patients as themselves or relatives, such as their parents or children. They disclosed that as they won’t anybody take inappropriate care from their relatives, they are careful about patients to prevent mistakes.

4.1. Consider myself as a patient: Participant 3 said: “I’ve tried to think from first that my family or I am lying on this bed. Right or wrong, I do not know, maybe it’s a bit too hard to imagine. I always consider that, for example, a young one who is patient lying on the bed is my brother. I won’t anything bad happen for my brother, so I do my best to care appropriately and prevent mistakes” [P3].

4.2. Consider relatives as a patient: Another one said: since I do not want any nurse to harm my family when they are sick by their carelessness or irresponsibility, I try to do my job with responsibility and integrity [P15].

In the observation period, sometimes we encountered with dishonesty in taking care. For example; patient’s
participant referred to the nurse station and asked her to visit his patient, she said I will come but did not do that. When the researcher asked the reason of her neglecting, she replied “we have many works to do in the shift especially patient’s file recording. If we go several times on patient’s bed, we have no time for recording files and other jobs. Based on participants declarations there are many challenges a heading of clinical nurses, which decline their motivation for taking care, based on integrity such as organizational and managerial affairs, high workload, lack of facilities, low income and etc. For example, one of them complained of challenges and said:” you also know our burdens, we have any support from the nursing organization in this system even a patient’s companion is more valuable than me as a nurse. It happened that a one of them insulted me but the supper visors did not support me. There are many examples like this. Why I scarified myself for this system (nursing)?”[P17]

4.3. Empathy with patients:

One of the participants stated that:” Sometimes patients may complain of pain or problems. I try hard to understand her and take immediate action to reduce or alleviate her pain” [P2].

5) Divine rewards

Study participants strongly believe that the financial aspect of their job did not affect their integrity. They believe that God returns the result of their honest care in their life in another way. This category was the outcome for the empathetic care for main participants and raises from “God returns the result of honest care,” “having God’s blessings,” “low worthiness of profession financial aspect” and “tranquility in life” subcategories.

5.1 Low worthiness of profession financial aspect: One of them said: I cannot say that the financial aspect is not important for me at all. It’s far from reality, but from the bottom of my heart, I do not care much about it. I believe that if I do my job with integrity, God will help me more than money in life. Money is a small part of life [P11].

5.2 God returns the result of honest care: Another one says: you may hear from some nurses that “they don’t pay enough money based on our work hardship, so why we should devote ourselves for patients, “ but I am totally disagree with this thought because the patient should not pay a fine for it. God will give us more than money.”[P10].

5.3 Tranquility in life: The same participant stated that: “I have tranquility in my life; it’s more valuable than money for me “[P10].

5.4 Having God’s blessings: Participant 5 says:” I worked nearly twelve years as a nurse in this ward and nine year as a head nurse. In both position I did my best for patients. I passed several hard situation in my life that I understand its just for God’s blessing in result of my professional integrity. [P5].

Discussion

The aim of present study was explaining the factors affecting professional integrity in nurses. Based on the result of this study, many factors included religious beliefs, inner call, philanthropic sense, empathetic care, divine rewards, were effective to internalizing professional integrity in nurses in Iran.

The religious beliefs is the first category in this study. Religious values and beliefs are the most widely documented personal influencers of ethical beliefs among health professionals. Participants discussed religious beliefs about God and his influence in their actions. Religious beliefs are recognized as a tangible expression of spirituality and the affirmation of higher power 18. These beliefs affect one’s ethical decision-making. Religious beliefs affect the performance of nurses. This is confirmed in a British study based on which, nurses’ spiritual beliefs contribute to their attitude towards patients’ suicide 19. The experiences of participants reveal that their beliefs influence their actions, similar to the previous study. Study participants mention beliefs about God’s presence in their life, Halal income, and attempt to acquire God’s satisfaction. These thoughts strongly affect their actions and prevent
them from ignoring integrity in their professional roles. Professional integrity involves one’s conscience, and when his/her belief systems differ with a situation, it can cause conflict with the decision-making 20.

The second category is inner call. Participants mentioned inner call as a main reason for internalizing professional integrity. The current study participants show feelings, such as “a doom of conscience” or “sorrow” after incompatibility due to deviating from integrity in care affairs. They were believed that working according to the integrity help them feel tranquility in their occupation. Nurses confront situations that require ethical consideration and judgment on routine affairs. When they can meet their expectations under certain care conditions and their moral decisions are in accordance with their conscience, they are at a lower risk of suffering from the feel of sin or mind disturbance21. Another study confirm the influence of conscious and inner feelings of moral obsession on their care roles. When nurses are unable to balance their moral integrity with ethical issues, the result can be moral distress22.

Philanthropic sense is the third category. Caritas’s idea, love, and affection are the core of all nursing care. According to Ericsson, the ability to love is the true humanity that is nurtured in the form of caring. Loving care indicates unselfishness and altruism; “altruism is selfless concern for the welfare of others” 23. Loving the profession is one of the prominent factors in all study participants. They emphasize that they really love helping others. They devote themselves in spite of difficulties in nursing profession in the country, such as issues mentioned by Farsi Z et al. as low levels of respect, low income, heavy workloads, high patients’ expectations, managerial issues, lack of staffs’ resources in the healthcare system 18. In our study, some declare even they spend a part of their wage to help patients, and some do nursing for some poor patients at home free.

Another category is empathetic care. In this study main participants who were nurses qualified by professional integrity revealed empathetic care in clinical setting in spite of many challenges. Based on experiences, empathy with patients has a very effective and accelerating role, in other words, nurses’ empathy with patients provides a shortcut to communication with patients. Based on another study in Iran, nurses’ empathy with patients is the core of the interaction between nurses and patients and causes nurses to understand the conditions, needs and feelings of patients in performing nursing intensive care and take steps to meet their needs24. In confirmation of the results of our research, empathy is defined as, “the ability to understand and share the feelings of another.” It is the capacity to put one’s self in another’s shoes and feel what that person is going through and share their emotions and feelings. It is the recognition and validation of a patient’s fear, anxiety, pain, and worry25. Besides of finding our study, result of a review study demonstrated empathetic also care lead to enhancing quality of care, eliminating of errors, and an increasing satisfaction of patient and nurse 26.

Divine rewards is the last category. Based on experiences of our study, God returns the result of honest care, having God’s blessing, low worthiness of profession financial aspect and tranquility in life are main sub- categories of this category. Based on a study nurses emphasized that many of the prayers of the patients’ companions lead to inner rewards for them, which include keep our nurses healthy and strong in all areas of their lives. Protect their families, their marriages, their children, bless them with so much compassion that it spills effortlessly into their patients’ lives, which are consistent with our research findings 27. However, in a qualitative study which is done in Netherlands nurses placed more emphasis on external rewards, which in that research three main reward categories were derived: financial, non-financial and psychological rewards. These differences may be due to the role of different religions in care as well as different cultures and contexts28.

Conclusion

This study shows that many factors are effective to internalization of professional integrity in nurses. Keeping up confident beliefs is as a main theme that
contain all of them. It a great role in acting in nursing according to the principles and in many cases it is beyond their responsibilities (we can call it self-devotion). This study is carried out in Iran; however, professional integrity has no boundary and it may be different in other countries and other cultures, thus, it can be investigated in all nurses and other allied health care professions in the all of world. Authorities, managers, and faculty members should pay special attention to the internalization of ethical values, such as altruism, conscience, empathy, and the presence of God in life to decrease the mistakes and promote quality of care.

Acknowledgments: We thank all participants who shared their experiences with the research team. This study was approved by the Research and Technology Deputy of Golestan University of Medical Sciences, Golestan, Iran

Conflict of Interest: The authors declare no conflict of interest in this study.

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Post-mortem Gross Detection of Early Myocardial Infarction using Triphenyl Tetrazolium Chloride Test

Ali Ayad Abdulridha¹, Saad Kadhim Kareem²

¹Scholar Researcher, Medico-Legal Directorate, Ministry of Health, Iraq, ²Professor of Forensic Medicine, College of Medicine, Al-Nahrain University, Iraq

Abstract

Background: Ischemic heart disease is the leading cause of mortality in the world. More than 17 million people die each year as a result of myocardial infarctions. Post-mortem histochemical detection of early myocardial infarction using 2,3,5-Triphenyl Tetrazolium Chloride (TTC) technique is based on the fact that ischemic myocardial cells lose their membrane integrity and release dehydrogenase enzyme into the blood stream, resulting in decrease or total depletion of these enzymes within the necrotic areas of the myocardium, and so, after immersion in TTC solution, enzyme depleted necrotic myocardium will not retain TTC dye and therefore appears as pale/yellow areas.

Materials and Methods: This prospective postmortem study was conducted at Medicolegal Directorate in Baghdad city during the period from March 2020 to December 2020. Myocardial specimens were isolated from 75 cases of sudden natural death and were subjected to 1% TTC solution staining for gross assessment, and subsequently, tissue samples were taken from suspected areas, processed and stained with H&E for histopathological evaluation and detection of a possible acute myocardial infarction.

Results: The mean age of studied cases was 50.3 ±12.57 years, ranging from 18 to 78 years, median age was 53 years. Males constituted the majority of cases (80%). Cases were predominantly within 40-59 years age group (58%). The postmortem interval was ranging from 2 to 17 hours (mean= 8.6 ±3.6 hours). After immersion in 1% TTC solution, 45 myocardial specimens showed macroscopic pale/yellow areas which were indicative of acute myocardial infarcts within the ventricular wall, while histopathological examination of specimens revealed features of acute myocardial infarction (AMI) in 62 specimens. TTC stain was found to have a diagnostic sensitivity of 69.4% and specificity of 76.9% in postmortem detection of AMI.

Conclusion: The TTC technique allows identification of early myocardial necrosis. It’s practical, reliable and valid method that promises to be of considerable value. It can be used together with histopathology for postmortem detection of visually unapparent acute myocardial infarcts.

Keywords: TTC, myocardial infarction, sudden death, postmortem diagnosis

Introduction

Ischemic heart disease (IHD) is considered as the main cause of natural deaths in both men and women. (1) Sudden natural deaths involve important challenges in forensic pathology, especially when death takes place shortly after coronary occlusion. Following myocardial ischemic damage, it usually requires 24 to 48 hours for the damaged area to be visually apparent, area of hyperemic borders with central tan softening maybe observed after 3-7 days. Wavy fibers, in association with interstitial edema are often described as the earliest light microscopic changes of necrosis as it can be detected in the infarcted region within 3 hours after coronary occlusion. (2) Triphenyl tetrazolium chloride (TTC) is commonly used in histochemical staining for detection of necrotic myocardial tissue, it’s based on the fact that ischemic myocardial cells lose their membrane integrity and release their enzyme contents into the blood stream, resulting in a marked decrease or total depletion of these enzymes in the ischemic areas of the myocardium. The postmortem histochemical detection of myocardial
infarction is relied on immersion of myocardial specimens in TTC solution, intact myocardial tissue will be visualized as brick red while dehydrogenase deficient infarcted zones will not retain TTC stain and therefore appear grossly as pale/yellow areas\(^{(3)}\). The current study aim to explore the reliability of Triphenyl Tetrazolium Chloride staining in gross delineation of necrotic myocardium. Furthermore, to facilitate postmortem macroscopic recognition of early myocardial necrosis for subsequent histopathological evaluation.

**Materials and Methods**

This prospective postmortem study was conducted at Medicolegal Directorate in Baghdad city during the period from March 2020 to December 2020. Seventy five cases of sudden natural death were investigated for a possible underlying acute myocardial infarction (AMI) as a cause of death. On a hard dissecting platform, serial 10mm-thick sections of the heart was obtained using a dissection knife, each section was thoroughly examined in order to identify the ischemic risk zones, the territories of coronaries perfusion were examined, with careful appreciation to zones at risk. Slices were photographed using digital camera with magnification. Specimens with no visually apparent infarcted zones were taken from each heart, washed with distilled water, the slices were placed in a sealed container with 1% TTC solution and incubated in 38°C degrees; slices were turned upside down after 15 minutes to obtain a uniform staining. After incubation for 30 minutes, heart slices were photographed and examined for unstained zones within myocardium, normal myocardium stained brick red whereas infarct zones appear pale/yellow or show very much reduced staining. Pale unstained zones were regarded as indicative for AMI, whereas slices completely stained brick red regarded as negative for AMI. After macrostaining with TTC solution, both TTC positive and negative specimens were incubated in 10% formalin. Sections were mounted on labelled microscope slides for routine histopathological examination. Microscopic observations of ischemic changes including myofibrillar waviness, hyper eosinophilia, coagulative necrosis, neutrophilic infiltration were considered as a diagnostic feature of AMI.

**Results**

This prospective study included 75 cases of sudden death autopsies conducted at Baghdad Medicolegal Directorate; their mean age was 50.3 ±12.5 years, ranging from 18 to 78 years, the median age was 50 years. Males constitute the majority of cases (n=60). Table 1 illustrates the frequency of cases according to age groups, 44 subjects were within 40-59 years age group and within this group, 35 subjects were males. Considering past history prior to death, 70.7% (n=53) of cases had at least a single pre-existing risk factor for developing IHD.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age Group</th>
<th>Total</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18-39</td>
<td>40-59</td>
<td>60-80</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>35</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>9</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>44</td>
<td>17</td>
<td>75</td>
</tr>
</tbody>
</table>
Approximate time of death was obtained from information provided by police autopsy inquest, in addition to the observed postmortem changes. The postmortem interval (PMI) was ranging from 2 to 17 hours (mean= 8.67 ±3.69).

Following immersion in 1% TTC solution, slices from 4 cases failed to retain the dye, and within the remaining 71 cases, 45 showed macroscopic pale/yellow areas which were indicative of acute myocardial infarcts within the ventricular wall (Figure 1). Microscopical histopathological examination of specimens revealed features of AMI in 62 specimens (Figure 2).

Figure 1: Triphenyl tetrazolium chloride (TTC) staining of transverse heart slices. Photographs on the left are myocardial sections before TTC staining. Same slices on the right after immersion in TTC solution showing areas of staining defect which are indicative of acute myocardial infarcts (arrows).
Figure 2: Photomicrograph showing acute myocardial infarction by H&E  A. Interstitial edema with myofibrillar waviness (10x).  B. Subendocardial MI showing vacuolated myocytes with coagulative myocytolysis (40x).

Table 2 summarizes the statistical values considering TTC and H&E staining of specimens. There was significant statistical differences (p value= 0.001). TTC stain was found to have a diagnostic sensitivity of 69.4% and specificity of 76.9%.

Considering the four specimens which fail to retain TTC stain, no significant correlations were evident when the stainability of TTC was compared with age of deceased’s or the PMI. The final postmortem diagnosis was ruled as AMI in 62 cases (82%), other cardiac diseases in 6 cases and respiratory infections in 4 cases. AMI was more prevalent in male and more profound within 40-59 years age group.

<table>
<thead>
<tr>
<th>Staining</th>
<th>Histopathology</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative for AMI</td>
<td>Positive for AMI</td>
</tr>
<tr>
<td>TTC Staining</td>
<td>Count</td>
<td></td>
</tr>
<tr>
<td>No staining</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>100% within TTC</td>
<td>25%</td>
<td>75%</td>
</tr>
<tr>
<td>100% within Histopathology</td>
<td>7.7%</td>
<td>4.8%</td>
</tr>
<tr>
<td>Negative for AMI</td>
<td>Count</td>
<td></td>
</tr>
<tr>
<td>100% within TTC</td>
<td>38.5%†</td>
<td>61.5%</td>
</tr>
<tr>
<td>100% within Histopathology</td>
<td>76.9%§</td>
<td>25.8%</td>
</tr>
<tr>
<td>Positive for AMI</td>
<td>Count</td>
<td></td>
</tr>
<tr>
<td>100% within TTC</td>
<td>4.4% ‡</td>
<td>95.6%†</td>
</tr>
<tr>
<td>100% within Histopathology</td>
<td>15.4%</td>
<td>69.4%*</td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td></td>
</tr>
<tr>
<td>100% within TTC</td>
<td>17.3%</td>
<td>82.7%</td>
</tr>
<tr>
<td>100% within Histopathology</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

† Negative predictive value ‡ Positive predictive value * Sensitivity § Specificity
Discussion

Triphenyl tetrazolium chloride (TTC) staining is a convenient procedure for detection and demarcation of myocardial infarct zones. It’s dependent on loss of dehydrogenases from the irreversibly damaged myocytes. In this study, MI was the leading post-mortem diagnosis of natural death autopsies and was more prevalent in 40-59 age group which is consistent with recent studies conducted in Iraq by Al-Qazzaz et al. (2012)(4) and Abdulrahman (2016). (5)

More than 70% of the examined individuals in this postmortem study had at least single risk factor for developing IHD. A review study by Aljefree and Ahmed (2015) showed high prevalence of risk factors for developing cardiovascular diseases in the countries of The Gulf Cooperation Council, which was attributed to urbanization, cultural, lifestyle, and environmental factors in these countries.(6) In Iraq, however, bad social and dietary habits, lack of awareness, poor preventive measures might be crucial contributing factors.

The main concept of this study is the visual macroscopic detection of AMI using TTC regardless the size and extent of necrotic area, a study by Kakimoto et al. (2013) relied on computerized software planimetry to detect minute foci of infarction as well as to quantify the size and ratio of infarct zones in comparison to the remaining viable ventricular mass. (7)

As summarized in Table 2, the comparative analysis of histopathological examination with TTC staining technique in the present study showed that out of the total 75 cases, AMI was evident in 62 specimens by H&E, and out of these cases, it was observed that specimens of 43 cases showed macroscopic demonstration of AMI by TTC staining technique and 16 specimens were TTC-stained negative for AMI. The overall TTC sensitivity and specificity were 69.4% and 76.9% respectively.

Adegboyega et al. (1997) reported that histochemical staining using TTC solution for gross detection of early infarction has diagnostic sensitivity of 77%. (8) A review article by Kundal et al. (2012) showed that the overall efficacy of TTC test in postmortem diagnosis of AMI is 88%. (9)

Another study by Shrigiriwar et al. (2019) had noticed that TTC staining technique is more superior to the conventional H&E staining in detection of AMI, the study included known cases of MI and IHD. TTC staining technique was positive for MI in 80% of cases while H&E method was positive in 33.3% of cases, the study further recommended the TTC staining technique to be included in natural deaths autopsies as it was considered more sensitive and efficient method than the H&E microscopic examination. (10) These results support the previous findings by Gupta et al. (2013) who suggested that TTC stain has a higher efficacy than microscopical evaluation by H&E in detection of AMI. (11)

Unfortunately till date, there is no local published data considering the use of TTC technique in postmortem practice, and it’s well known that postmortem diagnosis of AMI is essentially relied on histopathological examination by H&E to define necrotic zones, but unapparent infarcted zones are often missed during random sectioning.

There were 26 cases that showed no evidence of AMI by TTC staining even though H&E examination identified AMI in 16 cases in this group. Some studies have questioned whether staining of viable cells can mask necrotic ones in the highly irregular border zone. It is known that the formazan compound of TTC is lipid soluble and readily diffuses to adjoining cells/tissues, especially those rich in lipids, therefore necrotic tissue in the TTC stained slices might be estimated as being smaller than their actual size. (12)

Some observations suggest that TTC staining may be subjected to artefacts, it appears that superoxide dismutase has the potential to enable necrotic myocardium to retain its ability to stain as viable tissue for at least 24 h after the onset of the ischemic insult followed by reperfusion. (13-15) It’s possible that myocardial cells, which have become irreversibly injured but have not yet lost a sufficient amount of NADH will be stained,
Therefore, infarct size will be underestimated.

Following immersion in TTC solution, only 4 specimens fail to retain the TTC dye, this could be attributed to PH of the solution, a precise PH range of 7.4 is required for proper tissue staining. Photosensitivity of the Tetrazolium dye, prolonged exposure of TTC salts or TTC solution to light leads to inactivation of the tetrazolium salt. Another factor that may compromise the efficacy of the test is the prolonged storage of TTC salts. Kakimoto et al. (2013) found that TTC stainability decrease logarithmically when PMI is more than 1.5 days. Another limitation was the decrease in TTC staining quality for aged patients (more than 80 years). In the current study, however, no correlations were evident between TTC staining results and age or PMI in this study.

Conclusions

The overall Triphenyl Tetrazolium Chloride (TTC) sensitivity and specificity in this study were 69.4% and 76.9% respectively. TTC technique allows identification of early myocardial necrosis. It’s practical, reliable valid method that promises to be of considerable value. Random sectioning of heart for histopathological examination often miss the necrotic zones if the section does not include the unapparent infarct area, in these instances, TTC can be used together with histopathology for detection of early and unapparent myocardial infarctions.

Acknowledgment: Special thanks to colleagues and staff in Medicolegal Directorate with particular appreciations to members of Histopathology Department for their assistance in the implementation of this study.

Conflict of Interest: None

Funding: self

Ethical Clearance: Not required

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Fetal and Maternal Plasma Thyroid Hormone’s Levels in the Guinea Pigs

ALwan Ali Fadil¹, AL-Souz Mohammed Abdul Hadi Khalil², TareqHafdhiAbdtawfeeq Al-Khayat¹
¹Prof., ²Asist.Prof., AL-Farahidi University.Medical Technology College.Department of Medical Laboratory Technology, Baghdad, Iraq

Abstract

Background: All available data indicate that the thyroid of the guinea pig fetus is capable of functioning independently of that of the mother. Therefore fetal thyroid function is necessary for normal growth in utero. Intrauterine adaptation to the outside environment is important mechanism via which fetus increases the chance to thrive after birth. Aim and Objective of this work to assess the plasma level of thyroxin (T4) and Triiodothyronine (T3) through most period of pregnancy in fetal and maternal guinea pigs. Materials and Methods: Blood samples collected from 51 pregnant guinea pigs and their fetuses (93), by the Cobase 411 device produced by, Roche com. and it works with technology Electro, Plasma T4 and T3 were measured by ChemiLuminescence immune-assay. Results: Fetal plasma Thyroxin (T4) concentration was greater than that of the mother between Results 50 and 62 days of gestation while fetal plasma T3 concentration was less than that of the mother throughout gestation. In fetal guinea pig the reciprocal relationship between pituitary and thyroid is established at approximately day 34-35 of gestation. Conclusion: The results indicated that the fetal pituitary–thyroid system (PTS) is functioning at a relatively high level than that of the maternal, starting few days before the second half of pregnancy. Thyroid hormones are important for fetal growth and maturation event.

Keywords: Fetal, Maternal, thyroxin, Tri-iodiothyronine, Guinea pig.

Introduction

The follicle is the smallest functional unit of the thyroid. The iodine containing thyroid hormones (TH) are synthesized from a large glycolprotein thyroglobulin. The anterior pituitary gland secretes a thyroid stimulating hormone (TSH) which is under the control of hypothalamic neurohormone¹.

Human as well as animal studies have suggested that very high or very low maternal TH level during development may induce a shift in Hypothalamus-Pituitary-Thyroid (HPT) system². AL-Zubaidy³ showed that the fetal pituitary gland of guinea pig has the same morphology as in adult after 40 days of gestation.

In most species total plasma T4 concentrations are usually approximately 100 times as great as the total plasma T3 concentrations¹. The total plasma THs concentrations are about 80 ng/ml and 1.5 ng/ml, respectively⁴&⁵. Thyroid hormones are necessary for body growth, especially, brain growth and differentiation⁶. THs are also required for full maintenance of the function of the nervous system and the lack of these hormones lead to an impairment of

Corresponding Author:
Prof Dr. Ali Fadil ALwan,
AL-Farahidi University, College of Medical Technology, Department of Medical Laboratory Technology, Baghdad, Iraq: Tel ( +964 ) 077088628626:
E-mail: Ali.Fadil @alfarahidi. Edu.iq
brain function \(^{7,8}\). As well as their effects on energy metabolism, the calorigenic actions important for small animals to tolerate prolonged exposure to cold\(^9\). THs are involved in the maturation of various tissue and organs including lung maturation \(^{10}\).

Intrauterine adaptation to outside environment is important mechanism via which a fetus increases its chance to thrive after birth\(^9\).

The previous studies concerning the thyroid hormone level in guinea pigs in pregnancy and in fetal stage lack specificity. Therefore, this work aims to assess the plasma concentration of thyroid hormones throughout different periods of gestation.

**Methods**

Dunkin Hartley guinea pigs of both sexes were used throughout this experiment. Animals were obtained from the closed colony maintained in the Animal House and were housed (2 to 3 animals per cage), under temperature controlled conditions and fed pelleted diet. The experiment was carried out on a female guinea pigs were caged with a male and the day on which sperm was found in the vagina was taken as day one of pregnancy.

Blood samples were collected from pregnant guinea pigs and their fetuses at 45, 50, 55, 60 and 65-67 days of pregnancy. All animals were either killed quickly by a blow at the back of the neck or anaesthetized by an Intra Prenatally (I.P.) injection of sodium pentobarbitone (45 mg/Kg body weight). After 10 minutes, five milliliters of blood were collected from the mother by cardiac puncture. The fetuses were located by palpation and the abdomen and peritoneum were opened by a midline incision and the uterus was exposed. Blood was collected from the umbilical vessels of each fetus. All experiments were performed between 11.00 and 12.00 a.m. Blood samples were transferred to EDTA tubes and centrifuged immediately. The plasma was aliquoted and stored at \(-20\) C until assay.

Thyroid hormones levels were determined using electrochemiluminescence immunoassay technique performed by Cobas e411 Analyzer, Roche company, The intra assay coefficient of variation was determined and found to be 11.7%. The extraction efficiency was found to be 81.87\(\pm\) 0.54% (mean\(\pm\) SEM; \(n=10\)).

Statistics: Treated groups have been compared with normal as control. All result have been presented as the mean \(\pm\) the standard error of the mean, where \(n=\) the number of samples in each group. Significance limits have been determined using Student's t-test with \(p<0.05\) were considered significant.

**Results and Discussion**

**Fetal and Maternal T4**:

The T4 concentrations in fetal plasma are low at 45 days (18.5\(\pm\)2.4 ng/ml) and increased significantly (\(p<0.05\)) by 50 days (27.6\(\pm\)2 ng/ml). Concentrations then again rose significantly (\(p<0.0001\)) to reach the highest level at 55 days (36.7\(\pm\)1 ng/ml). At this time, the T4 concentration in the fetus was significantly greater (\(p<0.0001\)) than the corresponding maternal value (25.4\(\pm\)1 ng/ml). Measurements of fetal T4 concentrations from 50 days of gestation until term, the difference was only significant between days 50 and 62.

After 55 days of gestation fetal plasma T4 concentration declined to 34.9\(\pm\)2 ng/ml at 60 days, 30.0\(\pm\)2.3 ng/ml at 62 days and 25.0\(\pm\)2.5 ng/ml at 64 days by which time there was no significant difference from that of maternal values (Table-1). These results indicated that the fetal PTS is functioning at a relatively high level.

**Fetal and Maternal T3**:

Plasma T3 concentrations changed in similar manner to fetal plasma T4 concentrations. Fetal T3 concentrations were significantly less maternal T3 concentrations at all stages of gestation except at 60 days (Table -1). Fetal T3 levels increased from 0.6 \(\pm\)0.1 ng/ml at day 40 to 1.5\(\pm\)0.1 ng/ml at day 50 of gestation. Peak concentrations of T3 (1.7\(\pm\)0.1 ng/ml) occurred on days 55 and 60 of gestation after which fetal T3 concentrations fell significantly (\(p<0.05\)) to day 62 and fell significantly (\(p<0.001\)) again by day 64 (1.1\(\pm\)0.1 ng/ml). The lowest levels, (0.96\(\pm\)0.1 ng/ml) were found on days 65 and
There were no significant changes in maternal plasma T3 concentrations throughout last third part of gestation (Table-1).

**Table 1 :- Plasma T4 & T3 concentration (ng/ml) in the mothers and their Fetuses during gestation.**

<table>
<thead>
<tr>
<th>Gestation age/in days</th>
<th>Mother/T4</th>
<th>Fetal/T4</th>
<th>Mother/T3</th>
<th>Fetal/T3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng/ml)</td>
<td>(ng/ml)</td>
<td></td>
<td>(ng/ml)</td>
</tr>
<tr>
<td>40</td>
<td>16.7 ± 1.2</td>
<td>18.5 ± 1.7</td>
<td>1.7 ± 0.3</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>45</td>
<td>18.5 ± 1.3</td>
<td>18.5 ± 1.9</td>
<td>1.9 ± 0.5</td>
<td>0.981 ± 0.1</td>
</tr>
<tr>
<td>50</td>
<td>19.4 ± 0.8</td>
<td>27.6 ± 2.0</td>
<td>2.7 ± 0.3</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>55</td>
<td>25.4 ± 1.1</td>
<td>36.7 ± 1.0</td>
<td>2.4 ± 0.2</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>60</td>
<td>21.8 ± 1.6</td>
<td>34.9 ± 2.0</td>
<td>2.1 ± 0.3</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>62</td>
<td>20.3 ± 1.4</td>
<td>30.0 ± 2.3</td>
<td>1.9 ± 0.3</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>64</td>
<td>22.1 ± 2.0</td>
<td>25.0 ± 2.5</td>
<td>1.8 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>65-67</td>
<td>23.0 ± 1.0</td>
<td>25.0 ± 1.3</td>
<td>1.8 ± 0.1</td>
<td>0.96 ± 0.1</td>
</tr>
</tbody>
</table>

The figure between brackets indicate the sample size.

The period between 32/33 and 34/35 days of gestation can be considered as the earliest time in gestation when fetal thyroid of the guinea pig might begin to synthesis hormone. Mitskevich concluded that the onset of the fetal guinea pig functional activity was at about 35 days of gestation. In human Fisher reported the concentration of iodide in fetal thyroid is about 10-12 weeks of gestation. Iodine is required for the production of TH control by fetal pituitary TSH. In the present study the concentration of T4 was much higher than that of T3 in the plasma of the fetal guinea pigs. The results of the present study agree closely with those reported by and less well, with those of in the fetal guinea pigs. Low serum T4 concentrations have also been measured in 11-16 week human fetuses. Obregon reported fetal thyroid gland reaches maturity by week 11-12, and begin to secrete hormone by about week 16. THs are controlled by fetal pituitary TSH at approximately 20 weeks of gestation these authors commented that the low T4 values are primarily due to low T4 binding protein (TBP) concentration. In humans the increases in fetal plasma T4 concentration could be due to increased fetal thyroid gland secretions and probably TSH dependent. These results indicate that the fetal...
pituitary–thyroid system is functioning at a relatively high level than the maternal at this time, resulting in a high fetal T4 secretion rate. Mean fetal plasma T4 concentration exceeds that of the motherat 50-62 days of gestation. During this period, an adequate supply of maternal thyroid hormones must be sustained to ensure normal neurological development.

In present study plasma, T3 had was found to be lower in the fetus than in the mother throughout gestation (Table 1). The low fetal T3 concentrations are in agreement with those reported by but higher than those reported by. Low fetal T3 concentrations have been seen in human. Plasma T3 was measured at 64, 65 and 67 Days of gestation showed a significant decrease compared to that at 55 or 60 days, this results is in agreement with the results reported by. These results indicate that the T3 change in fetal guinea pigs is quite different from that of the human fetus. The low level of T3 could be due to decreased T3 secretion, decreased availability of T4 for de iodination, or a decreased rate of extra thyroid conversion of T4 to T3. In human, Castro have measured a high level of rT3 in the plasma of fetal guinea pigs at 62 days. It appears that conversion of T4 to T3 is reduced in the fetus whereas conversion of T4 to rT3 is well preserved. During pregnancy thyroxin binding globulin (TBG) concentrations increase because of estrogen stimulated TBG production and thyroid gland secrete hormones under the control of the HPS. In human fetal TSH, T4 and TBG gradually rise to adult level by 36 week.

Effect of hypothyroidism in pregnancy are anemia, low birth weight and mental retardation in neonatal. Increase T4 production and T3 is critical for fetal brain development. Fetal development adaptively refers to the process by which the fetus is prepared for the environment it is about to enter, this is important for optimal chance of survival and reproductive success for offspring. Present results indicated that pituitary and thyroid hormones secretions are important for fetal guinea pig differentiation and development of various organs and neonatal outcome.

**Conflict of Interest**: Nil.

**Ethical Consideration**: Ethical permission was taken from ethics review committee of institute.

**Sources of Funding**: The research was funded by the authors.

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Effect of Different Drugs on Diuretic Resistance Indices in Patients with Renal Impairment Using Intravenous Furosemide

Alyaa Abdalrazaq Abass¹, Ali Mohammed Hadi², Jubran K. Hassan³

¹Pharmacist, College of Pharmacy, University of Thi Qar, Iraq, ²Assist.Prof, Clinical Pharmacy, Department of Clinical Pharmacy, College of Pharmacy, University of Basra, Iraq, ³Assist.prof, Clinical Pharmacy, Department of Clinical Pharmacy, College of Pharmacy, University of Basra, Iraq

Abstract

Chronic kidney disease is a medical condition defined as a reduction in kidney function, made known by glomerular filtration rate of a less than 60 mL/min per 1·73 m², or signs of kidney impairment (albuminuria), or both for at least three months period, nevertheless of the original cause.

Diuretic resistance in the edematous patient is defined as a clinical state in which diuretic response is diminished or lost before the therapeutic goal of relief from edema has been reached.

This study aims to find the effect of different drugs on kidney functionindices and the relationship of using these drugs with the development or improvement of diuretic resistance.

The study is a cross-sectional observational study. It was carried out at Al-Hussein-Teaching Hospital in Thi-Qar. Nasiriya. Data from 104 patients were collected and analyzed using different statistical methods.

The results showed that there was a different odd of effects of the drugs commonly used to patients with CKD due to other comorbidities on diuretic resistance indices. There was a significant increase in the body weight and decline in serum urea in patients already with edema when they used calcium ampoule. Serum urea was not affected by any of the other studied drugs. There were two drugs affected positively on serum creatinine, which are clopidogrel and heparin. Albuminuria was highly improved in patients receiving aspirin and atorvastatin. Clopidogrel significantly increased GFR in these patients.

Key words: Aspirin, Atorvastatin, Calcium ampule, Clopidogrel, CKD, Heparin, Hydrocortisone, serum urea.

Introduction

Chronic kidney disease is a medical condition characterized by a reduction in kidney function, made known by rate of glomerular filtration of a lesser amount of than 60 mL/min per 1·73 m², or signs of kidney impairment (albuminuria), or both, for at least three months period, nevertheless of the original cause (¹).

The main causes of CKD globally are Diabetes (DM) and hypertension in all high, middle-income nations and in many low-income countries (²) Rate, occurrence, and advance of CKD also differ within countries by ethnicity and social causes of health, possibly through epigenetic influence (³).
Diuretic resistance is a clinical condition when diuretic effect is lessened or disappeared before the helpful objective of edema has been overtaken (4). The most demanding problems that the cardio nephrologist must mark in daily clinical practice is diuretic resistance, because of considerable burden on health care costs and hospital admissions (5).

Loop diuretics are agents used in CKD to decrease blood pressure and treat edema (6, 7), such as Furosemide, bumetanide, and torsemide, they bind to the extracellular surface of sodium-potassium-chloride symporters (NKCCs) of thick ascending limb cells along the loop of Henle by translocation pocket at the blocking ion transport directly. Hence, they increase the excretion of sodium, potassium, magnesium, and hydrogen (8-9).

Loop diuretics are used for medical conditions like in Oedematose disorders include (congestive heart failure (CHF), hepatic cirrhosis, nephrotic syndrome), renal insufficiency, hypertension in kidney disease, hypercalcemia, hypernatremia, syndrome of inappropriate antidiuretic hormone (SIADH) and renal tubular acidosis (10, 11).

The comorbid diseases which associated with CKD should be treated, like hyperlipidemia, diabetes mellitus and hypertension, in order to decrease the cardiovascular events and improve the kidney function so that the quality of life. These drugs like aspirin that is used for its antiplatelet action. It is well known to use in secondary prevention of stroke and myocardial infarction (12). Clopidogrel, which is a thienopyridine drug that, by blocking the adenosine diphosphate P2Y12 receptor, inhibits platelet aggregation. Clopidogrel is used more commonly to avoid potentially lethal thrombosis of newly inserted coronary stents (13).

Meanwhile in CKD, both thrombotic events and bleeding are at greater risk. The initial stages of CKD are predominantly associated with prothrombotic risk, while platelets can become unstable due to uremic-related toxin toxicity in their advanced stages, besides the prothrombotic condition, leading to an increased predisposition to bleed. For the treatment or prevention of thromboembolic diseases, CKD patients typically need anticoagulation therapy like heparin (14).

In (CKD), hyperparathyroidism occurs in a reduced parathyroid hormone calcemic response (PTH). Ca ampule helps to establish hyperparathyroidism and is presumed to be due to decreased bone efflux of calcium, so prevent osteoporosis (15, 16).

Atorvastatin is used in patients at CKD due to risk of or with cardiovascular disease. It had renal protective effects and given for dyslipidemia atorvastatin improved kidney function over time in a dose dependent manner (17, 18, 19). The patients also take corticosteroid as hydrocortisone and dexamethasone which are used for breathing problems to asthmatic patients or for skin or drug allergy (20).

All these drugs may affect positively or negatively on diuretic resistance indices, which are body mass index (BMI), serum urea, serum creatinine, estimated glomerular filtration rate (eGFR) and albuminuria or they may have no any significant effect.

The goal of this study is to find the effect of different drugs on kidney function indices in patients with CKD treated acutely by furosemide, as a method of detecting diuretic resistance and factors affecting it.

**Patients and Methods**

This research was conducted at (Al-Hussein-teaching hospital) in Thi-Qar, Nasiriya. Data were collected from patients after getting approval from the Ethical and Scientific Committees of the Faculty of Pharmacy/ Basra University in addition to the Scientific Committee of Researches of Thi-Qar Health Directorate.

This study is a cross-sectional observational study, carried out from October 2019 to June 2020.

The Inclusion criteria were patients age of 18 years and older of either gender, patients or relative should be able to communicate and willing to participate in this study, patients that take loop diuretic (intravenous furosemide) only. Also, patients with chronic kidney
disease (GFR ≤ 60), diabetes mellitus, asthma, Chronic obstructive pulmonary disease (COPD), hypertension (HT), cancer and had no contraindication to diuretics or other standard medications for heart failure and kidney diseases. Excluded patients were those with acute infectious diseases like pneumonia, complicated urinary tract infection (UTI), human immunodeficiency virus (HIV), Children or Patients who were <18 years. Patients excluded if they refused to participate. Patients who had cognitive deficits (physical or mental state), females who were pregnant or breastfeeding, Patients taking oral diuretics or other diuretics than loop diuretic and patients with Acute Surgery. Patients with recent myocardial infection (MI) within 3 weeks also were excluded, in addition to patients who already on anticoagulant therapy for other indications. Patients with nephrotic syndrome, urinary tract malformation, urolithiasis, kidney transplant, Hemodialysis also were added to the exclusion list.

In the present study, (104) patients were recruited. They were all agreed with the data collection formats used. The data collected from patients by their specific file and the laboratory analysis.

Statistical analysis used in this study included the use of SPSS version 2017 and Excel Soft ware 2019, the p value of (<0.05) is considered as statistically significant. Statistical programs were Chi Square for analysis of frequency, T- test used for analysis for continuous variables defined as Mean ± Standard deviation (SD), Pearson correlation was performed to find correlation among different variables.

Estimation of GFR is done by modification of diet in renal disease (MDRD) equation\(^{(21)}\) and BMI (kg/m\(^2\)) calculated as BMI= \(\text{weight}/\text{height}^2\)\(^{(22)}\).

Albuminuria is known as an indicator for renal dysfunction, the method used to detect albuminuria is Albumin-to-creatinine ratio (ACR). This method detects albuminuria by a spot urine sample. By dividing concentration of albumin in (mg) to concentration of creatinine in (gm), we can calculate ACR \(^{(23)}\).

### Results

One hundred and four patients were included in this study, number of females was 54 (51.9%) and that of male was 50 (48.1%). Mean age of patients was (67±13.5). Other data regarding patients in the study considering BMI, laboratory data, age distribution are involved in table (1).

General characteristics of patients enrolled in the study and the drugs used are detailed in table (2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>50 (48.1%)</td>
<td>0.7686</td>
</tr>
<tr>
<td>Female</td>
<td>54 (51.9%)</td>
<td></td>
</tr>
<tr>
<td><strong>Data of patients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>67 ± 13.5</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>25.3 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>S. creatinine (mg/dl)</td>
<td>3.2 ± 1.7</td>
<td></td>
</tr>
</tbody>
</table>

Table (1) General characteristics of patients enrolled in the study, N=104
Table (1) General characteristics of patients enrolled in the study, N=104

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helpless</td>
<td>72 (69.2%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Student</td>
<td>2 (1.9%)</td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>17 (16.3%)</td>
<td></td>
</tr>
<tr>
<td>Retired</td>
<td>8 (7.7%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Officer</td>
<td>2 (1.9%)</td>
<td></td>
</tr>
<tr>
<td>Worker</td>
<td>3 (2.9%)</td>
<td></td>
</tr>
<tr>
<td>Drugs Used</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Aspirin 100mg</td>
<td>53 (51%)</td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>8 (7.7%)</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>38 (36.5%)</td>
<td></td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>27 (26%)</td>
<td></td>
</tr>
<tr>
<td>Heparin</td>
<td>20 (19.2%)</td>
<td></td>
</tr>
<tr>
<td>Calcium ampule</td>
<td>33 (31.7%)</td>
<td></td>
</tr>
</tbody>
</table>

P value < 0.05 considered significant.

Table (2) General characteristics of patients enrolled in the study, N=104.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. urea (mg/dl)</td>
<td>117.6 ± 56.5</td>
<td></td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>10.4 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Age Distribution (years)</td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>&lt;=40</td>
<td>5 (4.8%)</td>
<td></td>
</tr>
<tr>
<td>41 – 50</td>
<td>6 (5.8%)</td>
<td></td>
</tr>
<tr>
<td>51- 60</td>
<td>18 (17.3%)</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>75 (72.1%)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>2 (1.9%)</td>
<td></td>
</tr>
<tr>
<td>18.5-25</td>
<td>42 (40.4%)</td>
<td></td>
</tr>
<tr>
<td>25-30</td>
<td>55 (52.9%)</td>
<td></td>
</tr>
<tr>
<td>&gt;=30</td>
<td>5 (4.8%)</td>
<td></td>
</tr>
</tbody>
</table>

P value < 0.05 considered significant.
1. Effect of aspirin on diuretic resistance parameters (BMI, serum urea, serum creatinine, eGFR and albuminuria severity).

Aspirin administration showed non-significant (p<0.05) effects on BMI, serum urea, serum creatinine, eGFR. However, it had significantly (p<0.05) lowered the severity of albuminuria (1.5 ± 1.2 with aspirin vs. 2.1 ± 1 without aspirin, p=0.0052). See table (3)

2. Effect of Hydrocortisone and dexamethasone on diuretic resistance parameters (BMI, serum urea, serum creatinine, eGFR and albuminuria severity).

Hydrocortisone or dexamethasone administration showed non-significant (p<0.05) effects on BMI, serum urea, serum creatinine, eGFR and albuminuria severity. See table (3)


Atorvastatin administration showed non-significant (p<0.05) effects on BMI, serum urea, serum creatinine, eGFR. Nevertheless, it had significantly (p<0.05) lowered the severity of albuminuria (1.4 ± 1.2 with atorvastatin vs. 2 ± 1 without atorvastatin, p=0.0367). See table (3).

Table (3) Effects of drugs used on BMI, serum urea, Serum creatinine, eGFR and albuminuria severity.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>With</th>
<th>Without</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aspirin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>25.5 ± 2.7</td>
<td>25.1 ± 3</td>
<td>0.4531</td>
</tr>
<tr>
<td>S. Creatinine (mg/dl)</td>
<td>2.8 ± 1.6</td>
<td>3.5 ± 1.7</td>
<td>0.0764</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>31.6 ± 28.6</td>
<td>23.8 ± 18.6</td>
<td>0.1253</td>
</tr>
<tr>
<td>Albuminuria severity</td>
<td>1.5 ± 1.2</td>
<td>2.1 ± 1</td>
<td>0.0052</td>
</tr>
<tr>
<td>S. Urea (mg/dl)</td>
<td>115.6 ± 60.8</td>
<td>119.7 ± 52.1</td>
<td>0.5163</td>
</tr>
<tr>
<td><strong>Hydrocortisone &amp; dexamethasone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>25.9 ± 2.1</td>
<td>25.3 ± 2.9</td>
<td>0.3319</td>
</tr>
<tr>
<td>S. Creatinine (mg/dl)</td>
<td>3.1 ± 2.1</td>
<td>3.2 ± 1.7</td>
<td>0.5436</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>38.1 ± 45</td>
<td>26.9 ± 22</td>
<td>0.4138</td>
</tr>
<tr>
<td>Albuminuria severity</td>
<td>1.1 ± 1.4</td>
<td>1.8 ± 1.1</td>
<td>0.6491</td>
</tr>
<tr>
<td>S. Urea (mg/dl)</td>
<td>87.1 ± 69.5</td>
<td>120.5 ± 54.7</td>
<td>0.2957</td>
</tr>
<tr>
<td><strong>Atorvastatin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>25.8 ± 2.4</td>
<td>25 ± 3.1</td>
<td>0.2229</td>
</tr>
<tr>
<td>S. Creatinine (mg/dl)</td>
<td>3.1 ± 1.8</td>
<td>3.2 ± 1.6</td>
<td>0.4032</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>31.9 ± 32.6</td>
<td>25.4 ± 17.9</td>
<td>0.0997</td>
</tr>
<tr>
<td>Albuminuria severity</td>
<td>1.4 ± 1.2</td>
<td>2 ± 1</td>
<td>0.0367</td>
</tr>
<tr>
<td>S. Urea (mg/dl)</td>
<td>114.4 ± 65.9</td>
<td>119.6 ± 49.9</td>
<td>0.8707</td>
</tr>
</tbody>
</table>

P value < 0.05 considered significant.
4. Effect of Clopidogrel on diuretic resistance parameters (BMI, serum urea, serum creatinine, eGFR and albuminuria severity).

Clopidogrel administration showed non-significant (p<0.05) effects on BMI, serum urea, and Severity of albuminuria. However, patients using clopidogrel showed significantly (p<0.05) lowered serum creatinine as compared with those non using it, (2.8 ± 1.7 with clopidogrel vs. 3.3 ± 1.7 without clopidogrel, p=0.0356). In addition, eGFR was significantly (p<0.05) higher with clopidogrel as compared with clopidogrel free group (33.8 ± 29 with clopidogrel vs. 25.5 ± 22.7 without clopidogrel, p=0.0276). See table (4).

5. Effect of Heparin on diuretic resistance parameters (BMI, serum urea, serum creatinine, eGFR and albuminuria severity.)

Heparin administration showed non-significant (p<0.05) effects on BMI, serum urea, eGFR, and severity of albuminuria. Patients using Heparin showed significantly (p<0.05) lower serum creatinine levels as compared with those non using it, (2.8 ± 1.3 with Heparin vs. 3.2 ± 1.8 without Heparin, p=0.0146). See table (4).

6. Effect of Calcium ampoule on diuretic resistance parameters (BMI, serum urea, serum creatinine, eGFR and albuminuria severity.)

Calcium ampoule administration showed non-significant (p<0.05) effects on Serum creatinine, eGFR, and Severity of albuminuria. However, patients using Calcium ampoule showed significantly (p<0.05) lowered serum urea as compared with those not using it, (103.3 ± 47.9 with Calcium ampoule vs. 142.7 ± 62.1 without Calcium ampoule, p=0.0242). In addition, BMI was significantly (p<0.05) higher with Calcium ampoule as compared with non (25.8 ± 2.7 with calcium ampoule vs. 24.4 ± 2.9 without calcium ampoule, p=0.0243). See table (4).

Table (4) Effects of drugs used on BMI, serum urea, Serum creatinine, eGFR and albuminuria severity.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>With</th>
<th>Without</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clopidogrel (Plavix)®</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26 ± 2.4</td>
<td>25.1 ± 2.9</td>
<td>0.5418</td>
</tr>
<tr>
<td>S. Creatinine (mg/dl)</td>
<td>2.8 ± 1.7</td>
<td>3.3 ± 1.7</td>
<td>0.0356</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>33.8 ± 29</td>
<td>25.5 ± 22.7</td>
<td>0.0276</td>
</tr>
<tr>
<td>Albuminuria severity</td>
<td>1.4 ± 1.1</td>
<td>1.9 ± 1.1</td>
<td>0.5253</td>
</tr>
<tr>
<td>S. Urea (mg/dl)</td>
<td>101.4 ± 52.9</td>
<td>125.6 ± 57.5</td>
<td>0.0572</td>
</tr>
<tr>
<td><strong>Heparin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26 ± 2.8</td>
<td>25.2 ± 2.9</td>
<td>0.2098</td>
</tr>
<tr>
<td>S. Creatinine (mg/dl)</td>
<td>2.8 ± 1.3</td>
<td>3.2 ± 1.8</td>
<td>0.0146</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>34.7 ± 37.4</td>
<td>26.1 ± 20.1</td>
<td>0.0786</td>
</tr>
<tr>
<td>Albuminuria severity</td>
<td>1.4 ± 1.1</td>
<td>1.9 ± 1.1</td>
<td>0.2341</td>
</tr>
<tr>
<td>S. Urea (mg/dl)</td>
<td>91.8 ± 52.3</td>
<td>124.4 ± 55.9</td>
<td>0.0518</td>
</tr>
<tr>
<td><strong>Calcium ampoule</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8 ± 2.7</td>
<td>24.4 ± 2.9</td>
<td>0.0243</td>
</tr>
<tr>
<td>S. Creatinine (mg/dl)</td>
<td>2.9 ± 1.6</td>
<td>3.6 ± 1.8</td>
<td>0.1940</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>27.9 ± 20.6</td>
<td>27.5 ± 31.4</td>
<td>0.4849</td>
</tr>
<tr>
<td>Albuminuria severity</td>
<td>1.7 ± 1.1</td>
<td>1.8 ± 1.1</td>
<td>0.0670</td>
</tr>
<tr>
<td>S. Urea (mg/dl)</td>
<td>103.3 ± 47.9</td>
<td>142.7 ± 62.1</td>
<td>0.0242</td>
</tr>
</tbody>
</table>

P value < 0.05 considered significant
Discussion

1. Effects of Aspirin on diuretic resistance parameters (BMI, serum urea, serum creatinine, eGFR and albuminuria severity).

The results of this study were not consistent with a study of Okada et.al 2016 concerning albuminuria. He found that there is non-significant effect of low-dose aspirin on albuminuria severity in patients with DM2 who had renal dysfunction (24) but the study done on patients not using intravenous furosemide. However, this study was consistent with a study of Pastori. et.al, 2016 who found that the low dose of aspirin had no effect on the eGFR in patient with CKD and hypertension, who was taking intravenous furosemide (25).

Aspirin is an active inhibitor of cyclooxygenase enzyme, at low dose (75mg-160mg) it acts as an antiplatelet agent, which demonstrates its action by inhibiting irreversibly platelet COX-1 enzyme, thereby stopping the development of thromboxane A2 (TXA2). It has been used in the Prevention of vascular thromboembolic event (26-27).

Aspirin may positively interfere with the severity of albuminuria, through interference in thromboxane A2 synthesis that affects the albuminuria severity according to studies (28).

TXA2 is a potent vasoconstrictor and platelet aggregator, its production increases in renal dysfunction, HF and hypertension; when aspirin inhibits cyclooxygenase, it will inhibit the production of TXA2, which may reduce deteriorating the renal function, and reduce of albuminuria (29).

2. Effect of Hydrocortisone and dexamethasone on diuretic resistance parameters (BMI, serum urea, serum creatinine, eGFR and albuminuria severity).

Corticosteroids are potent anti-inflammatory drugs and immuno-suppressants used in many conditions like asthma (30). Patients that use hydrocortisone and dexamethasone in low doses through injection in the present study as a combination with furosemide, showed non-significant (p<0.05) effect on kidney function and their use were to avoid allergic reaction and not intended to treat any possible adrenal or endocrine problems or any autoimmune problem.

Since all corticosteroid-treated diseases have inflammatory components at least, so that exacerbation of this inflammation is treated with a systemic corticosteroid, the impact on renal function was attributed to diseases themselves (31).

3. Effects of Atorvastatin on diuretic resistance parameters (BMI, serum urea, serum creatinine, eGFR and albuminuria severity).

In this study, the results regarding atorvastatin effects were consistent with result of Stefano Bianchi et.al 2003 who found that using atorvastatin would decrease the albuminuria severity (32), however, his study was done on patients using diuretics but intravenous furosemide was not specified.

Atorvastatin is a class of HMG-CO reductase inhibitor drugs, which decreases the glomerular damage and preserves the renal function by decreasing the deposition of lipid in the kidney (33).

Therefore, Atorvastatin had a role in reducing the renal injury that comes from elevation in oxidative stress levels, procoagulant and inflammatory biochemical levels in renal insufficiency (32), resulted in improvement of intra renal endothelial functions (34).

4. Effects of Clopidogrel on diuretic resistance parameters (BMI, serum urea, serum creatinine, eGFR and albuminuria severity).

The study of Tonelli et.al. 2007 found that patients who had CKD and HF would benefit from the addition of clopidogrel, as antiplatelet with or without aspirin, and this was consistent with our study that clopidogrel will reserve the eGFR (35). However, the study was done on patients using diuretics, the intravenous furosemide was not specified.
It was also consistent with finding of study of Gremmel et.al., 2013 who found that using clopidogrel had significant effect on serum creatinine and eGFR but the study was done on patients not using intravenous furosemide(36).

Using ADP receptor antagonist like clopidogrel may have beneficial effects on eGFR and creatinine. This indicate implicated role of platelets in deterioration of kidney function (37) may be through interference with renin and kidney perfusion pressure and hence eGFR (38).

5. Effects of Heparin on diuretic resistance parameters (BMI, serum urea, serum creatinine, eGFR and albuminuria severity.)

The clearance of heparin occurred by renal excretion, the unfractionated heparin doesn’t accumulate in the kidney like LMW heparin (39)Nagge, et.al.,2002. Heparins have many physiological effects beyond their anticoagulant property; Nagge study was done on patients not using intravenous furosemide (40).

Heparin, in animal model, was associated with reduction levels of parameters that indicate renal problems like urea, creatinine, reduce renal damage induced by ischemia and albuminuria and it may improve eGFR (40).

In this study, these finding came in agreement to those mentioned by previous studies. Blood urea was lower for patients using heparin (91.8 ± 52.3 with heparin vs. 124.4 ± 55.9, p=0.0518) but was non-significant for eGFR; there was improvement but again it was nonsignificant (34.7 ± 37.4 with heparin Vs. 26.1 ± 20.1, p=0.0786). However, serum creatinine was significantly (p<0.05) lowered in patients with heparin as compared with those not using it.

Histones might be implicated in the damage of kidney infrastructures, through inducing inflammation and the apoptosis (41). Heparin may attenuate these effects by neutralizing these histones and may help in reserving kidney function (41). In addition to that, heparin injection may affect level of midkine, a heparin-binding growth factor. Recently it was found to be implicated in many inflammatory processes and its expression was elevated in renal tubules (42). In acute and chronic kidney diseases, Pulsated elevation of midkine, after injection of heparin may has a beneficial effect on renal tubules and other endothelial cells by reducing apoptosis, and it enhances tubular cells regeneration. The effect of heparin and midkine on kidney function may be more complicated and not fully elucidated (43).

6. Effects of Calcium ampoule on diuretic resistance parameters (BMI, serum urea, serum creatinine, eGFR and albuminuria severity.)

In this study, High percentage of Patients had CKD, where its complications are hyperparathyroidism and alteration in the absorption of calcium and phosphate. This causes osteoporosis. Therefore, calcium ampule has a tendency to decrease the release of calcium from bone to blood in order to prevent osteoporosis and to replace calcium lost in urine by using of furosemide, which may increases calcium resorption and degradation of bone by action of parathyroid hormone (44).

One of complications of renal failure is the uremic encephalopathy where serum urea level increased significantly. It was found that PTH, one of hormones that had a direct effect on serum urea by regulating the calcium in the cerebral area. Calcium will decrease the uremic encephalopathy caused by elevation of serum urea (45). It was found also that any increase in PTH hormone would increase renin release (46). Addition of calcium may reduce renin release and study of Davies, et. al 2000 found that calcium intake decreases BMI and that was not consistent with our findings (47) and the study was done on patients not using intravenous furosemide.

Conclusion

Concomitant use of drugs with furosemide injection that is used to overcome edema associated with CKD has had different odd of effects on the activity of the diuretic on renal function itself. It was found in this study that drugs frequently used to treat comorbidities associated
with CKD were most likely helpful in potentiating the
effect of furosemide on renal function and we may be
able to say that they decreased likelihood of diuretic
resistance that usually associated with the diuretic in
CKD patients due to different causes.

From this study, we can get issue that no drug
affected positively on BMI. In case of calcium ampule,
the effect was negatively by increasing the body weight
in patients already with edema. The only drug that
affected positively on Serum urea was calcium ampule;
however, all other drugs had no significant effect on
serum urea.

Clopidogrel and heparin reduced significantly
Serum creatinine levels, meanwhile, they did not reduce
albuminuria severity, the effect that was positive from
two drugs, Aspirin and Atorvastatin only. The only
drug that affects the eGFR was clopidogrel. It affected
positively by increasing eGFR in these patients.

Acknowledgments: I would like to express my
deepest gratitude to al Hussein teaching hospital for
helping me throughout my study.

Limitations:
The limited number of patients where only 104
patients were involved, it was only one group and
there was no control group to compare the effect on
kidney function. In addition, lack of follow up for these
patients; all data collected were in the time of patient’s
admission only. In addition, the study was a single
center study.

Recommendations: We recommend by increasing
the number of patients and using more lab tests that
indicate diuretic resistance like urinary Na – K ratio,
Fractional Na excretion (FeNa), Spot urinary Na and
more marker for kidney to see the effect of drugs on this
organ.

Conflicts of Interest: The author had no conflicts
of interest.

Source of Funding: Self.

Ethical Clearances: Approval from the Ethical and
Scientific Committee of the Faculty of Pharmacy/ Basra
University in addition to the Scientific Committee of
Researches of Thi-Qar Health Directorate.

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Assessment of Relationship between Ectopic Pregnancy and Assisted Reproductive Technology in Baghdad City

Alyaa Khazaal Neamah1. Wisam Mashaan Muttaleb2
1Assistant Lecturer, 2Lecturer, Maternal and Neonate Nursing Department, College of Nursing/ University of Baghdad, Iraq

Abstract

Objectives: To find out the relationship between assisted reproductive technology, Some demographics characteristic and ectopic pregnancy among women with ectopic pregnancy Methodology: A descriptive study was applied to assess the relationship between ectopic pregnancy and Assisted Reproductive Technology among purposive sample of (60) women aged between (20-43) years old attended the hospitals in Baghdad city. Results: The study show that the most woman in the age group (32-37) years old, was primary school graduate, most of them have no gravidity, were treated with assisted reproductive technology previously for (5-12) month, Clomiphene citrate (clomid) is used in the rate (50%), suffering from lower abdominal pain and Darker or brown vaginal spotting. Conclusions: The study concluded that there is significant relationship between the use of Clomiphene citrate (clomid) and ectopic pregnancy and the most benefit treatment for ectopic pregnancy is methotrexate. Recommendations: The study recommended a furthers study on ovulation induction, training program about the effect of ovulation induction especially (clomid) on women health

Keywords: Assessment, ectopic pregnancy, assisted reproductive technology

Introduction

Ectopic pregnancy is a pregnancy in which the fertilize ovum not implanted in the endometrial of the uterine cavity, meaning outside the uterine cavity, most commonly in fallopian tube, the important of management as pharmacologic treatment with methotrexate, or surgery especially with tubal rupture (1).

The incidence rate of ectopic pregnancy is 1–2 percent in the general population, and ART lead to increase this rate in pregnancies than in spontaneous pregnancies about 2–5 %. Although the overall mortality has decreased over time and about 6% of cases suffer from ruptured EPs of all maternal deaths, the fallopian tube EP is the most common ectopic implantation site, others 10 % of it occur in the cervix, ovary, myometrium, interstitial portion of the fallopian tube, abdominal cavity or within a cesarean section scar (2).

Nowadays, by increase infertility rate in the world, the demand to use ART had increase which a common technique for infertility treatment. However, with the time the complications that come from this technology appear widely, such as ovarian hyperstimulation syndrome, Ectopic Pregnancy, and multiple pregnancy, have drawn more and more attention. Even though the incidence of EP is rare during IVF (approximately 2%–11%) (3).

Ovulation induction agents is one of the most drugs used for infertility treatment, in the United States there are more than 2 million women within age group (15–44 years) have taken fertility medications. On the other hand, Janee and David reported that the possible complications associated with ovulation induction become more than
before, like ovarian hyperstimulation syndrome, multi fatal gestations, preterm delivery, heterotopic/ectopic pregnancy, spontaneous abortion, and the theoretic risk of a possible increase in the incidence of ovarian cancer so we deal more with these complication of ART in this study (4).

Methodology

A descriptive study was applied to ass the relationship between ectopic pregnancy and Assisted Reproductive Technology among purposive sample of (60) women aged between (20-43) years old attended the hospitals in Baghdad city. Data is collected through using the questionnaire format which consisted of five parts, including demographic, reproductive history, Diagnosis of Ectopic Pregnancy, Assisted Reproductive Technology, Management Treatment, and complications of PCOS included short, the items of questionnaire are rated by (yes) scored as (2) and (no) scored as (1).

Data are collected through using study instrument and the interview technique in purpose of data collection after agreement of woman which suffering from ectopic pregnancy which implemented for the period of (20th October 2019 through 25th February 2020). The reliability and Content validity of the questionnaire are determined through a pilot study of (10) women with ectopic pregnancy which was excluded from the study sample, a panel of (6) experts reviewed the study tool for its validity, and internal consistency reliability ‘split-half technique’ is employed for the determination of the study instrument internal consistency. The correlation coefficient is ($r=0.89$) which indicates that the questionnaire is an adequately reliable measure. Descriptive and inferential statistical analyses were used to analyze the data by using frequency (F), percentage (%), mean of score (MS), assessment (ASS), and Bivariate Pearson correlation, through SPSS program the statistical procedures tested at $p \leq 0.05$.

Results

Table (1) Distribution of the Reproductive Information in the study sample

<table>
<thead>
<tr>
<th>List</th>
<th>Reproductive History</th>
<th>Yes</th>
<th>NO</th>
<th>M.S</th>
<th>ASS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>%</td>
<td>F</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>Gravidity</td>
<td>27</td>
<td>45</td>
<td>33</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>Treatment with ART</td>
<td>44</td>
<td>73.3</td>
<td>16</td>
<td>26.7</td>
</tr>
<tr>
<td>3</td>
<td>Years of Treatment with ART</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>Not Use</td>
<td>16</td>
<td>26.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.2</td>
<td>5-12 month</td>
<td>20</td>
<td>33.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.3</td>
<td>13-36 month</td>
<td>12</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.4</td>
<td>37-60 month</td>
<td>12</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

F: Frequency, %: Percentage, M.S: Mean of scores, Ass: Assessment, L: Low, M: Moderate, H: High
Table (2): Assisted Reproductive Technology

<table>
<thead>
<tr>
<th>List</th>
<th>Assisted Reproductive Technology</th>
<th>Yes</th>
<th></th>
<th>NO</th>
<th></th>
<th>M.S</th>
<th>ASS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>%</td>
<td>F</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Not Use</td>
<td>16</td>
<td>26.7</td>
<td>0</td>
<td>0</td>
<td>2.1</td>
<td>M</td>
</tr>
<tr>
<td>2</td>
<td>Ovulation induction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>Clomiphene citrate (clomid).</td>
<td>30</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>L</td>
</tr>
<tr>
<td>2.2</td>
<td>(HMG) (Pregonal).</td>
<td>6</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Artificial Insemination</td>
<td>8</td>
<td>13.3</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Relationships between Studied Variables and Ectopic Pregnancy

<table>
<thead>
<tr>
<th>Studied Variables</th>
<th>Pearson Correlation</th>
<th>P-value (2-tailed)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>0.0946</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>Education</td>
<td>-0.191</td>
<td>0.144</td>
<td>NS</td>
</tr>
<tr>
<td>Gravidity</td>
<td>-0.077</td>
<td>0.559</td>
<td>NS</td>
</tr>
<tr>
<td>Treatment with ART</td>
<td>0.005</td>
<td>0.972</td>
<td>NS</td>
</tr>
<tr>
<td>Years of Treatment with ART</td>
<td>0.017</td>
<td>0.899</td>
<td>NS</td>
</tr>
<tr>
<td>Use Clomiphene citrate (clomid)</td>
<td>-0.223</td>
<td>0.056</td>
<td>S</td>
</tr>
<tr>
<td>Use (HMG) (Pregonal)</td>
<td>0.029</td>
<td>0.825</td>
<td>NS</td>
</tr>
<tr>
<td>Use Artificial Insemination</td>
<td>0.167</td>
<td>0.261</td>
<td>NS</td>
</tr>
<tr>
<td>Management treatment</td>
<td>-0.069</td>
<td>0.899</td>
<td>NS</td>
</tr>
</tbody>
</table>

P-value: Probability level, S: Significant, NS: Not significant

Discussion

The study shows that the most woman in the age group (32-37) years old, was primary school graduate, most of them have no gravidity, were treated with assisted reproductive technology previously for (5-12) month, the most of them were used Clomiphene citrate (clomid) (50%), suffering from lower abdominal pain and Darker or brown vaginal spotting.

The most site of presence the ectopic pregnancy in
Ampullary of fallopian tube, more management used to treat EP is Methotrexate IM.

Also, there is no significant relationship between the studied variables in table (5) and ectopic pregnancy except use Clomiphene citrate (clomid) and used of artificial insemination had significant relationship.

The age is consider one factor that increase the incidence of ectopic pregnancy as the study done by Shayestehm et al 2014, reported highly improbable an increase in chromosomal abnormalities in the trophoblastic tissue be caused by advanced maternal age (19, 20yrs). In addition, the fallopian tube function decreased in effectively transport the ovum to the uterus during increase maternal age. However, these hypotheses remain to be tested (5).

In this study the educational level is important to women for knowing and following their health condition like the study that indicated the social and demographic factors determining the level of awareness of the signs and symptoms of ectopic pregnancy (6).

The study shows that more patient with ectopic pregnancy is treated with ART in the past and Use the ART put the woman at risk for development ectopic pregnancy as the study done by Cihan K. & Eray Ç. Mentioned that ART and ectopic pregnancy and associated risk factors have increased the incidence of an ectopic pregnancy, so the exact method that bind the ectopic pregnancy and ART still between ectopic pregnancy and ART still ambiguous. After the IVF the most common risk factors are ectopic pregnancy which include (Day of transfer, fresh or frozen-thawed cycle single or double transfer), also mentioned that ectopic pregnancy will happened at day 5 blastocyst transfer that the day 3 blastocyst transfer, and frozen-thawed cycle has lower risk of ectopic implantation than fresh cycle (7).

Table (2) shows that most women with ectopic pregnancy in Baghdad city had significance relationship with the use of Clomiphene citrate (clomid). In the summary of product characteristics of clomid (January 2020) mention that clomid increase the chance of ectopic pregnancy in women use it for treat fertility, Clomid uses lead to Ovarian Hyperstimulation Syndrome (OHSS) which is most common causes of EP. Clomiphene has direct effect on pituitary gland to release more amount of FSH and LH therefore the estrogen receptors are blocked this pose to increase the rate of EP in future confirm by (8).

In the study performed at Arash Women Hospital, Tehran, shows that long duration of treated with ovarian hyper stimulation (clomid) lead to increase the level of estrogen which consider as a direct cause of ectopic pregnancy (EP) (9).

In this study it was observed that there is a relationship between used of artificial insemination and ectopic pregnancy, as in the study conducted in Arak Taleghani Hospital, Iran. which showed that women’s who undergo artificial insemination, that used to treat some cases of infertility especially tubal factor infertility, after 30 days of this treatment suffer from ectopic pregnancy which was diagnosed by ultrasound (10).

The diagnosis of ectopic pregnancy associated with some signs and symptoms like miss one or two menstrual period and Darker or brown vaginal spotting which appear high in this study while the study in Nigeria showed that 80 % of study sample have abdominal pain when come to hospital, Also the study show that the most types of ectopic pregnancy was in the ampullary and isthmus site of fallopian tube (66.7%,18.3%) respectively, this result corresponding with result of the study of done in south-east Nigeria that showed (n:52/82) of women have ampullary ectopic pregnancy (11).

The study shows that 50% of women are treated by methotrexate IM and 35% of them treated by mixed of surgical and use of methotrexate drug methotrexate drug is a folic acid antagonist that inactivates dihydrofolate reductase and de novo synthesis of purines and pyrimidines, and therefore, cellular DNA. The most common action of methotrexate is increasing the trophoblastic cell division and not allow these cells to
become multiplying (12).

American society for Reproductive Medicine 2013, mention that the dose of MTX depended on the level of hCG in blood, so the dose may be single or double. This mentioned that 84.5% of women with ectopic pregnancy are receiving treatment by MTX and it was benefit to remove ectopic pregnancy (13). The most management of EP is Methotrexate IM. This result is disagreement with another study done by Togas Tulandi which fine that the surgical treatment is the best solution to EP (14).

Ectopic pregnancy is a potentially life-threatening condition. While surgical method is better way to treat the ectopic pregnancy. In the 1980’s discover that the early diagnosis is eased the initiation of methotrexate as a medical therapy for EP. With the routine use of early ultrasound, diagnosis of ectopic pregnancy can be established early, and medical treatment can be administered in most cases (14).

Conclusions

The study concluded that there is significant relationship between the use of Clomiphene citrate (clomid) and ectopic pregnancy and the most benefit treatment for ectopic pregnancy is methotrexate.

Recommendation:

The study recommended a future study on ovulation induction, training program about the effect of ovulation induction especially (clomid) on women health

Conflict of Interest: Nil

Source of Funding: the source of funding is our self

Ethical Clearance: is obtained from the Ministry of Health / Al-Russafa Health Directorate (Baghdad Teaching Hospital), and All women participants in the research - have been approved before the questionnaire is started.

References


Evaluating the Safety of Non-Steroidal Anti-inflammatory Drugs use in Asthmatic Patients: A Systematic, Critical Review of Literature

Amanj Baker Kurdi

Lecturer in Clinical Pharmacy, Department of Pharmacology and Toxicology, College of Pharmacy, Hawler Medical University, Erbil, Iraq

Abstract

Background: Evidence suggest avoidance of Non-steroidal anti-inflammatory drugs (NSAID) in aspirin-intolerant asthmatics due to the risk of triggering exacerbation attack; however, evidence around avoiding NSAIDs in all other asthmatic patients are unclear. This study aimed to evaluate the evidence surrounding the safety of using NSAIDs, including selective COX-2 inhibitors, among asthmatic patients.

Methods: A systematic review used Medline (OVID), Scopus and Embase from January-2008 to January-2019. Inclusion criteria included English, and human studies that evaluated the use of NSAID in asthmatics. Data was screened/extracted using a pre-designed data extraction form using Covidence®, then were critically appraised.

Conclusion: Of the 49 identified studies, eight were eligible. Prevalence of NSAID-induced asthma exacerbation was 9% (95%CI: 6.0–12.0%)-9.9% (95%CI: 9.4-10.5%). Asthmatics who were aspirin/NSAID intolerant had 37% higher risk of hospitalisation compared to tolerant patients (RR:1.37; 95%CI:1.12–1.67). Use of COX-2 inhibitors showed non-significant associations with worsening respiratory symptoms/exacerbation. Only low-quality evidence was found for the safety of topical NSAID. NSAIDs-induced respiratory reactions/symptoms is relatively uncommon with the majority of asthmatic patients could tolerate NSAIDs therapy. Asthmatic patients who suffer from aspirin-induced asthma or NSAIDs-exacerbated respiratory disease (NSAIDs intolerant) should be avoided NSAIDs prescribing but could be safely prescribed selective COX-2 inhibitors as an alternative.

Key words: Asthma; Non-steroidal anti-inflammatory drugs; safety; analgesics; exacerbation

Introduction

Asthma is a complex, heterogeneous airway disorder responsible for a variety of recurring symptoms, such as tightness of the chest, difficulty in breathing and wheezing, caused by bronchoconstriction, airway oedema, hyper-responsiveness and remodelling. Asthma is a prevalent chronic condition affecting over 300 million people, with its prevalence continues to increase with expectation that another 100 million people would be affected by 2025. In the UK, 5.4 million people currently suffer from asthma with one potentially life-threatening asthma attack every 10 seconds resulting in three asthma attack related deaths, on average, every day, giving the UK some of the highest asthmatic rates in Europe.
Multiple factors have been identified as potential risk factors for triggering acute attack of asthmatic exacerbations and these include poor medication compliance, allergies and exposure to some pharmacological agents such as non-steroidal anti-inflammatory drugs (NSAIDs). Non-steroidal anti-inflammatory drugs are one of the most commonly prescribed analgesics, accounting for approximately 5-10% of all annual prescribed medications. NSAIDs’ pharmacological effects are mediated via inhibition of prostaglandins via reversible inhibition of cyclooxygenase (COX) enzymes (both COX-1 responsible for the production of prostaglandins which maintain normal physiological functions and COX-2 involved only in production of pro-inflammatory prostaglandins). Based on their selectively toward COX enzyme, NSAIDs can be classified into standard NSAIDs (these are non-selective inhibiting both COX-1 and COX-2) such as ibuprofen, and indomethacin and Coxibs (these are selective COX-2 inhibitor) such as celecoxib and etoricoxib.

Use of NSAIDs in asthmatics might trigger an acute asthma exacerbation attack which is known as aspirin-induced asthma (AIA) or Non-steroidal anti-inflammatory exacerbated respiratory disease (NERD), which has an average prevalence of about 21% in adult asthmatics, even though it could vary between 4%-44%. AIA/NERD is a common cause of life-threatening asthmatic exacerbations, and although its exact pathophysiology has not yet been fully understood, current evidence suggest the involvement of leukotrienes. Although it has been suggested that the majority of asthmatics can tolerate NSAIDs, the current international guidelines, including the UK National Institute for Health Care and Excellence (NICE) recommend all asthmatics should be offered a NSAIDs provocation test before prescribing NSAIDs to assess patients’ tolerability to NSAIDs; and subsequently avoid NSAIDs in asthmatics who are intolerant to NSAIDs (i.e. AIA/NERD sufferers). However, due to issues with the availability and feasibility/practicability of undertaking provocation test for all asthmatics, these recommendations mean that NSAIDs should also be avoided in all asthmatics with unknown sensitivity status which constitutes the majority of asthmatic patients. Accordingly, avoiding NSAIDs in this large population of untested asthmatics for NSAIDs sensitivity leads to many untested asthmatics being unnecessarily denied the multiple advantages of NSAIDs and being prescribed alternative analgesics such as paracetamol, and opioids/opioid derivatives: this might be of particular concern since the latter could be associated with potentially problematic long-term adverse effects and harms such as dependence, risk of abuse and reduced quality of life.

Similarly, although evidence suggest the safety and efficacy of selective COX-2 inhibitors in many sufferers of AIA/NERD, due to their selective inhibition of COX-2, NICE continues to recommend their avoidance due to non-lethal intolerance in a small number of AIA/NERD patients. In order to avoid the unnecessary use of potentially harmful analgesics in asthmatic patients such as opioids, who could otherwise be prescribed NSAIDs, it is important to understand the current evidence around the safety of NSAIDs among asthmatic. Therefore, this study aimed to critically review and analyse the current scientific literature for the safety of NSAIDs in asthmatic patients including the safety and tolerability of selective COX-2.

Method

Data Source and Searches

This was a systematic review of the literature conducted and reported in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement checklist. The literature search was carried out in Medline (OVID), Scopus, and Embase from January 2008 to January 2019, using key terms related to asthma, NSAIDs, COX-2 inhibitors and aspirin induced asthma or NSAID exacerbated respiratory disease based on previously conducted systematic review in this area including the use of certain exclusion terms to limit the volume of irrelevant results. A population, intervention, comparison and outcome (PICO) approach was adopted to assist with the search and selection of the relevant articles (Table 1). Reference lists of the eligible articles were also manually searched to identify any further relevant articles.
Table 1. Population, Intervention, Comparison and Outcome definition used in the study

<table>
<thead>
<tr>
<th>Population</th>
<th>Intervention</th>
<th>Comparison</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>All asthmatics of every age group including those asthmatics who suffer from AIA/NERD and those tolerant to NSAIDs</td>
<td>Use of NSAIDs and selective COX-2 inhibitors</td>
<td>As an alternative to NSAID avoidance or use of COX-2 inhibitors as an alternative to NSAIDs</td>
<td>Safety of NSAIDs and selective COX-2 inhibitors in terms of inducing exacerbation of asthma</td>
</tr>
</tbody>
</table>

(Note): COX-2: Cyclooxygenase enzyme 2; NSAIDs: Non-steroidal anti-inflammatory drugs

Study Selection

Studies were considered eligible if they were conducted in human, published in English, without restrictions to study designs and studies that matched our PICO criteria (Table 1). All the identified records from the search strategy were exported from the databases and imported into Covidence® whereby duplicate records were removed. The author (AK) independently undertook titles and abstract screening for relevance, followed by selecting records for full-text screening and data extraction; each of the above stages were validated by an independent reviewer (MM). Any discrepancy was resolved through discussion until consensus was achieved.

Data Extraction, quality Assessment, synthesis and analysis

After identification of the eligible studies, data from these studies were subsequently extracted into a spreadsheet including information on the study characterises (study design, setting, sample size, population) and outcome measures including prevalence of NERD/AIA, differences in the risk of asthmatic morbidity in relation to NSAIDs and COX-2 inhibitors, differences in FEV1 after exposure to NSAIDs and COX-2 inhibitors, average doses provoking asthmatic response in NSAIDs and COX-2 inhibitors and the presence of symptoms induced by NSAID and COX-2 inhibitor exposure. Studies’ quality and risk of bias was assessed using the National Heart, Lung and Blood Institute’s quality assessment tool for the various types of study design including observational studies, systematic reviews, meta-analyses and before-after studies except from case reports; whereby studies’ were classified based on their quality into good, fair or poor depending on the total scores from each tool. The evidence from all the eligible studies were then critically reviewed and appraised using the Critical Appraisals Skills Programme (CASP) checklists for the appropriate study design/type.

Literature Review Results

Overall, 316 articles were identified from the initial literature search, of which only eight studies were eligible for inclusion. A total of 50,086 patients were included in these eight records.

Study characteristics

The eligible eight studies comprised of two meta-analysis, one retrospective cohort study, one before and after study, one expert opinion, and
three case reports covering both adult and paediatric population, and short/acute and long-term complications.

In terms of outcomes, the eight included studies provided a wide range of information concerning the safety and use of NSAIDs in the general asthmatic population and those who suffer from AIA/NERD. Overall, the evidence indicated that the use of NSAIDs is associated with higher risk of asthmatic complications/exacerbations in those who suffer from AIA/NERD (intolerant patients) compared to those are tolerant to NSAIDs (Table 3). Furthermore, asthmatics who are suffering from NERD showed poorer asthma control when taking NSAIDs and the average NSAIDs dose that found to provoke asthmatic reaction is even below the therapeutic NSAIDs dose. Moreover, asthmatic children, compared to adults, using NSAIDs were at higher risk of asthma associated morbidity and mortality. In terms of the safety of COX-2 inhibitors, the evidence from the meta-analysis showed no harms or safety issues with the use of COX-2 inhibitors among asthmatic including those who suffer from AIA or NERD; this is despite some case report studies showed contradicted evidence as they reported an increase in the onset of asthmatic symptoms and a reduced FEV1 upon exposure to COX-2 inhibitors but only among AIA or NERD asthmatics. In terms of the quality and risk of bias, two studies were classified as good quality, one study as fair quality, and one as poor quality.

Critical appraisal and analysis

Use of NSAIDs

The highest quality evidence about the safety use of NSAIDs in asthmatics came from a meta-analysis of 46 clinical trials and observational/population-based studies conducted by Morales et al which included data from 20,162 patients with NERD and NSAIDs-tolerant asthma (NTA). This study determined the prevalence of NSAID hypersensitivity (intolerance) measured either by oral provocation challenge tests (OPCTs)(9%) or a self-reported questionnaire (9.9%); the observed difference between NSAID hypersensitivity (intolerance) prevalence was attributed to the vague respiratory reaction definitions used within the questionnaires which likely resulted in overestimation of NSAID hypersensitivity (intolerance) prevalence. These estimates were significantly lower than what initially estimated in a previous systematic review since this study, unlike the previous study minimised bias by including only studies that used blinded and controlled OPCTs. Data on aspirin dose that provoked asthma symptoms in both adults and children was also assessed; then observational studies reporting asthma morbidity in those with NERD and NTA were compared. In regard to the former, the mean aspirin dose that provoked respiratory reactions in NERD patients was 85.8 mg (95%CI:73.9-97.6) which is a clinically relevant doses of oral aspirin given the 75-100 mg aspirin dose recommended by the US guidelines for the CVD prevention; although the potential risk reduction of using 75 mg vs 100 mg aspirin was not possible to quantify due to lack of data, sub-group analysis results suggest a dose-response relationship between aspirin dose and the extent of fall in FEV1. Having said this, it should be noted that there were considerable variations in individual patients susceptibility to the low aspirin dose suggested other factors might play a role as well; accordingly, it could be concluded that using low-dose aspirin would not trigger clinically significant adverse respiratory symptoms in all asthmatics with NERD even though the risk would be higher when higher loading aspirin dose (≥300 mg) is used; however, these risks should be weighed against the net clinical benefits of using aspirin in the management and prevention of CVD. In terms of asthma morbidity in those with NERD and NTA, compared to NTA, use of NSAIDs was associated with higher risk of all asthmatic morbidity including 50% higher risk of severe asthma and double the risk of uncontrolled asthma as well as 80%, and 40% higher risk of emergency visits and asthma hospitalisation, respectively, emphasising the importance of considering NERD diagnosis to direct the safe use of NSAIDs and aspirin among asthmatic patients. Although this meta-analysis provided valuable findings about the safety of NSAIDs use among asthmatic and it was of a good quality, it has certain limitations including investigating
the safety of aspirin only without considering the other NSAIDs which do not exclude the possibility of selective hypersensitivity to aspirin and issues with generalisability to all the other NSAIDs.

The higher risk of asthmatic morbidity among NERD was further confirmed by another retrospective cohort study conducted by Lo et al.\textsuperscript{22} who investigated risk of asthma exacerbation hospitalisation with the use of NSAIDs among 29,484 paediatric asthmatic patients of which 9,862 (33.4\%) and 19,622 (66.6\%) were NSAIDs users and non-users, respectively. After adjusting for potential confounders, use of NSAIDs was associated with 41\% higher risk of hospitalisation compared to non-user (aRR: 1.41; 95\%CI:1.3-1.53) which equates to 9.2\% vs. 6.3\% in NSAIDs users and non-users, respectively. This study was of fair quality but still suffer from certain limitations such as failure to consider the over-the-counter use of NSAIDs as well as defining NSAIDs users as only those who were prescribed NSAIDs and anti-asthmatic therapy on the same day, both of which might have resulted in under-estimation of NSAIDs use and hence its risk. Overall, this study has clearly demonstrated the apparent safety risk of NSAIDs use among paediatric asthmatic patients.

Unlike the other identified studies, one of the studies\textsuperscript{23} investigated the safety of topical NSAIDs among 11 NERD asthmatics; this was a “before and after” study in which asthma control was evaluated 6-months before and after starting and ceasing topical NSAIDs, respectively. The study findings indicated improvement in all the asthma control measurements after ceasing topical NSAIDs. However, this study was of poor quality with many methodological flaws, that question the validity of the findings, including a very small sample size, failure to disclose anti-asthmatic medications being taken by patients as well as considering the adherence to the topical NSAIDs. Accordingly, despite suggesting topical NSAIDs are hazardous in asthmatics with NERD, this evidence is inconclusive and as such a recommendation on the safety of topical NSAID use in NERD patients cannot be made.

Furthermore, exacerbation of asthma symptoms upon exposure to NSAIDs was further reported by two case reports by King \textit{et al.}\textsuperscript{25} and Tang and Zhang\textsuperscript{24}; however, evidence from case reports are only considered as hypothesis generating which in turn has been confirmed in subsequent studies such as those described earlier\textsuperscript{20,22}.

### Use of selective COX-2 inhibitors

The safety of using COX-2 inhibitors (e.g., celecoxib, rofecoxib) and selective NSAIDs (e.g., meloxicam) among NERD asthmatic was investigated in a meta-analysis by Morales \textit{et al.}\textsuperscript{21}. The meta-analysis included a total of 426 NERD asthmatic patients from 14 clinical trials. Compared to placebo, the study found no significant differences in respiratory symptoms, FEV\textsubscript{1}, and nasal symptoms after exposure to COX-2 inhibitors but only a very small increase in respiratory symptoms (risk difference:0.08; 95\%CI:0.02-0.14) after exposure to selective NSAIDs, suggesting the safety of using COX-2 inhibitors in asthmatic patients with NERD.

This study was of high methodological quality in which bias was minimised by including only blinded clinical trials; however, only low-moderate dose in patients with stable, mild-to-moderate persistent asthma was evaluated in this study; hence this observed safety might not be applicable to high doses of COX-2 inhibitors or those with unstable asthma or those who have experienced severe life-threatening reactions requiring intubation after aspirin or NSAIDs exposure. This is despite two case reports\textsuperscript{26,27} who reported respiratory reaction to COX-2 inhibitors in a uncontrolled 33-year old asthmatic with NERD and a controlled 25-year old NERD asthmatics, respectively; however, these hypothesis generating evidence were subsequently contradicted and disapproved by several clinical trials as summarised in the meta-analysis by Morales \textit{et al.}\textsuperscript{21}.

### Discussion

The current evidence from the literature indicated that the prevalence of AIA/NERD among asthmatic was relatively uncommon with a reported prevalence...
of approximately of 9%, suggesting that the majority of asthmatic patients could tolerate NSAIDs; hence their safe prescribing among asthmatic with unknown history of hypersensitivity to NSAIDs. These findings might question the current guideline recommendations\(^1\), \(^1\) of avoiding NSAIDs in asthmatics unless they have proven to be NSAIDs tolerant through either NSAIDs provocation challenge test or self-reported questioning; however, both of the latter are problematic since, firstly, the NSAIDs provocation challenge test is not widely used in routine clinical practice due to issues with availability, practicality, feasibility\(^1\), \(^1\); and secondly, due to variability of self-awareness of NSAIDs-induced symptoms in patients with asthma\(^2\) which makes the self-reported intolerance to NSAIDs unreliable. Collectively, these guideline recommendations\(^1\), \(^1\) imply denying and withholding NSAIDs prescribing from the majority of asthmatics despite NSAIDs effective anti-inflammatory, analgesic, and antipyretic effects resulting in doctors prescribing alternatives analgesics to NSAIDs such as paracetamol, and opioids/opioid derivatives\(^1\); however, this is concerning since some of these alternatives such as opioids/opioid derivatives are associated with potentially problematic long-term adverse effects and harms such as dependence, risk of abuse and reduced quality of life\(^3\).

Furthermore, there are high quality evidence for increasing asthma morbidity among AIA/NERD patients upon exposure to NSAIDs suggesting that NSAIDs should be avoided in these group of asthmatic patients. However, unlike NSAIDs, selective COX-2 inhibitors are shown to be safe in AIA/NERD patients and could be prescribed safely as an alternative to NSAIDs in AIA/NERD patients. Moreover, selective COX-2 inhibitors could also be a safe option for the general asthmatic patients who are unwilling to accept receiving NSAIDs due to the potential risk of their asthma exacerbation in response to NSAIDs exposure. There is lack of robust and high-quality evidence about the safety of topical NSAIDs among AIA/NERD patients leaving this an area for further research.  

**Strengths and limitations**

To our knowledge, this is the first comprehensive systematic evaluation of the safety of using NSAIDs, including selective COX-2 inhibitors, among patients with asthma as well as asthmatics who suffer from AIA/NERD, without restrictions to study types across multiple databases over a 10-year period. Since the search was conducted up until January 2019 it is possible that we have missed potential studies that have been published after January 2019 which might have impacted our study findings.

**Conclusion**

In summary, it could be concluded that NSAIDs-induced respiratory reactions/symptoms is relatively uncommon with the majority of asthmatic patients could tolerate NSAIDs therapy. Asthmatic patients who suffer from AIA/NERD should be avoided NSAIDs but could be safely prescribed selective COX-2 inhibitors as an alternative.

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**Conflicts of Interest:** Nothing to declare

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**Ethical Clearance:** Ethical clearance was not required as the study was a systematic review of studies that have been already published.

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Pt Kereta Api Indonesia (Persero) Strategy in Facing Corona Virus Disease-19 Pandemic

Ambar Mutiara¹, Djoko Suhardjanto², Yeni Priatna Sari²
¹Student, Masters in Management, Universitas Sebelas Maret, Indonesia, ²Lecturer at the Faculty of Economics, Universitas Sebelas Maret, Indonesia

Abstract

The purpose of this research is to study and find out the strategies implemented by KAI in dealing with the impact of the Covid-19 pandemic on KAI’s performance. The method of analysis in this study is a qualitative descriptive method by analyzing all the data related to the topic being studied, which is then presented with fairly comprehensive and comprehensive arguments. The results showed that the very fast growth in the last ten years is KAI’s performance that should be appreciated. However, the Covid-19 pandemic is a reminder that economic development (profit) must be accompanied by the principles of sustainability and environmental and social concerns. The current situation in the impact of the Covid-19 Pandemic is very unfavorable for achieving revenue targets for passenger and freight transport. KAI has carried out various strategies in dealing with the impact of the Covid-19 pandemic. Several things that have been done by KAI in dealing with the impact of the Covid-19 pandemic include: 1. Protecting passengers and employees of KAI by prioritizing health through the Covid-19 prevention protocol; 2. Maintain company liquidity by preparing several scenarios, if the pandemic ends in June, August or December; 3. Perform cost efficiency (operational expenditure) and reduced investment (capital expenditure) to balance the potential decline in income.

Keywords: Covid-19, Pandemics, Sustainability.

Introduction

PT Kereta Api Indonesia (Persero) is an Indonesian State-Owned Enterprise that provides railroad transportation services which are then called KAI. Various revolutionary efforts and steps in revitalizing business according to changing times and increasingly dynamic external conditions have been carried out. For a decade, KAI has continued to improve its performance in various aspects. In making continuous improvements and changes, companies are increasingly prioritizing commercial functions in addition to public service functions which are no less important. With the increasing company performance both in operational and financial management and consistency in management in implementing Good Corporate Governance (GCG), stakeholders’ trust in KAI will also increase.1

Starting from 2009, various important and significant efforts were made to improve overall company performance and financial performance in particular. KAI’s losses in 2008 amounting to 83 billion rupiah can be repaired directly in 2009 by recording a profit of 155 billion rupiah, until now in 2019 KAI has been able to achieve profits of up to 1.9 trillion rupiah. The 2008 revenue of 4.4 trillion rupiah was able to be improved in 2009 to 4.8 trillion rupiah, then KAI’s profit growth fluctuated from 2015 to 2018 and then in 2019, KAI’s

Corresponding Author:
Ambar Mutiara
Student, Masters in Management, Universitas Sebelas Maret. Street. Ir. Sutami No. 26, Surakarta, Indonesia
Email ID: mutiaraambar7010@gmail.com
total income could grow compared to 2018 reaching up to 26.2 trillion rupiah. For the development of company assets also grew significantly, from the position of assets in 2008 amounting to 5.8 trillion rupiah, growing to a value of 44.9 trillion rupiah until 2019.

During the 2009-2019 period, profit growth continued to increase, as did income and asset growth. The realization of an increase in financial performance is evidence that the management function is able to create value for the company, which is reflected in the ability to create profits. The success of marketing performance cannot be separated from the performance of human resources (HR) which is the main measure considering that KAI is a service provider and public transportation services where the HR factor is the most important variable in the delivery of services and services to customers. In 2019, the highest revenue contribution was obtained from passenger transportation and followed by freight transport.

Meanwhile, from the freight transportation segment, the largest contribution is obtained from coal transportation of the total freight revenue. The volume of goods transportation has started to increase since 2011, from 2015 to 2019 the revenue for goods transportation continues to grow, revenue for goods transportation comes from transportation of coal, containers, fuel, cement, bulk, plantation, general cargo, BHP and others. The success of commercial performance is inseparable from operational performance indicators, services, human resources and information technology as well as other supporting performance.

Human resources that are well managed will be part of the company’s strength and increase the company’s value. Human resources are the main capital for a company or it is called human capital. Human resource management in the railroad industry in Indonesia initially followed the production orientation that took place in the company. Many things have been done by KAI related to human resource management in supporting the company’s business, including implementing a new remuneration system, prioritizing performance over years of service, recruiting professionals, enforcing regulations and developing human resources including internal training, technical and management courses. The country, training abroad and almost all employees are given the opportunity to conduct comparative train studies abroad.

Various efforts in the train operation process have been made and achieved by KAI. From 2011 to 2019 KAI continued to increase its fleet of locomotives, trains and carriages in large numbers. One of the keys to the success of this process is the expertise in managing rail operations and traffic. The division of work areas begins with the relatively limited KAI rail network, most of KAI’s operational areas cover Java Island and partly on the island of Sumatra. The working area in Java Island is divided based on the Operational Area (Daop), while the working area in Sumatra is divided based on Regional Divisions (Divre). Train travel management stems from the existence of train travel schedules on certain routes and certain frequencies. Operational planning is defined from commercial targets, efforts to maximize the number of passengers carried out commercially are supported by operational reliability. The capacity of available operating facilities is used as the basis for production targets.

Behind the train operating system, it is inseparable from the main elements that regulate train travel to run efficiently and safely, namely the signal and telecommunication systems. Until now, the signaling and telecommunication systems have continued to experience improvement as evidenced by the investment in increasing signaling in the Jabodetabek area which has been implemented since 2018. Train travel management stems from the existence of train travel schedules on certain routes and certain frequencies. The scheduling process is an activity that connects various work units in the railroad industry.

All the successes that have been achieved by KAI have occurred due to the use of information systems and technology in each of its business processes. KAI continues to develop various applications in information
technology to facilitate services and information for railroad users. Every year there is always updating and reviewing of information technology plans so that the focus of KAI’s technology plans and strategies is always in line with the development of KAI’s business and corporate strategies. KAI is committed to carrying out good corporate governance (GCG) practices in all of its business activities. As a manifestation of this commitment, KAI has implemented various policies and guidelines such as GCG Guidelines, Code of Conduct, Guidelines for the Board of Commissioners and Directors, Information Technology Governance, Risk Management, Reporting of Alleged Violations, Internal Control Systems and Internal Control Systems, as well as various other policies made to support the consistent and sustainable implementation of Good Corporate Governance.

KAI’s revenue growth from year to year from 2009 to 2019 is a performance that will continue to be maintained. There are so many challenges that have been faced and all of them can be overcome by continuing to improve services accompanied by revenue growth that can always provide good performance for all stakeholders. Entering 2020 with high optimism, KAI plans revenue growth of around 20% in accordance with KAI’s long-term plan. In January and February, KAI’s performance started very well, but this got worse after the trend of KAI’s daily passengers began to decrease significantly since the announcement of the first Covid-19 patient in Indonesia as of March 2020.

This was followed by various Large-Scale Social Restriction policies that further limit the passenger speed of KAI and its subsidiaries. As of June 2020, KAI’s passenger volume per day has decreased by 45% and passenger transport revenue has decreased by 52% compared to normal conditions. KAI’s freight revenue until April 2020 still shows growth and supports KAI’s overall revenue to decrease in revenue. The pandemic resulted in company losses and reduced company performance, passenger revenue per day which had so far reached 23 billion fell to only 300 million per day, along with the number of passengers plunging from 1.2 million passengers per day to 200 thousand per day during the pandemic.6

Table I: Projection of Parent Profit / Loss Performance until December 2020

![Table I: Projection of Parent Profit / Loss Performance until December 2020](image-url)
Table II: Projection of Parent Profit / Loss Performance until June 2020

<table>
<thead>
<tr>
<th>NO</th>
<th>UR AI N</th>
<th>URAJAN</th>
<th>RKA S. MEI</th>
<th>RKA S. JUN</th>
<th>EFISIEN SI</th>
<th>PROYEKSI LABA (RUGI) S.D 30 JUNI 2020</th>
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<tr>
<td>1</td>
<td>Angkutan Menengah</td>
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<td>569.072</td>
<td>74.668</td>
<td>1.642.246</td>
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<td>Angkutan Kargo</td>
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<td>495.690</td>
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</tr>
<tr>
<td>3</td>
<td>Pembangunan Angkutan Sd</td>
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<td>3.210</td>
<td>73.616</td>
<td>423.849</td>
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<tr>
<td>4</td>
<td>Inspeksi Kualitas</td>
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<td>52.626</td>
<td>20.605</td>
<td>332.540</td>
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</tr>
<tr>
<td>5</td>
<td>Pengelolaan Sd</td>
<td>162.046</td>
<td>267.470</td>
<td>12.341</td>
<td>519.316</td>
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Table III: Projected Master License until June 2020

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<th>NO</th>
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<th>URAJAN</th>
<th>RKA S. MEI</th>
<th>RKA S. JUN</th>
<th>EFISIEN SI</th>
<th>PROYEKSI EFISIENSI S.D 30 JUNI 2020</th>
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<tbody>
<tr>
<td>1</td>
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<td>2</td>
<td>Angkutan Kargo</td>
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<td>2.706.038</td>
<td>4.143.743</td>
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<tr>
<td>3</td>
<td>Pembangunan Angkutan Sd</td>
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<td>939.049</td>
<td>74.365</td>
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<tr>
<td>4</td>
<td>Penumpang Kq Tanker</td>
<td>493.214</td>
<td>284.086</td>
<td>590.850</td>
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</tr>
<tr>
<td>5</td>
<td>Penumpang Kq Tanker</td>
<td>1.106.909</td>
<td>763.488</td>
<td>1.334.670</td>
<td>941.831</td>
<td></td>
</tr>
</tbody>
</table>

Effisiensi skenario optimis (full year) Pencapaian efisiensi ke setahun penuh 34% 47%
The Covid-19 pandemic has made an important history in having a bad impact on company performance, the government’s appeal to all affected communities is to carry out social and physical distancing, even the government imposes large-scale social restrictions (PSBB) in areas experiencing red zones in Indonesia. So this is the main reason for the decline in KAI’s performance. For KAI itself, researchers are motivated to discuss what strategies KAI is doing and how KAI survives in the face of the current pandemic. It is important to research this in order to provide a reference for academics in future research as well as for practitioners when faced with the same conditions. This research will discuss the impacts and sustainability strategies that will be carried out by KAI during the Covid-19 pandemic.

Research Methods

This research was conducted using qualitative methods, where this research aims to explain the impact and strategies the company undertakes in dealing with force majeure (the Covid-19 pandemic). Qualitative design does not use hypotheses, does not contain variables and does not compare variable relationships but looks for information on a single concept - central phenomena and deep understanding (Ghozali, 2016). The data source in this article uses secondary data. As for the method of data collection through observation of literature studies, by reading and reviewing and analyzing various literatures, both in the form of documents, journals and KAI data that issue information relevant to the research.7

Discussion

KAI plays an important role as mass transportation that is energy efficient and environmentally friendly. The products offered by KAI are passenger train transportation, freight train transportation, property management services related to trains, train-based tourism services, restaurants and logistics distribution. To answer these challenges, it is necessary to make efforts that are better and structured in company operations. Adverse impacts related to company performance are reflected in a very significant decline in both passenger and freight transport. In passenger transportation the following occurs.8

1. Reduction and cancellation of train trips;
2. Limitation of railway transportation capacity (social distance government policy);
3. The number of passengers who cancel the train trip;
4. The decrease in the number of train users interested as a result of the decrease in the activity of the number of trips of people who use the train.

Meanwhile, the decline in freight transportation was caused by a decrease in coal market demand that occurred in several countries, this has resulted in coal transportation partners canceling train trips. KAI’s response to the impact of the Covid-19 pandemic was to take several strategic steps, including: First, what was carried out by KAI’s management was to protect KAI passengers and employees or what is known as “Protect Our People”, namely prioritizing the health of train transportation users. Api and the KAI employees. In December 2019, when news broke out for the first time about the Corona virus in Wuhan China, KAI management made instructions for the directors regarding the protocol to prevent the spread of the Novel Corona Virus (2019-NCOV) at stations and on trains. Then after on March 2, President Joko Widodo announced the first positive case of corona in Indonesia and was followed on March 16, the Minister of Administrative Reform and Bureaucratic Reform (Tjahjo Kumolo) wrote a circular with an appeal to all state civil servants to work at home. At the same time, the Management of KAI issued directors regulations regarding the following decisions:

1. Establishment of a Task Force for Handling Novel Corona Virus;
2. Work Arrangements for Workers in order to anticipate the spread of the corona virus (Covid-19);
3. The process of procuring goods and services
during the emergency period of the Corona virus disease outbreak (Covid-19);

4. Prohibition of traveling outside the region and / or homecoming activities in an effort to prevent the spread of the corona virus diseases (Covid-19);

5. Provide incentives for workers who are at risk of being infected with the corona virus diseases (Covid-19);

6. The protocol for preventing the spread of the corona virus diseases (Covid-19) on extraordinary train trips according to the criteria for limiting people’s travel in the context of accelerating the handling of the corona virus diseases (Covid-19)

The second step taken by KAI’s management in saving the company is to maintain the company’s liquidity by preparing several scenarios if the pandemic ends in June, ends in August and when it ends in December 2020. KAI continues to prepare payments, by preparing sufficient bank loans to maintain liquidity. The three scenarios are prepared as a projection for the end of the pandemic, including the optimistic, moderate and pessimistic scenario with the following explanations:

1. An optimistic scenario, if the pandemic ends in June with an estimated passenger transport revenue reaching 2.8 trillion or equal to 33% of the 2020 target and freight transport reaching 6.3 trillion or equal to 74% of the 2020 target.

2. Moderate scenario, if the pandemic ends in August with passenger transport revenues reaching 2.2 trillion or equal to 26% of the 2020 target and freight transport reaching 6.1 trillion or 71% of the 2020 target.

3. The pessimistic scenario, if the pandemic ends in December with passenger transport revenues reaching 1.7 trillion or equal to 20% of the 2020 target and freight transportation of 5.7 trillion or 67% of the 2020 target.

These three scenarios are scenarios when the pandemic ends at these times and also by predicting the losses incurred by the company from each scenario. This then makes management take the third step, namely by carrying out efficiency in costs (operational expenditure) and reducing investment (capital expenditure). With the decline in company revenue for the products offered due to the impact of the Covid-19 pandemic, the company has taken initiatives in terms of cost savings to balance the potential decline in revenue due to the Covid-19 pandemic. In addition to this, the company also supports government policies regarding social distancing in which people reduce their mobility by reducing the number of passenger train trips and limiting the number of occupancy passengers on passenger trains.

The company also undertakes optimization initiatives in managing capital expenditure with the aim of managing cash flow. Optimization initiatives are carried out with the aim of reviewing the feasibility study of the intended investment by readjusting the assumptions used including the expected rate of return on investment.

The new normal scenario applied to passengers is the first step in restoring public confidence in KAI transportation services. The company will also continue to increase its role in the Corporate Social Responsibility (CSR) program as a form of the company’s concern for the community during the Covid-19 pandemic. (Lloret, 2016) according to him there are three domains that can control sustainability, including stakeholders, sustainable leadership and corporate governance.

**Conclusion**

KAI has carried out various strategies in dealing with the impact of the Covid-19 pandemic. Several things that have been done by KAI in dealing with the impact of the Covid-19 pandemic include: 1. Protecting passengers and employees of KAI by prioritizing health through the Covid-19 prevention protocol; 2. Maintain company liquidity by preparing several scenarios, if the pandemic ends in June, August or December; 3. Perform cost efficiency (operational expenditure) and reduced investment (capital expenditure) to balance the potential decline in income.

**Conflict of Interest:** Nil

**Ethical Clearance:** This research has been proven
in the thesis examiner board, at the Postgraduate Program, Masters in Management, Universitas Sebelas Maret, Surakarta, Indonesia.

Source of Funding- Self

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Novelty Predictor ZnO Nano practical for Cancer Treatment

Anmar Hameed Bloh¹, Hassan Khuder Naji², Antesar Rheem Obead²

¹Lecture, Department of Radiology and Sonography Technique, AL-Rafidian University College, Babylon University, College of Education, Science Dept., Hilla, Iraq

Abstract

Zinc oxide nanoparticles (ZnO NPs) are used to increasing the number of industrial products such as rubber, paint, coating, and cosmetic and one of the most popular metal oxide nanoparticles in biological applications. The ZnO NPs have become exceptional biochemistry, trade and industry, and less poisonousness. In biomedicine the ZnO NPs have been appeared a encouraging possible, specially in the playing field of anticancer and antiseptic grounds, which are included by their effective capability to initiate additional of reactive oxygen species (ROS) creation, produce the zinc ions, and the cell apoptosis have been induce. Furthermore, to keep the structural integrity of insulin is well known zinc. Consequently, the ZnO NPs have been efficiently technologically advanced designed for antidiabetic drug. The ZnO NPs appearance exceptional luminescent material goods in addition have straight them into one of the chief applicants for bio imaging. Now, in the biomedical fields the synthesis of ZnO NPs for facilitating of future research progress and focusing in biomedical fields will be helpful.

Key words: ZnONPs, Nanoparticles, cancer, Cytotoxicity, Reactive Oxygen Species (ROS),

Introduction

The cancer, is a circumstance of uninhibited cell difference, it has typically been preserved by chemotherapy, radiation and surgery through the historical numerous periods (¹). The treatments are surely effective in the construction of cancer cells, but then, they derived through the charge of an increasing frequency of adversative significances because of indiscriminate special effects focused to standard cells also (²). The treatments have been currently become out-of-date in cancer management because of the progress of Nano medicine, targeted medicine delivery and multi-target inhibitors (³). The field of biomedical application of nanotechnology that is Nanomedicine (NPs) are using to treatment disorder. For early detection of cancer and cancer treatment, nanomedicine, have been advanced imaging then beneficial abilities, has been the possible (⁴). It has the properties of active/passive aiming, in elevation solubility and bio obtainability, biocompatibility then multi-functional in excess of outdated cancer treatments (⁵). The main feature of nanomedicine includes mineral NPs. Numerous mineral NPs conjugated using anticancerous medications or else bio-active fragments (peptides, proteins, DNA, etc.) have previously been appropriate via the U.S. Nutrition in addition Medication Management (FDA) and European marketplaces, such as Feridex, , etc. (⁴). Moreover, mineral NPs appearance discriminating cytotoxicity to cancer cells (⁶). iron oxide NPs, titanium dioxide NPs, cerium oxide NPs, zinc oxide NPs, copper oxide NPs, silica NPs, etc is like of mineral NPs., are being widely researched and used for anticancer therapy (⁷). The unique features of the nanoparticles has its own, which creates them a original then effective instrument for anticancer treatment. The Iron oxide NPs is conjugated through anticancer medications are being to create magneto-sensitive NPs for discriminating targeting via magnetic fields in cancer management (⁸).
Similarly, in photodynamic therapy, titanium dioxide NPs are used for cancer treatment. For photosensitizer are used as a replacement, that is excited by radiation to induce Reactive oxygen species (ROS) generation in addition of apoptosis (9, 10) in radiation treatment for cancer treatment that the Cerium oxide NPs are used, that in selectively kill irradiated cancer cells even though posing no properties on the adjacent usual cells (11) for selective cytotoxicity in cancer cells, the Zinc oxide NPs are also used, anywhere they appearance cytotoxicity by zinc-dependent protein activity disequilibrium then ROS induction (12). The plant extract such as Ficus religiosa is using for synthesized Copper oxide NPs (13) or Acalypha indica (14) and the methods of synthesis are simple, non-toxic and eco-friendly (15). A good carrier for drugs in anticancer treatment is making of controllable pores of silica NPs (16). Furthermore, gold, silver then platinum NPs, have been known as precious metallic or noble metal NPs, be present to used for cancer treatment by way of medication distribution and therapeutic agents (8). The advantageous of low reactive nature of these noble elements is drug delivery purposes.

2-Zinc-mediated protein activity disequilibrium

In the human body, the one of the major trace elements is found in Zinc and is preserved in a certain concentration secret a cell (17). Modification the concentration of zinc in the cell might reason severe difficulties in numerous cellular methods, such as zinc is the co-factor of additional than 300 mammalian enzyme (18). One of the application of ZnO NPs in the intracellular release of zinc ions, increases from normal level, subsequent in zinc facilitated protein activity disequilibrium. The disturbs a extensive variety of critical cellular methods, containing (DNA replication, DNA damage repair, apoptosis, oxidative stress, electron transport chain, cellular homeostasis, etc., rendering cytotoxicity towards the cell) (14).

![Figure 1. A schematic representation of the mechanism of cytotoxicity of a nanoparticle](image)

The synthesis of ZnO NPs and the biological activity of nanoparticles are determined by features of superficial chemistry, size circulation, morphology of the constituent component, and solution reactivity of the constituent part. Accordingly, nanoparticles advance through organized structures similar in Scale, morphology, and functionality are important for many biomedical applications.
The ZnONPs will be responsible for a wide range of assets taking place in an equal rich of proportions then shape. In recent years, stable ZnONP methods have been widely spread and developed, consisting mainly of the biochemical precipitation process, sol-gel process, solid-state pyrolytic process, solution-free mechanochemical process, and biosynthesis process.

2.1. Precipitation by biochemistry. The main general method for preparing ZnO NPs is biochemical precipitation, which also involves double reaction chemicals: a highly concentrated zinc precursor such as zinc acetate (Zn(CH3COO)2•2H2O), zinc nitrate (Zn(NO3)2), or zinc sulfate (ZnSO4) and a precipitator solution such as sodium hydroxide (NaOH) or ammonium hydroxide (NH3•H2O) \(^{(19)}\). The precipitator is normally additional droplet astute to the dissolved zinc precursor that awaits the pH level stretch to around 10. Fully mix together the solutions to become a white zinc hydroxide intermediary. Eventually, the example of zinc hydroxide (Zn(OH)2) was transformed into ZnO after sintering at elevation temperature. Organized factors in the process consist mostly of zinc meditation for runner then precipitator, molar ratio of double mixtures, calcination temperature reaction.

ZnO NPs have been synthesized using the Zn(CH3COO)2•2H2O and NaOH biochemical precipitation mechanism at a molar ratio of 1:5. In a muffle furnace, intermediate products were calcined at 200 °C for 2 hours to obtain clean, fine ZnO powder of 18.67±2.2nm \(^{(20)}\). ZnSO4 and NaOH solution provided a simplified methodology of precipitation in ZnO NPs with a molar ratio of 1:2. Available at chamber temperature under vigorous stirring for 12h. The white precipitate achieved has been washed away numerous periods and divided through centrifugation \(^{(21)}\). The precipitate (ZnO) stayed dried out in an oven for 6h at 100 °C as a last step. By a flake-like construction the structured ZnO NPs provided a supply of proportions of about 100 nm. Where ZnO NPs are located via In addition to being modest and simply organized, the biochemical precipitation process is informal industrial. On the other hand, owing to the superficial effect of nanoparticles, the nanooxide precursor organized via the process of biochemical precipitation could simply proceed with agglomerates.

ZnO Nanoparticles’ biomedical applications, such as a novel form of low-charge then low-toxicity nanomaterial, have been of great importance in various biomedical fields, containing anti-cancer, antibacterial, antioxidant, antidiabetic, and anti-inflammatory activities, as well as being used for drug delivery and bioimaging applications \(^{(22)}\). Here we have summed up the recent

2.2. Anticancer Activity: the cancer, a circumstance of restrained malignant cell proliferation, is classically preserved via chemotherapy, radiotherapy, and surgery in the historical numerous periods. Even though altogether the treatments appear to be identical effective for killing cancer cells in concept, the send on discriminating treatment approaches too present a lot of thoughtful cross effects \(^{(23)}\). In recent times, nanosubstantial-established nano medicine, by high biocompatibility, certainly superficial functional, cancer targeting, then medication delivery dimensions, has confirmed the possible to overwhelmed the adjacent effects. For adults The Zn2+ is an important nutrient, then ZnO nanomaterials remain deliberated to be not dangerous in vivo. The ZnO NPs can be designated as biocompanionable and biodegradable nano platforms then can also be discovered for cancer treatment \(^{(24)}\).

2.3. Anticancer activity by inducing apoptosis of cancer cells. It has been known to be associated with cellular ROS generation in the mitochondrial electron transport chain, so anticancer causes that arrive addicted to cancer cells may break the electron transport chain and then release huge amounts of ROS \(^{(25)}\). Extreme ROS, however, may result in mitochondrial injury in the damage.
**Conclusion**

In feature, Nanoparticles, have been presentation accumulative application in cancer investigation for treatment. ZnO NPs can be good alternatives used for outmoded cancer treatment such as a transporter agent. The assessment has mostly attentive on ZnONPs, with the relative among zinc and cancer, zinc’s character in the humanoid body then the ZnO NPs using the natural science of the human body, and cytotoxicity to cancer cells.

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**Conflict of Interest** : There has been no conflict of interest of any kind with the authors of this work

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**Ethical standard** : The study was formally approved the research plan by the ethical committee board at the Babylon health directorate

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Comparative Study Thymol Oil and Some Gel Face Washing on Resistance Propionibacterium Acnes

Mais E.Ahmed¹, Jenan A.Ghafil², Walaa Najim³
¹Assistant Professor, ²Lecturer, College of Science, Department of Biology, University of Baghdad, Baghdad, Iraq, ³Instructor Institute of Laser Graduate Studies, Baghdad, Iraq

Abstract

Acne is elevated of skin secretion with hyperkeratosis causing Propionibacterium acnes. The goal of study was to assess the anti-inflammatory, and antibacterial potential of Thymol oil compare Gel face ,and is the main bacterium involved in the acne. The unpredictable use of antibiotics for the treatment of acne may end up within the advancement of bacterial resistance.

Key words: Thymol oil, P. acnes, oil, Gel face, Hyperkeratosis

Introduction

A Gram-positive P. acnes is, anaerobic bacteria which colonizing the lipid-rich sebaceous surface of the human skin [¹]. It has a place to the clinical gather of skin commensals, but can every so often cause genuine contaminations deciding the nearness of P. acnes contamination can be troublesome. Due to its less harmfulness, contaminations are less dolente [²].

Skin break out could be an incessant incendiary illness of the pilosebaceous joins together, fundamentally of the confront, nose, shoulders and upper arms. It is one of the most common dermatoses affecting high school populace [³]. Acne vulgaris is one of the most common skin disarranges, and dermatologists are still have long struggled to treat acne completely. It primarily influences young people, in spite of the fact that it may display at any age. It is nearly all inclusive malady happening in all races. The rate of seriousness of skin break out, top at (40%) in 14-17 year ancient young ladies and (35%) in boys matured 16-19 year (4). It influences the skin of the confront, neck and upper trunk. These specific sebaceous follicles have capacious follicular channels and voluminous, multi acinar sebaceous organs. Skin break out creates when these specialized follicles experience pathologic change that comes about within the arrangement of non-inflammatory injuries (comedons) and fiery injuries (papules, pustules and knobs [⁵]. The intemperate utilize of anti-microbial for long periods has led to expanded resistance in skin break out causing microbes i.e. P.acne and S. epidermidis against a number of anti-microbial utilized to treat skin break out [⁶].

The antibacterial properties of (EO) has long been recognized and broadly tried in vitro against a broad run of pathogenic microscopic organisms, counting both (G⁺ve) and (G⁻ve) bacteria [⁷]. One of the foremost copious bunches of normal compounds is spoken to by the fundamental oils, Fundamental oils (EOs) are fragrant sleek fluids gotten from plant fabric.

They can amplify the rack lifeof natural or prepared nourishments by decreasing microbial development rate or reasonability. A few of these substances are moreover known in its contributions to self-defense of plants against irresistible life forms [⁸].

Thymol a phenolic compound show in undamental oils, may be a common monoterpen and carvacrol isomer that extricated from thyme and the other sorts of plants, Thymol is less water dissolvable at unbiased pH, but it is as well dissolvable in some natural solvents.
and liquor \cite{9}.

The carvacrol and thymol antioxidant impacts of have been affirmed in a few considers, recommending their organization as nutritious components within the enhancement of novel useful nourishments. Thymol defensive nature against charms the field of drugs in dental caries \cite{10,11,13}.

**Material and Methods**

Isolates strain were identified by microscopy and microscopy tests for identification of \emph{P. acnes} \cite{16,18}. Directly transport from the pus and sterile disposable cotton swabs, transport media and carry to the laboratory.

Final identification by Viteck System 2 : All the presumptive isolates (on blood agar (BA) medium and Brain heart infusion (BHI) agar were tested by Viteck 2 system (Biomerix, France). This system used for diagnosis of \emph{Propionibacterium acnes}.

**Antibiotic sensitivity test \emph{P. acnes}**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount / ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esknol</td>
<td>100µl</td>
</tr>
<tr>
<td>Pharmacies</td>
<td>100µl</td>
</tr>
<tr>
<td>Bio Balance</td>
<td>100µl</td>
</tr>
</tbody>
</table>

**Formulation of Face wash Gel**:

**Antibacterial test was determined using -well diffusing method according to the CLSI Clinical Laboratory Standards Institute** \cite{26}.

**Extraction of essential oils**:

About 20g of herb soaked in distilled water. The volatile vapour that condensed at water temperature of 80°C was called essential oils. The distilled oils were labeled and placed in a fridge until ready for use.

**Result**

The cases Most were obtain patients between the ages \cite{16-21} years old in both sexes, and the distribution of acne among female was higher than that of male (14% and 4%) respectively.
Table (1) *P. acnes* infection according to age and gender groups

<table>
<thead>
<tr>
<th>Age group (year)</th>
<th>Sample No.</th>
<th>Male Positive No. (%)</th>
<th>Female Positive No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-15</td>
<td>17</td>
<td>3 (4.8)</td>
<td>4 (6.4)</td>
</tr>
<tr>
<td>16-20</td>
<td>20</td>
<td>13 (20.9)</td>
<td>16 (25.8)</td>
</tr>
<tr>
<td>21-25</td>
<td>13</td>
<td>2 (3.2)</td>
<td>2 (3.2)</td>
</tr>
<tr>
<td>Chi-square Value</td>
<td>-----------</td>
<td>9.135 **</td>
<td>9.852 **</td>
</tr>
</tbody>
</table>

** (P≤0.01)

Macroscopic examination

Colonies appeared as circular, opaque and glistening colonies with different Colors, where may be white, gray or yellow. Bacterial colonies showed weak or no hemolysis when grown on BA. Figure (1)

![Figure (1): P.acnes on A) BHIA B) Blood agar at 37c for 48 hrs](image1)

Microscopic examination

After stained by Gram stain, all presumptive of isolates *P. acnes* isolates were Gram positive, in different forms polymorphism cells Figures(2).

![Figure (2): P. acnes arrangements (1000 x).](image2)
Antibiotic sensitivity test (AST) of *P. acnes*:

Result os AST show in (Figure 3) the highest sensitivity *P.acne* was to Levofloxacin then Clindamycin, while appeared highly resistance to Azithromycin , erythromycin, and metronidazole.

![Fig 3](image)

**Figure (3):** Antibiotic susceptibility test of *Propionibacterium acne.*

Used the morphological and biochemical tests and PCR technique to identify the *P. acnes* in lesions. The Figure(4) presents the PCR product electrophoresis on a garose gel (1.5%).

![Fig 4](image)

**Figure 4.** Amplification of 16SrDNA (1400bp) bacterial isolates. Agarose (1.2%), 5 V/cm for 2 h, stained with Red safe and visualized under UV transilluminator. M. 100 bp DNA marker. Lane 1-10: bacterial isolates.
Table (2): comparative results used against 

\[ P. \text{acnes} \]

<table>
<thead>
<tr>
<th>Treatment used against P. acnes</th>
<th>Inhibition zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levofloxacin</td>
<td>9± 0.04 d</td>
</tr>
<tr>
<td>Thyme oil (commercial)</td>
<td>20 ± 1.26 ab</td>
</tr>
<tr>
<td>Eskolin</td>
<td>10.4 ± 0.37 d</td>
</tr>
<tr>
<td>Pharmacies</td>
<td>10.5 ± 0.42 d</td>
</tr>
<tr>
<td>Bio Balance</td>
<td>16 ± 0.79 bc</td>
</tr>
<tr>
<td>LSD value</td>
<td>4.282 **</td>
</tr>
</tbody>
</table>

** (P<0.01).

Discussion

The current results were coincides with previous results studied in Iraq of \( P. \) acne in different age (15). that acne prevalence was more in female than male, agree with most cases were in ages group between (15-20). Also agree with study in Iraq isolated \( P. \) acne \[17\]. These results for identification \( P. \) acne were in agreement with \[18, 19\]. Viteck 2 System was a rapid, sensitive, to distinguish clinical isolates (20). The result agree with \[21\] The most using treatment (erythromycin) were anti \( P. \) acne \[22\].

The antibiotics is topical application like clindamycin, tetracycline most common to treatment of
acne but the major problems growing in resistance of *P. acnes* to antibiotics caused [23].

The acne-gel with retinol had lower effect in agar dilution test, but shown that is highly effective at high concentrations against aerobic and anaerobic bacteria including *P. acnes* [24] the antiacne more acts of oregano EO that of other evaluated including commercialized over the counter acne treatment tea tree [25].

**Conclusion**

The antibiotics play an important treatment in acne administers. Resistance *P. acnes* treatment by antibiotic compares using natural oil most affections alternative commercial washing gel in pharmacy.

**Conflict of Interest:** The authors declare that there is no conflict of interest regarding this study.

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**Ethical Committee Approval:** This work was approval by the ethical committee of Department of Biology, College of Science, University of Baghdad.

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Primary Prevention Program of Obesity among Primary School Children

Amina Mohamed Ismail 1, Hanaa Abdel Hakeem Ahmed2, Frial Fouad Malika3
1Research Scholar, 2Professor, 3Lecturer of Community Health Nursing, Faculty of Nursing, Ain-Shams University

Abstract

Background: Childhood obesity is a serious medical condition that affects children and adolescents. It’s particularly troubling because the extra pounds often start children on the path to health problems that were once considered adult problems: diabetes, high blood pressure and high cholesterol. Aim of the Study: assess the effect of primary prevention program of obesity among primary school children through: assessing knowledge & practices of primary school children related to obesity, Designing, implementing primary prevention program of obesity among primary school children, evaluating the effectiveness of primary prevention program on knowledge & practices related to obesity among primary school children.

Subjects and Methods: Setting: The study was conducted in four primary governmental school of El-Salam city, Cairo governorate including: (El Hussien, Osman Ben Afan, Atef El Sadat and Gamal Abdel Naser). Size: the sample composed of 144 primary school children chosen randomly through multistage sampling. Tools: Different tools were used for data collection of study and it was written in simple language to suit the understanding level of the studied primary school children First tool: Structured interviewing questionnaire, second tool: Anthropometric Measurements, physical assessment sheet Results: clarified that only few percentage of primary school children had good Knowledge preprogram while this improved to more than one third post program implementation related to their total knowledge about childhood obesity, there was highly statistically significant difference in post-program compared to pre-program according to their total practice related to childhood obesity. Conclusions: primary school children improved their knowledge and practice regarding childhood obesity after program implementation. Recommendation: primary prevention program must be provided for all primary school children to prevent childhood obesity risks.

Keywords: prevention program, primary school children, childhood obesity

Introduction

Childhood Obesity is defined as excess body fat leading to health impairment (1) (2). For boys, obesity was defined as >20% fat mass (FM). For girls, the cut-off point for obesity was >25% (3). However, FM is extremely difficult to measure in young children, because accurate techniques require a high degree of the subject compliance (4). The ideal definition, based on percentage body fat, is impracticable for epidemiological use. The measurement of change in adiposity in children is challenging because of the effects of maturation and growth on lean muscle mass, fat mass, and hydration status (5). Although less sensitive than skin fold thicknesses, the body mass index (weight/height²) is widely used in adult populations, and a cut-off point of 30 kg/m² is recognized internationally as a definition of adult obesity (6).

Corresponding Author:
Amina Mohamed Ismail,
Ain Shams, Egypt
Email: Aminaesmail051@gmail.com
Body mass index in childhood changes substantially with age. For many years, establishing an international definition of overweight and obesity among children based on pooled international data for BMI linked with adult obesity cut-off point, remained a big challenge (7).

There are many factors that influence food intake and energy expenditure in man. The following gives a brief account of these factors known to be involved in occurrence of obesity (8).

- **Genetic Factors:**

  It is a matter of common observation that obesity runs in families. It has been suggested that this is probably due to transmission of an abnormal gene from parents to child (9).

- **Socioeconomic factors:**

  Socioeconomic factors have been shown by most studies, to be correlated in one way or another, with overweight.

  Obesity is common among poor women, perhaps because food rich in protein and fat are more expensive than starchy foods which provide the bulk of cheap meals (10).

- **Psychological factors:**

  Psychological disturbances, like obesity, are very common in our complicated world today. When both are present in the same individual one may aggravate the other. Some obese children with a psychoneurosis or a personality disorder who are in happy find solace in cream buns and chocolates in the same way as others take alcohol or barbiturates and so they become more fat (10).

- **Endocrine factors:**

  Davidson, and passmore, (2018) (11) discussed the effect of endocrine factors on childhood obesity formation and declared that obesity in women commonly begins at children girls, puberty, pregnancy or in the menopausal period which suggests an endocrine factor. Cushing's syndrome arising from over secretion of adrenal cortical hormones is characterized by a special form of obesity, in which excess fat is laid down over the trunk and abdomen and usually not on the limbs (12).

- **Eating habits:**

  Obesity may occur as a result of family customs in using concentrated high caloric foods and drinks, in having to maintain social relationships including rich party foods in addition to usual meals or, in eating excessive amounts of carbohydrate foods because they are cheaper than lower caloric fruits and vegetables (12).

Generally, the community health nurse is a generalist and serves all population groups working in the clinic's setting not only utilizing principles of primary, secondary, and tertiary prevention but also serves population groups in clinics and schools. Moreover, nurses are largest segment of health care providers where, they have great opportunity to enhance client care, as well as impact health care delivery. The nursing profession has the holistic approach to client care, including a nursing process that fosters the participation and growth of clients and their significant others (13).

Comprehensive school health services are being developed to assist in meeting the needs of each community’s population of children. Intervention at primary, secondary and tertiary prevention levels are incorporated through collaboration with a diverse group of educational personnel and health care providers in the community who have fewer financial resources. The school nurse can be a central person in coordinating services (14).

**Significance of the problems:**

Worldwide in 2016 more than one-third of all school children were obese. Rates of school age children obesity have increased greatly between 1980 and 2016. It has more than doubled in school age children and tripled in adolescents in the past 20 years. Obesity is growing worldwide and becoming an emotional, psychological and financial burden on individuals and communities by
becoming a leading risk for global health problems. It has affected both developed and developing countries, and people of all socioeconomic levels (14).

Egypt has the highest percentage of obese adults worldwide as, Around 19 million Egyptians, or 35 percent of the adult population are obese – the highest rate across the globe. In addition, over 10 percent, or 3.6 million, of children are also considerably overweight (14).

Egypt, previous studies indicated that obesity is an important public health problem among school children as the prevalence of obesity among Egyptian children was 14.7 and 15.08% in boys and girls, respectively.

Furthermore, especially in Egypt, there is very little basic awareness of the problem of obesity, and so there are neither nationwide movements nor adequate documentation of initiatives dealing with obesity (15).

**Aim of the Study**

_This study aimed to_: assess the effect of primary prevention program of obesity among primary school children through:

- Assessing knowledge & practices of primary school children related to obesity.

- Designing, implementing primary prevention program of obesity among primary school children.

- Evaluating the effectiveness of primary prevention program on knowledge & practices related to obesity among primary school children.

**Hypothesis:** Primary prevention program will improve knowledge and practices related to obesity among primary school children.

**Subjects and Methods**

_Subjects and methods of this study were portrayed under four main domains as following:_

1- _Technical Design:_

_Research design:_

Quasi experimental study design (one group pre and post-test) was conducted for this study.

**Research setting:** This study was conducted in 4 primary governmental school of El-Salam city, Cairo governorate including: (El Hussien , Osman Ben AFAN, Atef El Sadat and Gamal Abdel Naser). These schools were chosen by a systematic random sample (select a random start at fixed interval) as interval (K) determining by divided the total number of primary governmental schools in El-Salam education management (N) = 32 schools , by the desired sample size (n)= 4 primary school. K = total number of primary school (N)/ desired sample size (n) =32/4=8th (every 8th school, one school was involved in sample).

**Subjects of the study , sampling:**

- **Type of sampling**: Multistage sampling was used as the following:

  Ø Stage one: selection of 4 primary governmental school by systematic random sample.

  Ø Stage two: selection of classroom from each grade from first to sixth by simple random sample.

  Ø Stage three : selection 10% of school children age (6-12yrs) from each class room by simple random sample.

- **Size of sampling** : sample was carried on (144) school children in four primary school calculated as the following: Average density of classroom equal 60 child, 10% of them was chosen randomly equal 6 child from each class room of six grade, so total number equal 36 school children in all grades in one school. Total number in four schools= 36 times 4= 144 primary school children.

- **Exclusion criteria for sampling:**

  Ø All children found to be < 6, > 12 years of age.

  Ø Children whose exact birth date was not available.

  Ø Children without written informed consent.
Sick Children and whose with chronic diseases.

Tools of data collection:

Data will be collected by using the following tools:

**First tool:** Structured interviewing questionnaire, to assess obesity among primary school children, the investigator designed questionnaire after reviewing the related literature and written in simple clear Arabic language, it included the following three parts:

- **Part I : Socio-demographic** data of primary school children
- **Part II:** primary school children knowledge related to obesity about the following items: general knowledge related to childhood obesity, knowledge related to childhood obesity risk factors, knowledge related to healthy nutrition and and finally knowledge related to health hazards and complication of childhood obesity as

**Scoring system for knowledge:**

The total items of knowledge 25 questions the scoring system was followed according to school children answers calculated as the following: complete correct was scored 2, incomplete correct was scored 1, incorrect and did not know answers was scored zero for each items of knowledge.

The total scoring of knowledge was classified according to the following:

- **Poor knowledge** if less than 60%.
- **Average knowledge** if 60-75%.
- **Good knowledge** if more than 75%.

- **Part III:** It include checklist for assessing primary school children practices related to childhood obesity consists of six main items include the following:
  - First item: food practices
  - Second item: Practices for handling fluids
  - Third item: Sleep and rest practices
  - Fourth item: Physical activities and exercise
  - Fifth item: Electronic activities practices
  - Sixth item: Behavioral activities

**Scoring system for practice:**

The check list included six main items and 34 sub items, For check list practice items, predetermined according to literature review, done items was scored one and did not do items scored zero.

The total scoring of practice was classified according to the following:

- **Inadequate practice** if less than 60%.
- **Adequate practice** if more than 60%.

**Second tool:** Anthropometric Measurements, physical assessment sheet for primary school children and laboratory investigation was conducted by trained school nurses and investigator.

- **Anthropometric measurements:** was conducted according to guidelines suggested by The WHO Expert Committee (2015).

- **Physical assessment** include the following item: Signs and symptoms of obesity, Vital signs of the primary school, physical assessment from head to toes of primary school children

- **Laboratory investigation of primary school children** as (random blood sugar, hemoglobin, total Cholesterol level).

Content validity:

Tools of study was reviewed by three expertise in community health nursing to test the content validity. Content validity was checked before pilot study and actual field work.

Content reliability:

Was done by Cronbach’s Alpha coefficient test.
which revealed that each of the two tools consisted of relatively homogenous items as indicated by the moderate to high reliability of each tool.

v Operational design

The operational design of the study entailed three main phases:

· Preparatory phase.

· Pilot study.

· Field work.

· Preparatory phase

A review of the past and current available related literature covering the aspect of the research problem was done by the investigator through using available articles, magazines, Internet, journals and text books in order to be acquainted with the research problem and develop the study data collection tools and prevention program.

· Pilot study

A pilot study was conducted for 10% of total sample size equal (14) primary school children to evaluate the clarity of the tools and its reliability used according to the analysis of pilot study results. The modifications were done in the tool according to pilot study results in order to be more applicable and changes were fulfilled by correction, omission or addition of items, until the final shape of the tool was reached. The subjects of pilot study will excluded later from main study sampling.

· Field work:

- A written consent was taken from every primary school children and their parents to share in the study.

- The investigator was started with introducing herself and explaining the aim of the study and program for the selected studied sample and assured that the data collected would be confidential.

- The investigator will complete the tool by the interviewing the primary school children.

- The investigator will visit pre mentioned schools through school daytime to collect data from 7.30 am to 1 pm, three days in the week Monday, Tuesday and Wednesday.

- Though six months duration of the program from the start till finishes the program and makes evaluation stage.

- The investigator Used different teaching methodology such as discussion, session demonstration, booklet and poster.

Program construction:

· Primary Prevention program for primary school children was conducted in four phases:

· First phase: preparatory phase

A review of recent, current, national and international related literature in various aspect of the problem was done at this phase its aim is to design and develop the study tools and to be acquainted with various aspects.

· Second phase: assessment phase

By using questionnaire based on the assessment phase (pretest) was done for (144) primary school children and (post test) was done after primary prevention program implementation.

· Third phase: planning and implementation phase

This phase at the planning and implementing the primary prevention program and its content according to its objectives, primary prevention program was designed to assess the knowledge, practices related to obesity among primary school children through using multiple session range from 4-6 session every session ranged from 1-2 hours and meeting the primary school children three days per week (individualized or group).

The program session was divided three session theory and three session practices every week and the
teaching method was used the lecture group, discussion, and role-playing, teaching material was used is Arabic booklet and audiovisual materials.

**General objective**: to assess the knowledge, practices related to obesity among primary school children.

**The program content was included the following**:  
- Meaning of school children obesity  
- Causes of school children obesity  
- Identify risk factors for school children obesity  
- Detecting knowledge about obesity among primary school children and its consequences.  
- Determining practices about obesity prevention among primary school children.

Finally: the evaluation phase

This phase aimed to evaluate the effect of prevention program to improve school children knowledge and practices related to obesity, a post-test similar to the pre-test was administered to the study subjects immediately after completion of the primary prevention program and follow up after three months.

**Administrative design**

· First approval was obtained from the authorities of the faculty of nursing Ain Shames University.

· A written letters was sent to the director of the El-salam educational management include the aims of the study.

· Official permissions were obtained from El-salam educational management authorities.

**Ethical Consideration**

Informed consent was taken from the primary school children to participate in the study after explaining the objectives of the study, it will haven’t any harmful effects on them, the information would be confidential and they could withdraw from the study at any time.

**Statistical design**

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

§ Chi-square ($\chi^2$) test of significance was used in order to compare proportions between qualitative parameters.

§ Pearson’s correlation coefficient (r) test was used to assess the degree of association between two sets of variables.

§ The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

§ Probability (P-value):

- $P$-value <0.05 was considered significant.
- $P$-value <0.001 was considered as highly significant.
- $P$-value >0.05 was considered insignificant.

**Results**

This study targeted a sample of 144 primary school children conducted in four primary school in E l - Salam city in the year 2019 -2020.
Table (1): Distribution of primary school children according to their demographic data (N=144).

<table>
<thead>
<tr>
<th>Demographic characteristics of primary school children</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>76</td>
<td>52.8</td>
</tr>
<tr>
<td>Female</td>
<td>68</td>
<td>47.2</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 years - 8 years</td>
<td>72</td>
<td>50.0</td>
</tr>
<tr>
<td>9 years - 12 years</td>
<td>72</td>
<td>50.0</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>8.67±1.99</td>
<td></td>
</tr>
<tr>
<td>Class room grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First to third primary</td>
<td>72</td>
<td>50.0</td>
</tr>
<tr>
<td>Fourth to sixth primary</td>
<td>72</td>
<td>50.0</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slums</td>
<td>40</td>
<td>27.8</td>
</tr>
<tr>
<td>Urban</td>
<td>65</td>
<td>45.1</td>
</tr>
<tr>
<td>Rural</td>
<td>39</td>
<td>27.1</td>
</tr>
<tr>
<td>Number of sibling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3 siblings</td>
<td>96</td>
<td>66.6</td>
</tr>
<tr>
<td>3-5 siblings</td>
<td>25</td>
<td>17.4</td>
</tr>
<tr>
<td>&gt; five siblings</td>
<td>23</td>
<td>16</td>
</tr>
<tr>
<td>Daily Pocket money (LE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 LE</td>
<td>38</td>
<td>26.4</td>
</tr>
<tr>
<td>5-10 LE</td>
<td>92</td>
<td>63.9</td>
</tr>
<tr>
<td>&gt;10 LE</td>
<td>14</td>
<td>9.7</td>
</tr>
</tbody>
</table>

Table (1) shows that the mean age of the studied primary school children was 8.67, regarding gender 52.8% of them were male, regarding class room grade 50% of them were equal first to third primary and fourth to sixth, regarding residence 45.1% of them were urban, regarding number of sibling 66.6% of them had less than 3 siblings, as well as daily pocket money 63.9% of them take 5-10 LE.
Figure (1): Distribution of primary school children's parents according to their Body Mass Index (N=144).

![Graph showing distribution of BMI categories for fathers and mothers.]

Figure (1): reveals that body mass index of 50.0% of studied sample fathers were Overweight while 65.9% of their mothers were Overweight. As well as 31.3% of studied sample fathers were obese while 34% of their mothers were Obese.

Fig. (2): The relation between pre-program and post-program of primary school children according to their total knowledge about childhood obesity.

![Graph showing total knowledge distribution pre and post program.]

This figure clarifies that only 2.8% of primary school children had good Knowledge preprogram while this percentage improved to 38.2% post program related to their total knowledge about childhood obesity.
Figure (3): The relation between preprogram and post program total practice about obesity of primary school children. (N=144)

Figure (3): shows that, 61.8% of inadequate total practices of primary school children preprogram improved to 74.3% post program so, there was highly statistically significant difference in post-program compared to pre-program according to their total practice related to childhood obesity. With p-value <0.001 HS, X^2 116.156

Table (2): Distribution of primary school children according to their anthropometric measurements related to body mass index between pre-program and post-program.

<table>
<thead>
<tr>
<th>Anthropometric measurement:</th>
<th>Pre-program</th>
<th>Post-program</th>
<th>Chi-square test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Body weight (Kg) ≤20 kg</td>
<td>8</td>
<td>5.6</td>
<td>6</td>
</tr>
<tr>
<td>20-30 kg</td>
<td>84</td>
<td>58.3</td>
<td>98</td>
</tr>
<tr>
<td>&gt;30-40 kg</td>
<td>48</td>
<td>33.3</td>
<td>38</td>
</tr>
<tr>
<td>&gt;40 kg</td>
<td>2</td>
<td>2.8</td>
<td>2</td>
</tr>
<tr>
<td>Height (cm) ≤110 cm</td>
<td>11</td>
<td>7.6</td>
<td>9</td>
</tr>
<tr>
<td>&gt;110-120 cm</td>
<td>53</td>
<td>36.8</td>
<td>50</td>
</tr>
<tr>
<td>&gt;120-130 cm</td>
<td>55</td>
<td>38.2</td>
<td>57</td>
</tr>
<tr>
<td>&gt;130 cm</td>
<td>25</td>
<td>17.4</td>
<td>28</td>
</tr>
</tbody>
</table>
Continued... Table (2): Distribution of primary school children according to their anthropometric measurements related to body mass index between pre-program and post-program.

<table>
<thead>
<tr>
<th>Classification of weight BMI for age and sex</th>
<th>Underweight (5th percentile)</th>
<th>Normal weight (5th - 85th percentile)</th>
<th>Overweight (&gt;85th -95th percentile)</th>
<th>Obesity(&gt;95th percentile)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9</td>
<td>80</td>
<td>33</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>6.3</td>
<td>55.6</td>
<td>22.9</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>98</td>
<td>31</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>67.4</td>
<td>21.5</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p-value >0.05 NS; *p-value <0.05 S; **p-value <0.001 HS

Table 2: reveals that primary school children, regarding body weight 58.3% of them were from 20-30kg, regarding height 38.2% of them were from >120-130cm preprogram, this percentage changed to 68.1%, 39.6% respectively after implementation of program.

Also shows that 22.9% of primary school children were Over weight (>85th -95th percentile) preprogram, changed to 21.5% after program. Also clarifies that 15.3% of them were Obesity(>95th percentile) preprogram changed to 6.9% after program.

This table shows that, there was statistically significant difference in post-program compared to pre-program according to their classification of weight BMI for age and sex, with p-value <0.05 S.

**Discussion**

The current study was carried out to evaluate the effect of primary prevention program on knowledge and practices related to obesity among primary school children. As regarding socio demographic characteristics, the present study showed that, the mean age of the studied primary school children was 8.67, regarding gender, slightly more than half of studied sample were male, regarding residence, less than half of them were urban, regarding number of sibling, more than two thirds of them had less than 3 siblings, as well as daily pocket money, less than two thirds of them take 5-10 LE Table (1).

This finding was agreed with (2), who studied (Socio-demographic factors associated with overweight and obesity among primary school children in semi-urban areas of mid-western Nigeria). And found that the mean age of the studied primary school children was 8.5, and also found that more than half of studied sample were male.

The current study disagreement with (3) who reported that in a study about Overweight and obesity in the Eastern Mediterranean Region and shows that the students aged 25 or older are at high risk and those over 30 years are at extra ordinary risk, also in contrast to (4) in a study about Socioeconomic status and obesity,” Epidemiologic Reviews., which revealed that more than one third of students affected by obesity were over age, with mean age of thirty years, which indicated that obesity and age increased the risk.

This finding agrees with the results of the study about overweight and obesity among school Children in Jordan: Prevalence and Associated Factors by (5) who find that the daily pocket money was associated with
overweight, while family monthly income associated with obesity.

The current study findings disagree with (6) who studied obesity and relation with family size cross-sectional USA study reported that the prevalence of obesity in the United States is lower among those of small family number and stated that there exists a negative correlation between family size and obesity prevalence for student. This could be due to the fact that smaller families had better food availability.

The current study finding revealed that body mass index of half of studied sample fathers were overweight while slightly less than two thirds of their mothers were overweight. As well as less than one third of studied sample fathers and mothers were obese Figure (1).

This finding congruent with (7) who studied (Prevalence and factors associated with body mass index in Brazilian children aged 9-11 years), and found that there was significant associations of individual, family, and school/family environment factors in children. Therefore, the aim of this study was to assess the individual anthropometric and behavioral, family, and school/family environment factors associated with BMI in children aged 9-11 years.

This current study clarified that only few numbers of primary school children had good Knowledge preprogram while this improved to more than one third post program implementation related to their total knowledge about childhood obesity. Figure (2)

This findings is in the same line to (A study to assess the effectiveness of Eduative supportive interventions on Knowledge, regarding Obesity among primary school children in selected schools of Mehsana city) carried out by (17) who stated that After given educative supportive intervention, half of school children had improve the knowledge. That was an effective technique in inducing the total knowledge level of primary school children regarding obesity.

Childhood obesity is determined by both genetic and environmental factors. With a dramatic increase in childhood overweight and obesity, knowledge and attitudes of the children themselves towards childhood obesity need to be placed on the frontline. Most studies have focused on knowledge and perception of parents/caregivers and health care professional about child obesity. The present study is among few that have focused on assessment of knowledge about child obesity and perceptions about body weight among primary school children and also implementation of prevention program to improve their knowledge.

the relation between primary school children total knowledge may related to obesity and dietary habits. Knowledge only not enough to change behaviors, we need also strong motivates. This may be due to less of health educations program conducted by health care providers to prevent childhood obesity.

The current study result revealed that primary school children, regarding body weight more than half of them were from 20-30kg, regarding height more than one third of them were from >120-130cm preprogram, this percentage changed to more than two thirds and more than one third respectively after implementation of program.

Also showed that less than one quarter of primary school children were Overweight (>85th -95th percentile) preprogram, changed to 21.5% after program. Also clarifies that more than one tenth of them were Obesity (>95th percentile) preprogram changed to less than one tenth after program implementation.

This table showed that, there was statistically significant difference in post-program compared to pre-program according to their classification of weight BMI for age and sex, with p-value <0.05 S. table 2

**Conclusion**

The current study findings and research hypothesis concluded that implementation of primary prevention program for primary school children was efficient in improving primary school children knowledge regarding childhood obesity, with highly statistical significant differences between pre and post prevention program
There were highly statistically significant difference in post-program compared to pre-program according to their total practice related to childhood obesity. with p-value <0.001 HS, $X^2 = 116.156$

RECOMMENDATIONS

In the light of these findings it can be recommended that:

- The primary prevention program for primary school children regarding obesity must be provided for all primary school children to improve their knowledge and practice.

- An orientation program for all parents to improve their knowledge about childhood obesity.

- Distribution of different illustration instructional booklets and brochures for primary school children using simple information including preventing childhood obesity.

- Official and policies support for school including modification of curriculums to meet primary school children needs regarding childhood obesity.

- Further researches are needed to study the childhood obesity prevention to find out the suitable solution to prevent this problem.

Ethical Clearance: The study was approved from ethical and research committee faculty of nursing Ain Shams University, Egypt.

Source of Funding: Self-funding

Conflict of Interest – Nil

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Neuroprotective Effect of Moringa Oleifera Extract on Acetamiprid Induced Neurotoxicity and Apoptosis in Albino Rats

Amr A. Abdallah¹, Marwa A. Ibrahim², Eman A. Ibrahim³, Hossam El Din H. Abdelhafez¹, Naglaa F. Mahmoud⁴

¹Associate Prof. of Mammalian Toxicology Department, Central Agricultural Pesticides Lab. (CAPL), Agricultural Research Center, Dokki 12618, Giza, Egypt, ²Professor of Biochemistry and Molecular Biology Department, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Giza, Egypt, ³Associate Prof. of Plant Biochemistry Department, National Research Centre (NRC), 33 EL Bohouth st. (EL Tahrir st.), Dokki 12622, Giza, Egypt, ⁴Associate Prof. of Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Cairo University, El Manial 11956, Cairo, Egypt

Abstract

Acetamiprid (ACMP) can cause neurotoxicity and induces neuronal apoptosis. The ameliorative effects of Moringa Oleifera (MO) seed extract against the neurotoxicity induced by ACMP in adult male albino rats were investigated. Forty male albino rats were divided into 4 equal groups. The first group served as a control, the second group administered ACMP 26.8mg/kg b.w. (representing 1/10 LD50) by gavage. The third group was given combination of ACMP 26.8 mg/kg b.w. and MO seed extract 150 mg/kg b.w. the fourth group was given MO extract only 150 mg/kg b.w. for 28 consecutive days. Results proved the efficacy of MO extract as a neuroprotective agent through the reduction of the oxidant parameter malondialdehyde (MDA) content by 26.9%, significant increase of reduced glutathione content (GSH) by 21.4%, and elevation of catalase (CAT) and superoxide dismutase (SOD) activities by (62.2% and 40.8%) respectively, compared to ACMP intoxicated group. Consequently, gene expression analysis for Bax and NBN genes was downregulated by 54.5% and 36.3% respectively in MO treated rats (group III) compared with ACMP intoxicated group. Moreover, restoration of the pathological tissue injuries was noticed. In conclusion, MO proved to be an effective neuroprotective and anti-apoptotic agent against ACMP-induced toxicity.

Keywords: Acetamiprid; Moringa Oleifera; Neurotoxicity; Apoptosis; Neuroprotection.

Introduction

Acetamiprid (ACMP), is one of the recently developed neonicotinoid group of pesticides, widely used against different variety of insect pests (¹). It was the second manufactured insecticide in this group after imidacloprid (²). Acetamiprid operates as a selective nicotinic acetylcholine receptor (nAChR) agonist in the central nervous system of insects (²). Over the last ten years, the expanded usage of neonicotinoid insecticides has been criticized because of the hazard associated with their toxicity (³, ⁴). ACMP was reported to have toxic effects on the thyroid, liver, respiratory and reproductive functions (⁵). Acute poisoning after ingestion of acetamiprid in humans has been documented (⁶). However, there is no satisfactory information on the neurotoxicity potential of acetamiprid on human health.
Acetamiprid may lead to oxidative damage by producing reactive oxygen species in the target tissues (7). Exposure to acetamiprid induce imbalance of oxidative stress status, and disruption of mitochondrial membrane permeability in rat’s brain through generating reactive oxygen species (8). The brain is liable to the damage caused by oxidative stress imbalance because of its comparatively low antioxidant capacity, its abundance in peroxidable fatty acid and its high-energy needs; this irreversible molecular corruption is the main cause of neuronal cell death and neurodegenerative diseases (9).

Several phytochemical expressed considerable protective effects against neurotoxicity in animal models by restoring the antioxidant capacity (10). Many plants contain natural antioxidant compounds, which used as curative agents for neuronal diseases by increasing memory; alertness and brain function in general. Moringa oleifera (MO) named a miracle tree by consisting of phytochemical, vitamins, essential amino acids and mineral. It has many therapeutic benefits such as anti-inflammatory, antipyretic, antiulcer, antidiabetic, anticancer and antimicrobial agent (11). MO extract has a role in preventing Alzheimer’s disease by reducing hyperphosphorylation and amyloid-b pathology (11). This study aimed to evaluate the neurotoxic effects induced by acetamiprid in terms of oxidant/antioxidant status, apoptosis markers and histopathological changes in male rats with studying the ameliorative effect of MO seed extract.

Materials and Methods

Chemicals:

Acetamiprid insecticide neonicotinoids (20% SP) was obtained from Starchem. Agro. Pesticides company, Egypt.

Preparation of Moringa oleifera seed extract:

Powdered seed of moringa oleifera L (1kg) was extracted with hexane (1L×5) by soaking at room temperature. The hexane extracts were concentrated under reduced pressure at 60c. The total oil from hexane were weighed and stored at -10 until analysis. The residue of moringa oleifera after hexane extract was re-extracted with ethanol. Components of MO seeds extract determined by colour test method for phenols, flavonoids and tannins (12), carbohydrates and steroids (13).

Animals:

Forty albino rats aged 12-weeks (170±10g) were obtained from the breeding unit of the Toxicology and Forensic Medicine Department, Faculty of Veterinary Medicine, Cairo University. Animals were maintained at the animal care facilities of Central Agricultural Pesticides Laboratory (CAPL) in plastic cages under controlled temperature (23±2°C), 12-h light/dark cycle and average humidity (50±5%). Water and food were available ad libitum. Rat’s acclimatization to the environmental condition were done for two weeks preceded the experiment.

Experimental Design:

Animals were divided into four groups with 10 animals each. The first group was used as a control. The second group (group II) treated with acetamiprid 26.8mg/kg b. w. (representing 1/10 LD50) by gavage. The third group (group III) given combination of acetamiprid (26.8 mg/kg b.w.) and MO seed extract (150 mg/kg b.w.), the fourth group (group IV) was given MO extract only (150 mg/kg b.w.). After treatment for 28 days, rats were anesthetized using diethyl ether, killed by capitation and the brain of each rat was collected, washed and kept for further analysis.

Lipid peroxidation and antioxidants parameters measurements:

Tissue preparation:

Brain were removed, washed with cold saline buffer, immediately stored at -80°C. For obtaining enzymatic extract, tissues were homogenized in ice cold 50mM sodium phosphate buffer (pH7.4) containing 0.1mM ethylenediaminetetraacetic acids (EDTA) yielding 10 % (W/V) homogenate. The homogenates were centrifuged at 2000 r.p.m for 30 min at 4°C. The supernatant samples were separated and used for measurement of
malondialdehyde (MDA)\textsuperscript{(15)}, reduced glutathione content (GSH)\textsuperscript{(16)}, catalase enzyme activity\textsuperscript{(17)}, superoxide dismutase (SOD) enzyme activity\textsuperscript{(18)}.

**Quantitative real-time PCR for Bax and NBN genes:**

Total RNA was extracted from fresh brain tissue using RNase Mini Kit (Qiagen) following the manufacturer’s guidelines. cDNA synthesis was done by reverse transcription of 10 μg RNA samples. Real-time PCR performed using a Real-Time PCR System (Applied Biosystems, USA) by the following primers: forward 5′-CTTCCAGGACAGCAGTGAGGA-3′ and reverse 5′-TCTTTCGAGCATGGTGACCT-3′ for NBN gene; forward ACCAAGAAGCTGAGCGAGTG and reverse CCAGTTGAAGTTGCCGTCTG for Bax gene. The cDNA amplification obtained by 40 cycles of denaturation at 95°C for 45 s, annealing at 63°C for NBN and 59°C for Bax gene for 45 s and extension at 72°C for 45 s. The 95°C step was extended to 5 min during the first cycle. 2% agarose gel electrophoresis stained with SYBR Safe DNA gel stain (Invitrogen) was used to confirm the size of the amplicons. As a reference gene, the β-actin gene amplified in the same reaction. Each measurement was repeated 3 times, and the values were used to calculate the gene/β-actin ratio, with a value of 1.0 used as the control (calibrator). The normalized expression ratio was calculated using the method described by Livak and Schmittgen\textsuperscript{(19)}.

**Histopathological examination:**

After routine histological laboratory procedures, sections of 5 μm paraffin-embedding sections were prepared and stained with hematoxylin and eosin (H&E) for histopathology\textsuperscript{(20)}.

**Statistical Analysis**

Data coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data was summarized using mean and standard error of the mean (SEM) for quantitative variables. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test. P-values less than 0.05 were considered as statistically significant.

**Results**

Phytochemical analysis of ethanol extract of Moringa oleifera seeds:

The total oil content of seed of moringa oleifera was 30%. Phenol, tannins, flavonoids, carbohydrates and steroids bioactive components are present in ethanol extract of seed moringa oleifera L (table 1).

<table>
<thead>
<tr>
<th>Table (1): Components of MO seeds extract analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethanol phytochemical test</strong></td>
</tr>
<tr>
<td>Phenol test</td>
</tr>
<tr>
<td>Flavonoids Test</td>
</tr>
<tr>
<td>Tannins Test</td>
</tr>
<tr>
<td>Carbohydrates</td>
</tr>
<tr>
<td>Steroids</td>
</tr>
</tbody>
</table>

+ Present, - absent

Effects on MDA level, GSH, SOD and catalase activities:

As shown in table 2, significant increase in the brain level of MDA in ACMP-treated rats was observed (p ≤ 0.05) as well as significant reduction of enzymatic activity for SOD and CAT and GSH concentration compared to the control. MO extract treated group (group III) showed significant increase of all antioxidant parameters (GSH by 21.4%, CAT by 62.3%, and SOD by 40.8%) and reduction of oxidant parameter (MDA) by 26.9%, in comparison to ACMP-treated group.
Table 2: Ameliorative effects of MOLE against ACMP-induced oxidative stress in different experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>GROUP I</th>
<th>GROUP II</th>
<th>GROUP III</th>
<th>GROUP IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT</td>
<td>24.51±0.48b</td>
<td>18.97±0.93a</td>
<td>30.81±2.11c</td>
<td>23.7±0.45ab</td>
</tr>
<tr>
<td>SOD</td>
<td>5.66±0.05b</td>
<td>4.06±0.34a</td>
<td>5.72±0.45b</td>
<td>4.9±0.1ab</td>
</tr>
<tr>
<td>GSH</td>
<td>36.59±0.94b</td>
<td>30.87±0.52a</td>
<td>37.47±1.35b</td>
<td>30.02±2.23a</td>
</tr>
<tr>
<td>MDMA</td>
<td>24.41±0.67ab</td>
<td>31.49±1.14c</td>
<td>23±0.45a</td>
<td>27.52±1.49bc</td>
</tr>
</tbody>
</table>

Values are presented as mean ±SEM: Mean values with different superscript letters from (a-c) in the same raw are significant different P values between each 2 groups at (P≤0.05).

NBN and Bax genes mRNA expression:

Quantification of the mRNA expression of NBN and Bax genes by real-time PCR was performed to evaluate the proapoptotic activity of acetamiprid by controlling gene expression of apoptotic pathways. The apoptosis level was significantly elevated in acetamiprid intoxicated group with a significant up-regulation of mRNA expression level for BAX gene by more than five-folds (marker of cell apoptosis) and NBN gene by four-folds compared with the control group (figure 2 A). Gene expression analysis for Bax and NBN genes in the brain tissue of MO extract treated group (group III) revealed significant downregulation by 54.5% and 36.3% respectively compared to ACMP intoxicated group.

Figure 1: Effect of MO extract on NBN and Bax genes expression level in brain rats intoxicated with acetamiprid pesticide. (A) Fold change of mRNA expression of Bax and NBN genes in different experimental groups using qPCR. Data showed as mean ± SEM. Groups having different superscripts letters are significantly different from each other. (B) The results of qPCR analysis were proved by agarose gel electrophoresis analysis of the PCR product compared to β-actin.
Histopathological examination:

As illustrated in figure 3, no histopathological changes of the neurons in the hippocampus were recorded in the control group (A). Degeneration and nuclear pyknosis were noticed in the neurons of group II (B). Degeneration and nuclear pyknosis were reported in few neurons of group III (C). Neurons of group IV (D) showed no histopathological abnormalities.

Figure 2: (A). Photomicograph of neurons of the control group showed no histopathological changes and normal histological structure (B). Photomicograph of neurons in ACMP treated group (II) showed degeneration and nuclear pyknosis in the neurons of subiculum of the hippocampus (C). Photomicrograph of neurons of ACMP + MO extract treated group (III) showed nuclear pyknosis and degeneration in few neurons of subiculum of the hippocampus (D). Photomicrograph of neurons of MO extract treated group (IV) showed no histopathological abnormalities and preserved normal structure of the neurons.

Discussion

The present study proved that the dried seeds of MO are great source of polyphenol compounds, such as phenolic acids and flavonoids. The flavonoids contained in MOLE are quercetin, catechin and kaempferol as reported by others \(^{(21)}\) in addition to tannins, flavonoids, carbohydrates, steroids bioactive components and vitamins \(^{(22)}\). Therefore, MOLE has a role as antioxidant, anti-apoptosis and anti-inflammatory.

ACMP–induced oxidative stress damage evaluated by quantification of MDA content, GSH, SOD and CAT activity. In the present work, Oxidative stress mediated by ACMP induced depletion in SOD, GSH, and CAT activities and induction of lipid peroxidation. This finding reflects one of the causative mechanism implicated in ACMP-induced toxicity \(^{(23)}\). Acetamiprid can cause increased reactive oxygen species (ROS) production in the cells. Excess ROS lead to increased lipid peroxidation (LPO) of the cell membrane followed by cellular damage, accumulation of MDA level and antioxidant depletion \(^{(24,25)}\). Several studies have reported the imbalance between oxidant / antioxidant status after Acetamiprid exposure \(^{(26,27)}\). The observed improvement
in the oxidative stress parameters in ACMP treated group in this study attributed to the coadministration of MO extract. The antioxidant activity of MO extract because of its content of bioactive polyphenols and flavonoids as (quercetin, catechin and kaempferol) against oxygen free radicals and its ability to prevent oxidative damage, polyphenols components of moringa seed extract such as catechin can act as an antioxidant by scavenging the free radicals and chelating the sulphyrus metal ions\(^{28}\). Moreover, quercetin contains a hydroxyl group with antioxidant capacity and other flavonoids, which suppress the production reactive oxygen species\(^{29}\).

Determination of mRNA expression level of NBN and Bax genes was performed to assess ACMP role as apoptotic and neuronal cell death provoking factor in the neurodegenerative diseases\(^{30}\). As revealed in figure 1A, ACMP intoxication induced significant upregulation of NBN and Bax genes expression level. However, there was a significant decrease of their expression level in MO extract treated group. Apoptosis is regulated by Bcl-2 family and caspase family of proteins\(^{31}\). Bax gene is a pro-apoptotic factor of the Bcl-2 gene family; encodes BCL2L4 protein that, upon activation of Bax protein, its function is to bind and induce mitochondrial outer membrane (MOM) permeabilization, leading to release of cytochrome c, followed by caspase pathway activation, hence, apoptotic cell death is accelerated in response to cell death signals\(^{32-34}\).

NBN gene plays an important role in genomic stability and repair of DNA double strand breaks. The NBN gene encodes for a protein called nibrin. This protein is responsible for several crucial cellular processes, including the repair of damaged DNA. Increased NBN gene expression indicates DNA damage\(^{35, 36}\). As proved by other authors\(^{37, 38}\), exposure to ACMP mediates apoptosis through disruption of the oxidative stress pathway. Similarly, recent studies\(^{39, 40}\) reported that, acetamiprid treatment leads to loss of mitochondrial membranes integrity and cell death through the induction of necrosis concomitantly with the generation of ROS. As shown in our results, ACMP induced neuronal cell death was significantly reduced by MO extract treatment, as revealed by significant downregulation of mRNA expression for both NBN and Bax genes in the brain tissue (figure 1A). Moringa extract used in this work as a protective agent against ACMP neurotoxicity. Treatment with MO extract significantly reduces apoptosis markers induced by ACMP; it improves mitochondrial functions, the flavonoids content of MO extract as luteolin has a powerful antioxidant activity and a protective role against DNA damage. Moringa extract contains tannins, steroids and phenols, which have free radical scavenging and anti-inflammatory capabilities\(^{41, 42}\). Moreover, MO extract contains Vit E (a-tocopherol) which inhibit programmed cell death by disrupting the activation of free radical cascade reactions in the lipid layer of the outer cell membrane\(^{43}\). Recently, Khan et al.\(^{44}\) stated that, quercetin content in MO extract could control apoptosis pathway in the mitochondria by interrupting the activation of caspases-3 and cytochrome-c.

The present study showed histopathological changes of the brain cells including nuclear pyknosis, shrinkage and degeneration in response to ACMP. The generation of ROS induced by acetamiprid result into damage of different membrane components of the cell. On the other hand, the histopathological changes were improved with MO extract treatment in comparison with ACMP intoxicated group. Results obtained by Kou et al., proved the neuronal protection effect of moringa extract\(^{45}\). Moringa Oleifera components can cross the blood brain barrier so it have beneficial effects on the neuronal system of the intoxicated-rats\(^{45}\).

Conclusion

The current findings revealed that, ACMP exposure might induce neurodegenerative disorders by induction of oxidative stress damage and apoptosis. The present study explored the role of MOLE to alleviate the ACMP-toxic effects. The protective role of MOLE is mediated through the regulation of antioxidant, and antiapoptotic signaling pathways.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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Desmoplastic Fibroma of the Mandible in an Infant: A Case Report

Amr M. Ibrahim¹, Hassan Sadek², Raafat Tammam³, Mohamed R. Omar⁴
¹Lecturer of Oral Pathology Division, Basic Dental Sciences Department, Faculty of Dentistry, Deraya University, ²Lecturer of Oral & Dental Pathology Department, Faculty of Dentistry, Al-Azhar University, ³Professor of Oral & Maxillofacial Surgery, Consultant of Oral & Maxillofacial Surgery International Medical Center (IMC), ⁴Associate Professor of Fixed prosthetics Department, faculty of Dentistry, Assiut University, ⁵Associate Professor of Prosthetic Dentistry Department, faculty of Dentistry, Deraya University, Lecture of Oral Surgery Department, Faculty of Dentistry, Deraya University

Abstract

Desmoplastic fibroma (DF) is a benign tumor of connective tissue origin that is locally aggressive and of high recurrent rate. (¹) The mandible is one of the most commonly affected site with a predilection at the posterior part of the mandible, most often in the ramus-angle region. (²) DF of either the mandible or the maxilla usually occurs and is more common during the second and third decades of life. Bone destruction may progress with a tendency to invade the surrounding soft tissues. In this case report, we define the clinical, radiological, and histological features of a rare case of DF of the mandible in an eleven-months-old male. The management of the lesion consisted of mandibular resection and immediate reconstruction with rib graft. Good healing, no evidence of recurrence, excellent esthetic and functional results after six months follow up. We hope that our case report will shed new light on this special entity.

Keywords: Case report; Desmoplastic fibroma; Infant; Mandibular resection; Right mandible.

Introduction

Desmoplastic fibroma (DF) is a benign tumor of connective tissue origin that is locally aggressive and of high recurrent rate. (¹) The mandible is one of the most commonly affected site with a predilection at the posterior part of the mandible, most often in the ramus-angle region. (²) DF of either the mandible or the maxilla usually occurs and is more common during the second and third decades of life. (³) It presents as a painless firm swelling over the span of weeks to months. (⁴) A slight female predilection has been identified among jaw tumors (54:45 % = F: M). (²) The initial diagnosis of DF is often indicated by clinical and radiographic features, but a definite diagnosis can be made only by histopathological analysis. On histopathological review, DF composed of spindle cells with minimal cytological atypia and abundant production of collagen as defined by the World Health Organization. (⁵) Recurrence depends on the management protocol. (⁶) The aim of this report is to discuss the clinical, radiological, and histological characteristics and debate the optimal treatment of this rare case.

Case Report

Eleven-months-old white boy was referred by the family physician to an Oral and Maxillofacial Surgery unit, International Medical Center, Egypt, with facial asymmetry due to firm, painless swelling on the right
side of the mandible before three months. The parent of the infant reported no history of trauma and no deficits in neurologic exam were noted in regard to V and VII cranial nerves. The overlying skin was normal, no regional lymphadenopathy or tenderness were evident (Fig. 1, A).

Cone beam computed tomography (CBCT) scan of the lesion revealed a radiolucent expansile lytic lesion extending from the right ramus and angle of the mandible to the right canine area, causing destruction of both buccal and lingual cortical plates, no periosteal reaction was noted (Fig. 1, B).

Primary bone sarcomas such as osteosarcoma and chondrosarcoma, as well as other intraosseous soft-tissue sarcomas, were the differential diagnoses based on perforation of the mandibular cortex and aggressive behavior of the lesion. DF and other aggressive benign spindle cell tumors should be ruled out.

Under local anaesthesia, an incisional biopsy was performed, and the tissue mass obtained was submitted for histopathological examination. A non-capsulated spindle cell tumor with a short fascicular pattern was found in an abundant collagen with thin walled, dilated vascular channels on microscopic examination. There was no evidence of necrosis, pleomorphism, atypia, or mitotic activity (Fig. 2, A & B). The differential diagnosis included neurofibroma, DF, and low-grade sarcomas such as low-grade leiomyosarcoma or fibrosarcoma based on histopathologic features.

Immunohistochemical study was performed using β-catenin, Smooth muscle actin (SMA), S-100 protein to confirm the diagnosis, and Ki-67 to evaluate the proliferative activity. The nuclei of the tumor cells were positively labeled for the adhesion protein β-catenin (Fig.3). Negative staining was noted for both SMA except in the wall of the blood vessels (in Fig 4…), which considered as positive control, and S-100 protein (Fig. 5). The nuclei of the tumor cells showed partially positive staining with Ki-67 (only 4 % of tumor cells) (Fig. 6).

Based on the clinical, radiological, histopathological and immunohistochemical features, DF, was rendered. The patient was subsequently referred to the Oral and maxillofacial surgery unit for further management. Wide supra-periosteal mandibular resection was performed under general anaesthesia and the surgical defect was immediately reconstructed with autogenous rib graft (Fig. 7). The patient was reviewed at One year clinical and radiographical follow-up. The surgical site healed well, no signs of recurrence were detected with satisfactory functional and esthetic outcome (Fig. 8 & Fig 9).

(Fig. 1) A. clinical photograph showing facial asymmetry due to a non-tender, firm, and prominent swelling in the right mandible. B. CBCT scan displays destruction of buccal and lingual cortices.
(Fig. 2) A. Photomicrograph of a cellular neoplastic proliferation of fibroblasts in a vascular stroma. (H&E stain, ×100).

B. Photomicrograph showing monomorphic, wavy, spindled cells with elongated nuclei in a background of collagenous stroma. (H&E stain, ×200)

(Fig. 3) Immunohistochemical staining of β catenin antibody showing diffuse positive staining of tumor cells for (Streptavidin Peroxidase, ×400).
(Fig. 4) Immunohistochemical staining of SMA antibody showing negative staining of tumor cells, and strong positive staining in the wall of blood vessels. (Streptavidin Peroxidase, ×400).

(Fig. 5) Immunohistochemical staining of S-100 protein antibody showing negative staining of tumor cells (Streptavidin Peroxidase, ×400).
(Fig. 6) Immunohistochemical staining of Ki-67 antibody showing positive staining of tumor cells.
(Streptavidin Peroxidase, ×400).

(Fig. 7) Clinical photograph immediate after surgery showing wide mandibular resection.
(Fig. 8) Clinical photograph after one year showing normal facial appearance.

(Fig. 9) CBCT scan displays jaw bone reconstruction by rib bone graft showing no features of recurrence after one year.

**Discussion**

DF can affect any bone, but it is most common in the mandible (22%), femur (15%), pelvic bones (13%), radius (12%), and tibia (9%). Gnathic bone DF affects about 84% of patients under the age of 30, with a mean age of 16 years. While particular etiologic factors for DF are unknown, possible associations with trauma, endocrine factors, genetic aberrations, or multifactorial aetiology have been proposed. (4)

The maxilla-mandibular DF typically presents as a firm painless swelling that is slowly growing, rarely associated with loose teeth, it does not impact on chewing/swallowing. Facial asymmetry and irregular alignment of the teeth may be the only manifestations of the lesion. A radiolucent, multilocular lytic lesion expanding bone without any indication of periosteal reaction is a typical radiographic appearance. (7) In CT scanning, it has the characteristic “internal explosion” appearance of the lytic lesion. (8)
Histopathologically, DF is characterized by spindle shaped cells with minimal cytological atypia and abundant collagen production, the absence of a capsule, and the infiltrative nature of the lesion. (5)

To confirm the diagnosis and differentiate DF from low-grade fibrosarcoma and other sarcomas, several immunohistochemical biomarkers are essential. Low-grade fibrosarcoma did not show positive reaction with β-catenin which can support the diagnosis. In addition, low-grade fibrosarcoma and other sarcomas showed higher Ki-67 immunoreaction versus benign spindle cell tumors such as DF. In our case the tumor revealed highly positive reaction with β-catenin, which is in accordance with the results of Nedopil et al. (9)

Disagreement results were reported by Kahraman et al., where they did not detect any positive reaction with β-catenin when they studied a series of twenty-two cases of DF in jawbones, they suggested that β-catenin staining may not be used as a corroborating the diagnosis of DF. Immunohistochemical staining difference of jaw bone DF from other soft tissue and bone lesions may be related to the origination of jaw bone from the neural crest. (10)

Proliferation markers like Ki-67 can be used as a surrogate marker for evaluating the tumor’s aggressiveness. A low rate of predicted cell turnover in DF has been reported. (11) Our Ki-67 staining results are in agreement with those reported in earlier studies. (9, 11)

Other benign spindle cell tumors and low-grade sarcomas, especially fibrosarcoma, were included in the differential diagnosis. As previously stated, a positive β-catenin and a low Ki-67 immunohistochemical reactions will aid in determining a definitive diagnosis. This finding is in agreement with Mohammadi et al. (12)

Due to the aggressive behavior of DF, different treatment protocols have been proposed. (13) Higher recurrence rate (37%-72%) of DF after enucleation and curettage was reported by Gersak et al. (14) While Böhm et al., showed no recurrences after resection with wide surgical margins. (15) Radiotherapy is not recommended due to the lesion’s potential for mutagenic transformation into fibrosarcoma. (16) Therefore, wide mandibular resection was our treatment of choice.

Mandibular reconstruction is needed because the resection approach will result in loss of mandibular continuity and serious growth anomalies. In our case, reconstruction of the defect was carried out by an immediate autogenous rib graft.

Despite the fact that research suggests the therapy is successful after a 6-year recurrence-free follow-up, (1) our patient was reassessed six months after the surgical procedure. The surgical site healed well, no sign of recurrence, excellent esthetic and functional results were found. The patient parent are advised for long term follow up.

**Conclusion**

DF of the jaw is a rare fibrous tumor with an aggressive behavior and high recurrence rate. We report a rare case of DF on the right side of the mandible with good healing, no evidence of recurrence, excellent esthetic and functional results after an One-year follow up. The best treatment choice to reduce the recurrence rate of this benign aggressive tumor may be wide local resection.

**Recommendation:** The need for long term follow up is advised.

**Declaration of patient consent**

The authors certify that all required patient consent forms have been received by his parent. For his photographs and other clinical details to be published in the journal, the parents gave their informed consent prior to their infant inclusion in the study.

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**Conflicts of Interest:** There are no conflicts of interest.
References


Morphometry and Sexual Dimorphism of Olfactory Fossa in Relation To Crista Galli in North Karnataka Region – A Multidetector Computed Tomographic Study

Anandagouda V Naikanur1, Balappa M Bannur2, Sanjeev I Kolagi3, Naseema Begum4

1Ph.D Scholar 2Professor, Department of Anatomy, Shri B M Patil Medical College, Vijayapura, 3Professor and Head, 4Associate Professor, Department of Anatomy, S Nijalingappa Medical College, Bagalkot

Abstract

Background: The aim of this study was to establish the morphometry and sexual dimorphism of olfactory fossa and its correlation with the types of crista galli in North Karnataka region.

Method: Two hundred and twenty Multidetector computed tomographic (MDCT) scans were collected from North Karnataka region and analyzed by using RadiAnt DICOM Viewer. The depth and width of olfactory fossa, their side symmetry, types of crista galli and correlation between the depth of olfactory fossa with types of base of crista galli were calculated in both genders. Statistical analysis was done by using Student unpaired ‘t’ test.

Conclusion: Keros type II of olfactory fossa was most common in the present study. There was significant difference (p˂0.05) in olfactory fossa depth when compared between right (mean=4.64mm) and left sides (mean=4.07mm) in females. There was no statistically significant difference found between the gender and the sides of depth and width of olfactory fossa. There was no statistically significant correlation found between the types of olfactory fossa and types of crista galli. The morphometry of olfactory fossa and crista galli would help neurosurgeons to assess these vulnerable regions of ethmoidal skull base in surgeries.

Key-words: Cribriform plate, Crista galli, Keros classification, Olfactory fossa.

Introduction

The Ethmoid bone lies in the anterior cranial fossa at the base of the cranial cavity. The cribriform plate, median perpendicular plate and two lateral labyrinths are the parts of the ethmoid bone. The orbital plate of the frontal bone and its roof joins with lateral lamellae of the cribriform plate (LLCP). [1]

The olfactory fossa is a depression in the anterior cranial fossa whose floor is formed by the cribriform plate. It is the most vulnerable site in the whole anterior skull base. It is medially bounded by crista galli and laterally by lateral lamella of the cribriform plate.[2] The depth of the olfactory fossa is determined by the height of the LLCP which is the thinnest area of the ethmoidal skull base. Thin LLCP and low ethmoidal skull base are the potential sites of injuries during endoscopic sinus surgeries.[3] Keros has classified the depth of olfactory fossa in to three types. Type I (1 to 3 mm), type II (4 to 7mm) and type III (8 to16 mm). [4]

Keros has also described the width and depth of olfactory fossa at different points and noted that type III Olfactory fossa exposes thin LLCP to injuries during
The width of olfactory fossa increases evenly from rostral to the occipital end. Measured below the wing of crista galli, the mean width is 3.8 mm on right side and 3.6 mm on left side. In the posterior one third, it is 5.2 mm on the right side and 5.1 mm on the left side at the rostral end.\textsuperscript{5}

The Crista galli (CG) is a triangular median process projecting upwards from the centre of the cribriform plate. It has a thin and slightly curved posterior border and a much thicker and shorter anterior border. It is a compact bone, but can also be pneumatized in some individuals.\textsuperscript{6} Thicker the crista galli, smaller the volume of olfactory fossa. Hajiioannou has classified the base position of crista galli into 3 types,

Type I= the base of the crista galli is located at the level of the cribriform plate.

Type II= less than 50% of the height of the crista galli is located below the level of the cribriform plate.

Type III= more than 50% of the height of the crista galli is located below the level of the cribriform plate.\textsuperscript{7}

Aim

The aim of this study was to establish the morphometry and sexual dimorphism of olfactory fossa and its correlation with the types of crista galli in North Karnataka region.

Objectives

a) To determine the gender differences in depth and width of olfactory fossa on both the sides.

b) To determine the dimensions of pneumatisation of crista galli and Hajiioannou types of crista galli in both the genders.

c) To determine the correlation between olfactory fossa depth and Hajiioannou’s classification of base of crista galli in both the genders.

Materials and Method

A prospective hospital based radiological study was done on two hundred and twenty Multidetector Computed Tomographic (MDCT) scans of the patients of all the districts of North Karnataka region of Karnataka state, India, after institutional ethics committee clearance. The study was carried out from April 2018 to September 2019.

Normal Paranasal MDCT scans of patients above the age of 16 years belonging to both genders were included in the study.

MDCT scans of Patients below the age of 16 years and MDCT scans of patients with nasal or paranasal trauma, congenital abnormalities of face, tumours or conditions involving bone destruction and surgeries were excluded from the study. While taking the MDCT of paranasal sinuses, patients were informed and instructed about the procedure before obtaining informed written consent. Axial MDCT images of 3mm thickness were taken from CT scanner (Siemens Somatom) by using bone window.

Direct coronal scan showing the maximum depth of the olfactory fossa at the centre of infraorbital foramen was taken as reference point. Depth of the olfactory fossa was determined by the length of the lateral lamella of cribriform plate (CP). The height of CP point was subtracted from the height of medial ethmoidal roof point (MERP) to measure the length of the LLCP on both sides in both genders. Figure 1 shows Line A which represents a direct horizontal line connecting the middle of the inferior orbital foramina on both sides. Line B represents direct vertical line connecting line A and to the site of communication of fovea ethmoidalis and the lateral lamella of the cribriform plate of the ethmoid bone (LLCP). Line C was drawn as a direct vertical line connecting line A to the most lateral bony boundary of the cribriform plate of the ethmoid bone at its communication with the lateral lamella which will be CP height. The height of the ethmoid roof (h) was considered as the depth of the olfactory fossa. “h” was calculated as the result of subtraction of length of line C
(“c”) from the length of line B (“b”) in millimeters (h = b - c). “h” will be representing the direct vertical height of the lateral lamella of the cribriform plate of the ethmoid bone.

FIGURE 1: Showing coronal section of Multidetector computed tomographic scan of the patient at the level of infraorbital foramen.

A(77.5mm) -Line joining two infraorbital foramen,

B (32.9mm) -Line joining medial ethmoidal roof point to line A and

C (24mm) -Line joining cribriform plate to line A.

Olfactory fossa depth was classified according to Keros classification i.e, type I (0 to 3 mm), type II (4 to 7 mm), type III (8 to 16 mm) on both sides in males and females. The side symmetry of the types of depth of olfactory fossa was then compared in both genders as shown in figure 2.

FIGURE 2: Types of depth of olfactory fossa in coronal section of MDCT scan on both the sides in the present study.
A- Line joining two infraorbital foramen,
B- Line joining medial ethmoidal roof point to line A and
C- line joining cribriform plate to line A.

The width of the olfactory fossa was measured from fovea ethmoidalis (fe) to the lateral margin of the crista galli at the level of centre of infraorbital foramina as shown in figure 3.

FIGURE 3: Measurement of width of left olfactory fossa in coronal section of MDCT scan of the patient.

The dimensions of the pneumatization of crista galli like anteroposterior and lateral to lateral diameter and types of base of crista galli according to Hajiioannous’s classification (figure 4 & 5) were noted in both coronal and sagittal section of MDCT scans.

FIGURE 4: Sagittal section of MDCT scan of the patient with type I crista galli.
FIGURE 5: Sagittal section of MDCT scan of the patient with type II crista galli.

Results and Discussion

With regard to less data available in North Karnataka region, the present study was carried out to find out any correlation between the Keros types of olfactory fossa and types of crista galli.

Comparison of the mean±SD of MERP, CP, depth of OF, and width of OF, and their significance in the present study on both sides in both genders is shown in table 1, 2 and 3.

Table 1: Comparison of MERP, Cribriform plate (CP), depth of OF, and width of OF and their significance on both sides in males in the present study.

<table>
<thead>
<tr>
<th>Males n * =131</th>
<th>Right side Mean±SD †</th>
<th>Left side Mean±SD</th>
<th>P ‡ value</th>
<th>Significance</th>
<th>95% CI §</th>
</tr>
</thead>
<tbody>
<tr>
<td>MERP</td>
<td>ht **</td>
<td>27.34±3.39</td>
<td>27.23±3.36</td>
<td>0.79</td>
<td>NS ††</td>
</tr>
<tr>
<td>CP ht</td>
<td>22.4±3.12</td>
<td>22.79±3.02</td>
<td>0.3</td>
<td>NS</td>
<td>-1.13-0.35</td>
</tr>
<tr>
<td>OF depth</td>
<td>4.85±2.04</td>
<td>4.47±1.79</td>
<td>0.11</td>
<td>NS</td>
<td>-0.08-0.84</td>
</tr>
<tr>
<td>OF width</td>
<td>5.15±1.91</td>
<td>4.86±1.67</td>
<td>0.19</td>
<td>NS</td>
<td>-0.14-0.72</td>
</tr>
</tbody>
</table>

* (n)=number, † (SD)=standard deviation, ‡ (p)=significance, § (CI)=class interval, ** (MERP)= medial ethmoidal roof point, †† (ht)=height and ††† (NS)=not significant
Table 2: Comparison of MERP, Cribriform plate (CP), depth of OF and width of OF and their significance on both sides in females in the present study

<table>
<thead>
<tr>
<th>Females n*={89}</th>
<th>Right side Mean±SD†</th>
<th>Left side Mean±SD</th>
<th>P‡ value</th>
<th>Significance</th>
<th>95% CI§</th>
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</thead>
<tbody>
<tr>
<td>MERP ht **</td>
<td>25.43±3.29</td>
<td>25.3±2.99</td>
<td>0.78</td>
<td>NS ††</td>
<td>-0.8-1.06</td>
</tr>
<tr>
<td>CP ht</td>
<td>20.83±3.14</td>
<td>21.28±2.78</td>
<td>0.31</td>
<td>NS</td>
<td>-1.32-0.042</td>
</tr>
<tr>
<td>OF depth</td>
<td>4.64±2.09</td>
<td>4.07±1.77</td>
<td>0.05</td>
<td>S ‡‡</td>
<td>0.002-1.14</td>
</tr>
<tr>
<td>OF width</td>
<td>5.16±1.6</td>
<td>4.84±1.43</td>
<td>0.16</td>
<td>NS</td>
<td>-0.13-0.77</td>
</tr>
</tbody>
</table>

* (n)=number, † (SD)=standard deviation, ‡ (p)=significance, § (CI)=class interval, || (MERP)= medial ethmoidal roof point, ** (ht)=height, †† (NS)=not significant and ‡‡ (S)= significant.

Table 3: Comparison of MERP, Cribriform plate (CP), depth of OF and width of OF and their significance on both sides in males and females in the present study

<table>
<thead>
<tr>
<th>n*={220}</th>
<th>Males (n=131) Mean±SD†</th>
<th>Females (n=89) Mean±SD</th>
<th>P‡ value</th>
<th>Significance</th>
<th>95% CI§</th>
</tr>
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<tbody>
<tr>
<td>MERP</td>
<td></td>
<td>ht **</td>
<td>27.34±3.39</td>
<td>25.43±3.29</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>27.23±3.36</td>
<td>25.3±2.99</td>
<td>0.000</td>
<td>S</td>
<td>1.054-2.797</td>
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<tr>
<td>CP ht</td>
<td>22.4±3.12</td>
<td>20.83±3.14</td>
<td>0.000</td>
<td>S</td>
<td>0.725-2.42</td>
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<td></td>
<td>22.79±3.02</td>
<td>21.28±2.78</td>
<td>0.000</td>
<td>NS ††</td>
<td>0.72-2.3</td>
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<tr>
<td>OF depth</td>
<td>4.85±2.04</td>
<td>4.64±2.09</td>
<td>0.46</td>
<td>NS</td>
<td>-0.35-0.76</td>
</tr>
<tr>
<td></td>
<td>4.47±1.79</td>
<td>4.07±1.77</td>
<td>0.09</td>
<td>NS</td>
<td>-0.07-0.88</td>
</tr>
<tr>
<td>OF width</td>
<td>5.15±1.91</td>
<td>5.16±1.6</td>
<td>0.96</td>
<td>NS</td>
<td>0.49-0.47</td>
</tr>
<tr>
<td></td>
<td>4.86±1.67</td>
<td>4.84±1.43</td>
<td>0.92</td>
<td>NS</td>
<td>-0.4-0.44</td>
</tr>
</tbody>
</table>

* (n)=number, † (SD)=standard deviation, ‡ (p)=significance, § (CI)=class interval, || (MERP)= medial ethmoidal roof point, ** (ht)=height, †† (S)= significant and ‡‡ (NS)=not significant.
Among 220 MDCT scans, the mean MERP height was 26.57mm on the right side and 26.45mm on the left side. The cribriform plate height was 21.77mm on the right side and 21.28mm on the left side. The depth of the olfactory fossa was 4.76mm on the right side and 4.31 mm on the left side. The width of the olfactory fossa was 5.15mm on the right side and 4.85 mm on the left side.

Among 131 males, keros type I OF was found in 25 cases (19.08%) on the right side and 31 cases (23.66%) on the left side. Keros type II OF was found in 86 cases (65.64%) on the right side and 91 cases (69.46%) on the left side. Keros type III OF was found in 20 cases (15.26%) on the right side and 9 cases (6.87%) on the left side.

Among 89 females, keros type I OF was found in 23 cases (25.84%) on the right side and 31 cases (34.83%) on the left side. Keros type II OF was found in 54 cases (60.67%) on the right side and 51 cases (57.3%) on the left side. Keros type III OF was found in 12 cases (13.48%) on the right side and 7 cases (7.86%) on the left side.

Keros type II of olfactory fossa was most common in the present study. There was significant difference (p˂0.05) in olfactory fossa depth when compared between right (mean=4.64mm) and left sides (mean=4.07mm) in females. There was no statistically significant difference found between the gender and the sides of depth and width of olfactory fossa.

In the present study, symmetry of Olfactory Fossa depth was found in 94 cases (71.75%) in male patients and 54 cases (60.67%) in female patients. Asymmetry of Olfactory Fossa depth was found in 37 cases (28.24%) in male patients and 35 cases (39.32%) in female patients. There was no statistically significant difference found in the symmetry and asymmetry of olfactory fossa depth between the genders in the present study.

In the present study, type I Crista galli was found in 84 cases (64.12%) in male patients and 57 cases (64.02%) in female patients. Type II Crista galli was found in 47 cases (35.87%) in male patients and 32 cases (35.95%) in female patients. Type III Crista galli was not found in male and female patients. There was no statistically significant difference found between types of crista galli between the genders in the present study.

There was no correlation between the keros types of olfactory fossa and types of crista galli in the present study. Comparison of cases of keros type of olfactory fossa present in all types of crista galli in the present study on right side and left side in males and females is shown in chart 1 and chart 2 respectively.

![Chart 1](chart1.png)

**CHART 1 - Percentage of cases of keros types of olfactory fossa present in various types of crista galli in both genders on right side in the present study.**

- x axis = keros types I, II and III
- Y axis = number of cases of Keros types I, II and III of OF in seen in CG types I, II and III
CHART 2 - Percentage of cases of keros types of olfactory fossa present in various types of crista galli in both genders on left side in the present study.

x axis = keros types I, II and III

Y axis = number of cases of Keros types I, II and III of OF in seen in CG types I, II and III.

There was statistically significant difference in olfactory fossa depth when compared between right and left sides in females as shown in table no 2 and chart 3. In males, there was no such difference as shown in table 1. Keros type II olfactory fossa was predominant (64%) among the cases and keros type III olfactory fossa was rare (10%) in the present study as shown in table no.4.

CHART 3 - Comparison of MERP, CP, depth of OF and width of OF and their significance on both sides in females in the present study

n = number, CP = cribiform plate, MERP = medial ethmoidal roof point, NS = not significant and S = significant.
x axis = MERP, CP, OF

Y axis = number of cases
Comparison of keros types of depth of olfactory fossa in the present study and in various studies is shown in table 4.

Table 4: Keros classification of Olfactory fossa among different studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Keros I (%)</th>
<th>Keros II (%)</th>
<th>Keros III (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>India</td>
<td>24.99</td>
<td>64.08</td>
<td>10.00</td>
</tr>
<tr>
<td>Original keros 4</td>
<td>Germany</td>
<td>26.3</td>
<td>73.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Shama AM 2</td>
<td>Egypt</td>
<td>56.5</td>
<td>40.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Kalpanoglu et al 8</td>
<td>Turkey</td>
<td>13.4</td>
<td>76.1</td>
<td>10.5</td>
</tr>
<tr>
<td>Solares et al 9</td>
<td>USA</td>
<td>83.1</td>
<td>15.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Souza et al 10</td>
<td>Brazil</td>
<td>26.3</td>
<td>73.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Paber et al 11</td>
<td>Philippine</td>
<td>81.08</td>
<td>17.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Bista et al 4</td>
<td>Nepal</td>
<td>86.0</td>
<td>12.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Among 220 patients, the mean width of the olfactory fossa was 5.15 mm on right side and 4.85 mm on left side in the present study. There was no significant difference in the width of olfactory fossa between the sides in the present study. The study done by Daniel H. Coelho et al revealed that the mean width of OF was 4.57 mm on the right side and 4.49 mm on the left side by Digital photography. [12]

In the present study, type I crista galli was found in 64.07% of cases, type II CG was found in 35.91% of cases and type III CG was not found. In the study done by Hajiioannou, type I crista galli was found in 28.3% of cases, type II CG was found in 63.6% of cases and type III CG was found in 8.1% of cases.

In the present study, the width, height and length of pneumatized Crista galli was 3.95 mm, 9.3 mm and 7.02 mm respectively. In the study done by Gorazd Poje, [7] the width and length of pneumatized Crista galli was 5.1 mm and 8.75 mm respectively. In the study done by Ranko Mladina, [6] the width, height and length of pneumatized Crista galli was 3.75 mm, 10.35 mm and 7.8 mm respectively.

Strengths and limitations of the study:

With regard to less data available in North Karnataka region, this study will help neurosurgeons and endoscopic surgeons to assess olfactory fossa during various skull base and endoscopic sinus surgeries.

This study was carried out only in North Karnataka region and there were limited number of patients during the study period. Only adult patients were included in this study.

Conclusion

The dangerous keros type III olfactory fossa was rare in the present study when compared to the most common keros type II olfactory fossa. There was statistically significant difference in olfactory fossa depth when compared between right and left sides in females. In males, there was no such difference. The width of olfactory fossa was not significant when compared between two sides in males and females. There was no statistically significant correlation found between the keros types of olfactory fossa and types of crista galli. The knowledge regarding the keros types of
depth of olfactory fossa, width of olfactory fossa and types of crista galli will help the neurosurgeons to assess these vulnerable regions of ethmoidal skull base that are difficult to access during skull base surgeries.

**Ethical Clearance** - It was taken from Blde (deemed to be university) Shri B M Patil medical college, hospital and research centre, Vijayapura, Institutional ethical committee and SNMC-Institutional ethics committee on human Subjects research, Bagalkot.

**Source of Funding** - Self

**Conflict of Interest** - Nil

**References**


Health Beliefs Related to Breast Cancer and Breast Self-Examination among Indian Women: Effect of Educational Program

Apoorva Dev.M¹, Githa Kishore², Faezeh khodadadi³
¹Asst. Professor, Department of Pharmacy Practice, PES University, ²Professor, Department of Pharmacy Practice, Visveswarapura Institute of Pharmaceutical Sciences, ³Intern, Pharm D, PES University, Rajiv Gandhi University of Health Sciences, Bengaluru

Abstract

Background: Breast cancer is believed to be the most widespread carcinoma all over the world and the most frequent type of carcinoma among females in India. Approximately 40% of breast lesions which lead to breast cancer is detectable by breast self-examination (BSE). The identification of the barriers of early detection and the baseline knowledge on breast cancer symptoms, risk factors, BSE method and also the effectiveness of a health education intervention among females in Bangalore, India are the main purposes of this research.

Methodology: The study was composed of totally 500 female participants from five different institutions in urban and rural of Bangalore in 2020. Assessing baseline knowledge on breast cancer and BSE using self-administered questionnaires as well as administering interventional health education in the form of a lecture, pamphlets and illustration of proper method of conducting breast self-examination using video tutorials.

Conclusion: The baseline knowledge about breast cancer and BSE practice among participants for early detection was found to be insufficient. However, we witnessed a great progress after immediate and follow up intervention. Periodic interventions are essential to prevent relapsing of certain types of barriers.

Key Words: Breast cancer, Breast self-examination, Educational intervention, Early Diagnosis, Follow up.

Introduction

Cancer is considered to be the number one cause of death in many countries. Countless number of people are annually diagnosed with cancer all over the world, and more than half of the patients ultimately lose their lives due to this awful disease. Breast cancer is considered as the most common cancer in women. Globally, it was estimated that 2.1 million females were diagnosed with breast cancer in 2018, the last year of incidence data collection ¹. It is a significant collective hurdle in both low-health expenditure and mid-level countries. In India, the rate of breast cancer in women, was 27.7% in 2018 with mortality rate of 23.5% ². It is the most wide-spread cancer among women who were residing in the urban areas of India ³. Patients who were diagnosed at an early stage of breast cancer have a better prognosis and good survival rate ⁴. However, the majority of the women in India present with an advanced stages of breast cancer, which declines the survival rates in India, which stems from the failure of early detection programs, causing a great number of females presenting with late-stage disease ⁵.
Lack of education on breast cancer and breast screening techniques used for early diagnosis is recognized as one of the major barriers to early detection of breast cancer. In the United States, the survival rate has increased which might be related to improvement in preventive measures and diagnosis of breast cancer in early stages. However, the mortality rate of breast cancer is increasing in most regions of the world particularly in low-income countries. Among different types of breast screening, Breast self-examination (BSE) is an easy, fast and free, non-invasive procedure that requires no special materials/equipment. It is also an effective diagnostic method for breast cancer that only takes five minutes to inform. There is evidence that women who correctly practice BSE on monthly basis are more likely to detect breast cancer in the early stages of its development, and early detection has been confirmed to influence early treatment and to yield a better survival rate. These findings highlight the importance of imparting proper knowledge on breast cancer and BSE and the barriers to early detection and implementation of preventive strategies, including education of the target population about breast cancer and BSE. Hence, this study is planned with the aim to assess and identify the barriers of early detection and the baseline knowledge on breast cancer symptoms, BSE method and risk factors of breast cancer and also the effectiveness of education on breast cancer and BSE in the Indian metropolitan women residing in Bangalore, Karnataka.

Material and Methods

Participants:

The study included women from Bangalore in the age group of 17 to 65 years and with no history of breast cancer. Non-random sampling method was used in this study and 500 women who gave their consent to participate in the study were included.

Study tools, techniques and procedure:

The research was carried out in order to investigate the knowledge of participants that was in association with breast cancer symptoms, risk factors, BSE technique and timing as well as obstacles for finding medical assistance. The efficacy of educational interference (in form of lecture, pamphlets and demonstration of suitable way of implementing BSE using video tutorials) in three phases (Pre-intervention phase and the post intervention phase itself is divided into two sections; immediate phase and three-month period). The following four steps describe the complete research procedure:

Step I:

A pre-designed, structured, and self-administered questionnaire was utilized for data collection. The first section of the questionnaire constituted demographic details of participants [Table 1]. The second section was comprised of 19 questions, the first 16 questions concentrated on knowledge evaluation of symptoms and risk factors of breast cancer and BSE method and training [Table 2, 3, 4]. The other three questions described the obstacles for seeking medical help [Table 5]. At the pre-intervention phase, participants were evaluated for baseline knowledge of breast cancer and BSE and then pamphlets were given out in which importance of breast cancer sign and symptoms, risk factors of breast cancer, importance of early detection by BSE and the proper method of conducting BSE were discussed. The study participants’ communication addresses were collected to avoid a loss to follow up.

Step II:

This was followed by a brief workshop as well a presentation concerning the sign and symptoms, risk factors of breast cancer and its screening methods with more focus on BSE method. The attendees were shown a brief instructive video. The content of the film revolved around the significance of BSE, ways of executing and the best time for BSE. Enquiries from the participants have been invited.

Step III:

After education, first post-interventional assessment was done to evaluate the increased awareness level of breast cancer and BSE using the same questionnaire.
among respondents.

**Step IV:**

After a three-month period, second post-interventional assessment regarding breast cancer and BSE knowledge was done.

**Statistical analysis:**

The statistical software for social sciences (IBM SPSS statistics 20) was used to analyses data. Descriptive statistics (i.e., frequencies, percentages, mean, standard deviation) were used to describe the demographic features and the Chi-square test was used to explore associations. P-value less than 0.05 was considered significant in all analyses.

**Ethical statement:**

Before entering the study, all the study participants were required to give their informed consent regarding the study benefits, risks, significance. The signed consent of every participant was recorded before the administration of the questionnaire. The study was approved by the Ethics committee of PES College of Pharmacy (PESCP/2015-16/PPD-PHD-01).

**Result:**

**Sociodemographic data:**

The survey involved a community-based research that included 500 women. The median age of women who participated in the study was 23.41 ± 5.063 years. The majority of the respondents 438 (86%) were in the age group of 17 to 25 years. The vast majority of the respondents 359 (71.8%) were students. Only 37 women (7.4%) were married and 463 (92.6%) were unmarried [Table-1].

**Awareness of Breast Cancer symptoms and risk factors:**

[ Graph 1] provides an evaluation of the knowledge of breast cancer manifestations and risk factors, and showed that the perception of common breast cancer symptoms (pain, discharge or bleeding from the nipple, a lump or thickening in the breast) and seven risk factors (the use of HRT, alcohol consumption, smoking, pregnancy in old age, overweight, positive family history and physical activity) improved significantly after the intervention. 71.4 % of them did not have any knowledge whatsoever that pain cannot be attributed as one of the main causes of breast cancer. In addition, at the pre-intervention stage, roughly 51.4% and 31.8% of women stated that only discharge and lump formations were the signs of breast cancer respectively and the remaining half had no knowledge of the fact that lump or thickening of breast is one of the major symptoms of breast cancer [Graph 1].

**BSE Skill Evaluation:**

After the comparison of correct percentage of pre-training and post-training responses in regards to the knowledge of immediate and a three-month follow-up BSE method and timing, it was determined that there was a statistically significant difference between the pre-training and post-training knowledge levels. When comparing the percentage of pre-training and the three-month follow-up awareness, an improvement in the correct response percentage was determined in the three-month follow-up period. The overall BSE knowledge increased from approximately (38.5%) to (83%) i.e., a twofold improvement was seen in the BSE knowledge even after three months of the intervention, due to the impact of health education provided for the respondents [Table-2], which has been found to be statistically significant (P < 0.05).

**Barriers in seeking medical help:**

The most significant obstacles to find medical care were multiple being too scared to go and visit doctor 361 (72.2%), followed by lack of confidence to speak about symptoms 357 (71.4%) and being too ashamed to go to see a physician 342 (68.4%) [Table-3].
### Table-1: Demographic details of participants

<table>
<thead>
<tr>
<th>Age group</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 to 25 Years</td>
<td>438 (87.6)</td>
</tr>
<tr>
<td>26 to 35 Years</td>
<td>47 (9.4)</td>
</tr>
<tr>
<td>36 to 45 Years</td>
<td>12 (2.4)</td>
</tr>
<tr>
<td>46 to 55 Years</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>56 to 65 Years</td>
<td>1 (0.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Marital status</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>37 (7.4)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>463 (92.6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUC</td>
<td>12 (2.4)</td>
</tr>
<tr>
<td>Diploma</td>
<td>58 (11.6)</td>
</tr>
<tr>
<td>Bachelor Degree</td>
<td>324 (64.8)</td>
</tr>
<tr>
<td>Master</td>
<td>72 (14.4)</td>
</tr>
<tr>
<td>Doctorate</td>
<td>34 (6.8)</td>
</tr>
</tbody>
</table>

### Table-2: Assessment of knowledge BSE – Method and Timing

<table>
<thead>
<tr>
<th>BSE is recommended to be done monthly</th>
<th>Before intervention</th>
<th>Immediate, post-intervention</th>
<th>3months, Post-intervention</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct N (%)</td>
<td>*207 (41.5)</td>
<td>460 (92)</td>
<td>447 (89.4)</td>
<td>**0.000</td>
</tr>
<tr>
<td>Suitable time to do BSE is 7th day after the start of menstruation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correct N (%)</td>
<td>*146 (29.3)</td>
<td>412 (82.4)</td>
<td>394 (78.8)</td>
<td>**0.000</td>
</tr>
<tr>
<td>Axilla should be examined while doing BSE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correct N (%)</td>
<td>*141 (28.3)</td>
<td>436 (87.2)</td>
<td>416 (83.2)</td>
<td>**0.000</td>
</tr>
<tr>
<td>BSE could be done while taking bath</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correct N (%)</td>
<td>*141 (28.3)</td>
<td>424 (82.8)</td>
<td>416 (83.4)</td>
<td>**0.000</td>
</tr>
<tr>
<td>BSE is done in front of the mirror only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correct N (%)</td>
<td>*227 (45.4)</td>
<td>409 (81.8)</td>
<td>394 (78.8)</td>
<td>**0.000</td>
</tr>
<tr>
<td>BSE could be done in the supine position</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correct N (%)</td>
<td>*293 (58.7)</td>
<td>435 (87)</td>
<td>418 (83.6)</td>
<td>**0.000</td>
</tr>
</tbody>
</table>
**Correct response  ** A comparison between before education and 3 months after education

<table>
<thead>
<tr>
<th></th>
<th>Before intervention</th>
<th>Immediate, post-intervention</th>
<th>3 months, Post-intervention</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Too ashamed to go and see the doctor</td>
<td>342 (68.4)</td>
<td>8 (1.6)</td>
<td>42 (8.4)</td>
<td>**0.000</td>
</tr>
<tr>
<td>Too scared to go and see the doctor</td>
<td>361 (72.2)</td>
<td>11 (2.2)</td>
<td>10 (2)</td>
<td>**0.000</td>
</tr>
<tr>
<td>Not feeling confident talking about my symptom with the doctor</td>
<td>357 (71.4)</td>
<td>28 (5.6)</td>
<td>24 (4.8)</td>
<td>**0.000</td>
</tr>
</tbody>
</table>

** A comparison between before education and 3 months after education

Graph-1. Percentage of correct answers to knowledge assessment about breast cancer symptoms and risk factors.

**Discussion**

Study participants were evaluated for their knowledge of breast cancer under four different concerns, such as warning signs and risk factors of breast cancer, BSE method and timing and barriers to seek medical help in case of any warning sign.

Knowledge of women on breast cancer symptoms and risk factors:

It is sad to say that the prior knowledge of women participants regarding breast cancer manifestation and risk factors was determined as medium-low level. After the training however, these values increased significantly. This indicates another important aspect of our research, which highlights the importance & effectiveness of the training regarding the knowledge level among female participants after the training on breast cancer and BSE."
Regarding risk factors, Smoking (57.8%) was the most reported risk factor while the vast majority of participants (80.6%) were not aware of the fact that obesity and having children later on in life (over 40) were also important risk factors for breast cancer [Graph 1]. Prusty RK et al., approved that women in Mumbai were weakly aware of breast cancer risk factors. Obesity was the most common cited risk factor (19%) while early menstruation (5.6%) and late menopause (10%) were among the least common risk factors. Another study by Millat WA, respondent with family history of breast cancer had a slightly higher level of awareness regarding BSE compared to respondents with no family history.

### Knowledge on BSE Method and Timing:

Our participants were also questioned about BSE practice. It was observed that the majority of the participants in the present study had moderate to poor knowledge when it came to BSE practice. However, it has been significantly improved up to 90% after the education, which highlights the positive changes on the BSE knowledge due to the training regarding BSE [Table 2]. According to Abdul Nazer Ali’s research, the general awareness score on BSE knowledge was moderate at baseline and improved drastically after educational program. A similar study by Mehrnoosh assessed the knowledge of women at baseline, 6, and 12 months and reported that education modules of BC and BSE method improved the knowledge scores in the intervention group.

### Barriers to seek medical help:

In our study, we found various barriers. During the pre-intervention phase it was found that almost 45% of women did not have the confidence to notice a change in their breast, 68% were embarrassed to consult a doctor, 72% were scared, and almost 72% were not confident enough to visit the physician for discussion. Acquiring finally helped to remove obstacles such as embarrassment, fear and lack of self-confidence. After all, cultural characteristics may be treated as the main barriers that can be influential. After education, it was noticed that above 65% of women got confidence and were freed of negative attitudes towards their physician for consultation. Education brought a significant change in their attitude and improved their confidence levels towards breast health [Table 3]. According to Saleha Qasim study, shows the most commonly prominent reasons of not visiting a doctor in time were: busy schedule, embarrassment, anxious to see a doctor, finding it hard to speak with the doctor.

Some limitations need to be addressed, when interpreting the results, first of all, a great number of the participants (87.8%) were identified at the age group of 17-25 years and may not be representative of all female of different age group; hence it is recommended to conduct further studies using a wider range of age. We therefore highly recommend that the appropriate and practical training programs should be provided for women through India’s health care system.

### Conclusion

We witnessed participants’ awareness towards breast cancer manifestation, risk factors and BSE for early detection insufficient at the pre-intervention phase, but we found a great advancement in the first and second post-intervention phase. The study emphasizes the fact that breast cancer awareness needs to be raised and individuals must be educated about the significance of BSE practice, which allows breast cancer to be detected in a much earlier stage. The presented data demonstrates a significant perceptual barrier and baseline knowledge regarding breast cancer and BSE among Indian females and demonstrates the ability to provide guidance for seeking the techniques to enhance awareness among Indian females according to their needs.

### Acknowledgment:

We thank the participants of the study for their valuable time and interest shown in our study.

### Conflict of Interest:

The authors declare that they have no conflict of interest.

### Funding support:

The authors declare that they have no funding support for this study.
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Mental Illness of Pregnant Women in Al-zahraa Teaching Hospital, Wasit Al-Kut 2019

Areej Sabah Abdulridha1, Baidaa Abdulkareem Alwan1, Mahdi Abdulkarim Hamood2
1Instructor, Obstetrics and Gynecology Department, College of Medicine, Wasit University, 2Consultant Psychiatrist, Ministry of Health Wasit Health Department

Abstract
Maternal mental health considered as an important subject among leading public health experts. It has been shown that women are two to three times more likely to be diagnosed with mental illness compared to men. Chronic stress, experiencing war, and history of abuse are the most effective factors that associated with mental illness. Iraq has experienced years of challenging circumstances due to the political and social environment as well as due to physical barriers to health care services. This paper presents findings of previous literatures in relative to mental illness among Iraqi women. In the current study, we are aiming to determine the mental illness symptoms and effects in pregnancy during a period of all trimesters. Moreover, we try to assess risk factors’ effects on mental illness symptoms in order to have better understanding of the occurrence and associated factors in pregnancy. Type of study comparative cross-sectional study with analytic component. A cross section study was conducted from 2018/9/5 to 2019/8/25. Observations of present study show that there is a relationship between some factors like domestic violence, unplanned for pregnancy, previous miscarriage, psychological trauma before or during pregnancy. Our study mainly focusing on women with maternal mental illness and inform strategies that would help to reduce and manage maternal mental problems in order to promote their general health status.

Key words: Mental illness, Pregnant, Teaching Hospital, Kut 2019.

Introduction
Mental illness is a syndrome characterized by clinically significant disturbance in an individual’s cognition, emotion regulation, or behavior that reflects a dysfunction in the psychological, biological, or developmental processes underlying mental functioning. Mental illness are usually associated with significant distress or disability in social, occupational, or other important activities. Mental health problems may be a result of an excessive stress. As with cancer, diabetes and heart disease, mental illnesses are often physical as well as emotional and psychological. Furthermore, mental illnesses may be caused by a reaction to environmental stresses, genetic factors, biochemical imbalances, or a combination of all mentioned factors. Notably, with proper care and treatment many individuals learn to cope or recover from a mental illness or emotional disorder. Although the incidence of mild mental health problems is not significantly different during pregnancy, the risk of bipolar or severe depressive illness is greatly increased postpartum and this period represents perhaps the highest risk period in a woman’s life for the development of a psychiatric disorder. Furthermore, women with previous serious mental health problems are at high risk of recurrence during both the antepartum and postpartum periods. Previously, it has been illustrated that the most common types of mental illness are Anxiety disorders, that including panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, and phobias. Moreover, Depression, bipolar disorder, and other mood disorders have also been described as types of mental illness.
illness. In addition, same study has referred to eating, personal and psychotic disorders as other types of mental illness. Moreover, Neurotransmitters (brain chemistry), are naturally occurring, as brain chemicals that carry signals to other parts of your brain and body. When the neural networks involving these chemicals are impaired, the function of nerve receptors and nerve systems change, leading to depression. Common risk factors: Family history of mental health problems, Complications during pregnancy or birth, Personal history of Traumatic Brain Injury, Chronic medical condition such as cancer or diabetes, especially hypothyroidism or other brain-related illness such as Alzheimer’s or Parkinson’s, Use of alcohol or drugs, Poor nutrition and lack of sleep. Furthermore, there is a wide range of symptoms could be categorized as indicators of mental illness. For instance, anxiety: excessive worry or fears. Persistent sad or low mood, unusual or illogical thoughts, unreasonable anger or irritability and poor concentration. Previously, it has been shown that diagnosing a mental health disorder is a multi-step process. During a first appointment, doctor may perform a physical exam in order to look for signs of physical issues that could be contributing symptoms. Some doctors may order a series of laboratory tests trying to screen for underlying or less obvious possible causes. Moreover, doctor may ask to fill out a mental health questionnaire. Patients may also undergo a psychological evaluation. You might not have a diagnosis after your first appointment. In some cases, doctor may refer patient to a mental health expert, because mental health can be complex and symptoms may vary from person to person, it may take a few appointments to get a full diagnosis. However, these are widely believed to be underestimates, due to poor diagnosis (especially in countries without affordable access to mental health services) and low reporting rates, in part because of the predominant use of self-report data, rather than semi-structured instruments such as the structured clinical Interview for DSM-IV (SCID); actual lifetime prevalence rates for mental disorders are estimated to be between 65% and 85%. In the current study, we are aiming to determine the mental illness symptoms and effects in pregnancy during a period of all trimesters.

**Methodology**

Describe mental illness in Iraqi women during pregnancy a cross section study was conducted in 2019 depending on many references. The time of study from 2018/9/5 to 2019/8/25. The study included women in 2018 and 2019 who are visited Al-Zahra hospital's gynecology outpatient clinic, to identify women who are needed for psychological support or suffer from sign and symptoms of mental illness during pregnancy.

Risk factors for mental illness during pregnancy, risk of history of psychological trauma on pregnant women was also measured. In this study 250 cases collected in age group ranged from 19 – 35 years, in any gestational age. We collected samples from Al-kut depending on questioners. Actually, pregnant women have been asked many questions that relative to signs and symptoms of mental illness and depend on psychiatry classification the questioner contain 20 questions. If the number of positive questions is seven in number or below there is no mental illness. If the number of positive questions is eight or above there is mental illness.

**Results**

We took 250 cases from Al-kut to estimated prevalence of mental illness during pregnancy and its risk factors.

<table>
<thead>
<tr>
<th>Score 20 sign and symptoms of mental illnesses</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal</td>
<td>87</td>
<td>34.8</td>
<td>34.8</td>
<td>34.8</td>
</tr>
<tr>
<td>abnormal</td>
<td>163</td>
<td>65.2</td>
<td>65.2</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>
Our observations show that there was no strong correlation between gestational age and signs and symptoms of mental illnesses (table 2).

**Table 2. Correlation of sign and symptoms of mental illnesses and gestational age.**

<table>
<thead>
<tr>
<th>Correlations</th>
<th>gestational age</th>
<th>score 20 sign and symptoms of mental illnesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person Correlation</td>
<td>1</td>
<td>.039</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.536</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Correlations</th>
<th>score 20 sign and symptoms of mental illnesses</th>
<th>education level</th>
<th>occupation</th>
<th>income</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person Correlation</td>
<td>1</td>
<td>-.063-</td>
<td>-.143-*</td>
<td>-.099-</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.321</td>
<td>.024</td>
<td>.120</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td></td>
</tr>
</tbody>
</table>

Most of cases in the present study were in low education level, however there was no strong correlation between education level and mental illnesses even the results show that prevalence of mental illnesses in low education more than high education level (table 3).

**Table 3. Correlation between educational level, occupation and income with mental illnesses.**

<table>
<thead>
<tr>
<th>Correlations</th>
<th>score 20 sign and symptoms of mental illnesses</th>
<th>education level</th>
<th>occupation</th>
<th>income</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person Correlation</td>
<td>1</td>
<td>-.063-</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.321</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Correlations</th>
<th>education level</th>
<th>occupation</th>
<th>income</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person Correlation</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>250</td>
<td>250</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Correlations</th>
<th>occupation</th>
<th>income</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person Correlation</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>250</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Correlations</th>
<th>income</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person Correlation</td>
<td>1</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>250</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level (2-tailed).
In our study were 203 cases unemployed (81%) and (19%) employers. We found that there is a high risk of developing signs and symptoms of mental illnesses among unemployed pregnant women (table 3).

In the present study, prevalence of sign and symptoms of mental illnesses in low income more than high income (table 3), there was 55 cases with low income (22%), 180 cases with moderate income (72%), and 15 cases with high income (6%).

In the is study, there was 167 cases with no any complication (66%), 83 with complication most of them hypertension and bleeding (33%). We found that there is high risk of developing mental disorders in complicated pregnancy comparing to those with un complicated pregnancy (table 4).

Table 4. Correlation between mental disorders and pregnancy complications

<table>
<thead>
<tr>
<th>Correlations</th>
<th>score 20 sign and symptoms of mental illnesses</th>
<th>complication during pregnancy</th>
</tr>
</thead>
</table>
| score 20 sign and symptoms of mental illnesses | Person Correlation 1 | .158*  
|mother | Sig. (2-tailed) | .012  
|N | 250 | 250  
| complication during pregnancy | Person Correlation .158* 1 |  
| | Sig. (2-tailed) | .012  
|N | 250 | 250  

* . Correlation is significant at the 0.05 level (2-tailed).

Additionally, we indicated that 240 cases with history of good relationship (96%), and 10 cases with poor relationship (4%), there was very high score of signs and symptoms of mental illnesses among women with poor sexual relationship (table 5).

Table 5. Correlation between domestic violence and sexual relationship with mental illness.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>score 20 sign and symptoms of mental illnesses</th>
<th>sexual and relationship history</th>
<th>domestic violence</th>
</tr>
</thead>
</table>
| score 20 sign and symptoms of mental illnesses | Person Correlation 1 | .149* | 163*  
| | Sig. (2-tailed) | .018 | .010  
|N | 250 | 250 | 250  
| sexual and relationship history | Person Correlation .149* 1 |  
| | Sig. (2-tailed) | .018  
|N | 250 | 250  
| domestic violence | Person Correlation .163* 1 |  
| | Sig. (2-tailed) | .010  
|N | 250 | 250  

* . Correlation is significant at the 0.05 level (2-tailed).
Results of our study revealed that there are 213 cases with no history of domestic violence (85%), 37 cases with history of domestic violence (14%). We found a strong correlation between domestic violence and symptoms of mental illnesses among pregnant women (table 5).

Twenty hundred and thirteen pregnant women with no history of psychological disorders before pregnancy (85%), 37 cases with history of psychological disorder (14%) there was strong correlation and prevalence of mental illnesses in women with history of psychological disorders before pregnancy than those with no history (table 6).

Table 6. Correlation between psychological disorders before pregnancy, family history of psychological disorder and social support with mental disorders.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>score 20 sign and symptoms of mental illnesses</th>
<th>psychological disorder before pregnancy</th>
<th>family history of psychological disorder</th>
<th>social and family support</th>
</tr>
</thead>
<tbody>
<tr>
<td>score 20 sign and symptoms of mental illnesses</td>
<td>Person Correlation</td>
<td>1</td>
<td>.304**</td>
<td>.124</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>.050</td>
<td>.024</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>psychological disorder before pregnancy</td>
<td>Person Correlation</td>
<td>.304**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>250</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>family history of psychological disorder</td>
<td>Person Correlation</td>
<td>.124</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.050</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>250</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>social and family support</td>
<td>Person Correlation</td>
<td>.143*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>250</td>
<td>250</td>
<td></td>
</tr>
</tbody>
</table>

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

Present study also shows that 243 cases with no family history of mental illnesses (97%), 7 cases with family history (3%). All women with family history of mental illnesses suffer from some signs and symptoms of mental illnesses but not related to pregnancy (table 6).

In the current study, we determine that 196 pregnant women with good family support (78%), 41 with moderate support (16%), and 13 cases with poor family support (6%). It was strong correlation between family and social support in relative to developing of signs and symptoms of mental illnesses in those with poor or moderate family and social support (table 6).

Discussion

This cross-section study presents detailed description of women’s maternal mental health in Al-kut. Many systematic reviews have suggested that
women are at high risk of mental illnesses because of social and cultural customs. There are also significant barriers to receive mental health services due to infrastructure challenges and perception of stigma at population level. WHO estimates that prevalence rate for mental illnesses is higher in low income countries (20%) compared to women in high income countries (16%), this provides to be true in Iraq, especially since 2003. Previous studies have demonstrated that Iraqi women present high rates mental illnesses compared to women in other countries. Actually, global prevalence rate for maternal depression and anxiety is between 15-20%. Moreover, it has been shown that depression and anxiety are highly indicated during antenatal period. The most relevant factors associated with antenatal depression or anxiety (ranked according to the number of studies that have found the factors to be significant predictors, and presenting also the number of studies who did not) are: lack of family or social support, history of abuse or domestic violence, personal history of mental illnesses, unplanned pregnancy, high perceived stress, present or past pregnancy loss or complication, low education level, low income, dissatisfied relationship with partner. Consistently, numerous previous studies revealed that young women are significantly associated with antenatal depression and anxiety. Whereas, 10 studies found that old age to be a significant risk factor. Another 10 researchers did not find any association with age. In agreement with previous studies, current study shows that there is no significant of age correlation. Strikingly, no studies regarding the psychopathology of the partner as potential risk factor for antenatal mental illness. In our study, we found that majority of the participants are in low income group. Our interpretation is that low income increases the likelihood of poor living conditions, financial struggle and influences interpersonal relationship. Moreover, we also found among pregnant women observed that patients being educated but not employed could predispose to depression during pregnancy (antenatal depression) and its associated Risk factor. Among women in Bangalore. In the current study and in agreement with aforementioned studies, we showed that the chance of getting depressed is significantly high in case of unplanned pregnancy. Furthermore, our study shows significant correlation between domestic violence and antenatal depression as well as anxiety. The linkage between poor social and family support and antenatal depression has been well documented. In fact, low social support may increase mental stress by inducing feeling of insecurity, predispose to substance abuse, and promotes interpersonal conflict. Other studies have reported that negative life event may lead to persistent higher levels of depressive symptoms since positive life event would reduce the severity of depression over time. It is important to mention that body mass index was not linked with the risk of prenatal depression in this study, even other research have indicated an interconnection between obesity and depression, the causal pathway could include inflammation, hormonal imbalance or sleep disturbance.

**Conclusion**

In summary we can conclude that there is a correlation between the mental illness in pregnant women and the following risk factors; domestic violence, not planned for pregnancy, house wife, previous miscarriage, history of psychological trauma before pregnancy. These factors affect the mental health of the mother.

**Conflict of Interest:** None of the authors have any conflict of interest

**Ethical Clearance:** Taken from institutional ethics committee before beginning the study.

**Source of Funding:** Self

**References**


Challenges in Maternal and Child Health Routine Data Administration in Indonesia: A Qualitative Study

Arief Priyo Nugroho¹, Diyan Effendi¹, Zulfa Auliya Agustina², Asep Kusnali¹, Siti Maimunah³, Irfan Ardani¹, Ratna Widyasari¹

¹Researcher, National Institute of Health Research and Development, Ministry of Health of Indonesia, Jl. Percetakan Negara No. 29 Jakarta, Indonesia, ²Researcher, Health Technology Innovation, Ministry of Health of Indonesia, Indrapura 17, Surabaya, ³Legal Drafter, National Institute of Health Research and Development, Ministry of Health of Indonesia, Jl. Percetakan Negara No. 29 Jakarta, Indonesia

Abstract

Background: Maternal and child health (MCH) routine data is essential in making a good health-related policy. However, the quality of MCH routine data in Indonesia is doubted, and thus the Indonesian government relies heavily on the survey data for policymaking. This condition raises questions about where the problems exist in routine data recording stages. This study aims to explore the barriers and strategies of MCH routine data recording by the administrators in the primary healthcare center.

Method: This study was qualitative research conducted in Buru Regency, Ambon City, Purworejo Regency, and Surakarta City from May to November 2020. The data collections were intended to understand administrators’ efforts to deal with the data recording problems. Data triangulation was performed through in-depth interviews with primary healthcare center staff and observations on daily routine data administration practices.

Results: The study demonstrated challenges in the MCH routine data administration context. The first problem is behavioral contexts lead to incorrect input and delay data submission. Second, technical determinant shows the lack of integration that leads to repetitive data recording and data fragmentation. The third was the organizational problem such as lack of inter and intra-departmental coordination in data sharing, infrastructure, and human resource shortage.

Conclusion: The findings elucidate the problem of administrative structures in the implementation of routine data policy. A comprehensive response to cope with routine data policy implementation context is needed. Existing maternal and child healthcare routine data requires structural administration refinement that provides a context for implementing reliable routine data recording of maternal and child health.

Keywords: Maternal and Child Health, routine data, administrative structure

Introduction

Routine data is inherent in the health efforts being carried out. Therefore, routine data would describe the achievements of health programs. Health routine data in Indonesia is sourced from the healthcare facilities reports. However, in practice, routine data generated by the bodies responsible for the health services are not reliable. Routine data generated in Indonesia is still questioned for its validity.

The question of validity arises when routine data has not maximally supported the health policy or there is a huge gap between reported data and the concurrent data source such as survey data.[¹,²] In Indonesia, there is a gap between the health-facilities-sourced data and...
the survey data. For instance, the health-facilities-sourced data reported that KN1 coverage was 97.4%, in 2019 but the data of Basic Health Survey (Riskesdas) showed KN1 coverage was 84.1%.[3] This data gap has potentially caused an error in the government’s response to health problems for pregnant women.

The presence of data gaps shows that routine data management is experiencing various obstacles. Routine data registration is facing inadequate supervision and alternate workload by local Midwives were cited as factors resulting in inconsistent reporting of data.[4]

This paper focuses on how the MCH data are routinely recorded and reported by the administrator in the primary healthcare center. This administration scheme has a primary role in MCH routine data. It shows how the data recording is administrated and explains the context of routine data policies implemented.

**Method**

This study used a qualitative approach which was conducted from May to November 2020. The data were collected through focus group discussions (FGDs) and in-depth interviews with the related stakeholders. The FGDs and interviews were designed to get a deeper understanding of the context of the MCH routine data problems both from the policymaker and implementer perspectives.

The research was carried out in four areas: Buru Regency, Ambon City, Purworejo Regency, and Surakarta City. The selection of these regions was intended to capture the diversity of region-related problems in carrying out routine data recording.

<table>
<thead>
<tr>
<th>No.</th>
<th>Regency/City</th>
<th>Population (Indonesia census 2010)</th>
<th>Maternal and Child Health Coverage (K4, KF &amp; KN)</th>
<th>PHDI Rank 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Buru Regency</td>
<td>108.235</td>
<td>Low</td>
<td>414</td>
</tr>
<tr>
<td>2.</td>
<td>Ambon City</td>
<td>330.355</td>
<td>High</td>
<td>241</td>
</tr>
<tr>
<td>3.</td>
<td>Purworejo Regency</td>
<td>694.404</td>
<td>Low</td>
<td>82</td>
</tr>
<tr>
<td>4.</td>
<td>Surakarta City</td>
<td>500.642</td>
<td>High</td>
<td>67</td>
</tr>
</tbody>
</table>

Note: K4 = 4th antenatal care visit; KF = post partum visit; KN = neonatal visit; PHDI = Public Health Development Index

Data triangulation was performed through in-depth interviews with primary healthcare center staff and observations on daily routine data administration practices. Both FGDs and in-depth interviews were audio-recorded. The recordings were transcribed and analyzed into behavioral, technical, and organizational determinants categorization.

**Results and Discussion**

Routine Data Circumstances in Four Regions

The implementation of MCH routine data recording in the four regions that were the locus of the study had the same budgeting conditions. Routine data recording is still relatively minimal, having a separate budget
and supporting existing activities. There is no budget specifically allocated for data recording. It is still embedded in the activities that are carried out.

Most of the budget came from deconcentration funds from the central government. Although in the regional budget, several were allocated for routine data management, in general, the funds allocated specifically for recording routine MCH data were still insufficient.

The implementation of routine data recording on maternal and child health had also experienced constraints due to the lack of human resources. The staff in charge of data at the Primary healthcare center often had to carry out more than one task. Apart from recording routine data, she was also responsible for other tasks such as delivery services. As such, the workload increased and there was an impression that the human resources in the primary healthcare center were insufficient.

<table>
<thead>
<tr>
<th>Region</th>
<th>Buru Regency</th>
<th>Ambon City</th>
<th>Surakarta City</th>
<th>Purworejo Regency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resources</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Resources</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Budget</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Infrastructure</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>±</td>
</tr>
</tbody>
</table>

Table 2. Resources in implementing MCH routine data recording

Routine data recording infrastructure was relatively minimal to support the implementation of maternal and child health routine data recording. Despite having very little budget allocation, the Surakarta city government had adequate infrastructure support. This infrastructure adequacy was caused by the implementation of the ‘one data’ policy in Surakarta.

MCH Routine Data Recording Problems

Behavioral problems

In routine data recording, problems often encountered in this study were input errors and delays in data transmission. The two problems by the implementer were included in the category of behavioral problems. Behavioral problems in routine data recording were caused by high and varied workloads. First, the high workload is caused by a large number of recording applications and diverse types of data. Second, the staff in charge of data recording not only had the function of recording routine data but also responsible for delivering health services. Also, there were other duties and functions which were the responsibility of the staff who carry out data recording, such as administrative duties as treasurers.

“There are many cohorts recordings, so there is a possibility that it will be wrong” (PHC A1)

“Yes, it was already reported but forgotten not to be written in the cohort. The midwives have the cohort book but sometimes they forget to write in the cohort book. I’ve done the monitoring and evaluation and rarely found that condition. Maybe there was a delivery or other activity, so they forgot to write in the cohort.” (PHC A2)

Experiences in other regions in Indonesia also found similar problems with the routine data recording.
Other countries also face similar experiences in carrying out routine health data recording. This problem is considered a daily problem encountered in routine data implementation. This daily problem cannot be underrated because it affects the quality of the routine data being produced.

Technical problems

Incorrect input and delay submission of data experienced by routine data recording implementers occurred due to inadequate technical support in the routine data recording system. Technically, the routine data recording application was not integrated, so there was data fragmentation. Data fragmentation caused by the unintegrated recording application made performance inefficient. Often the same data must be inputted into two or more different applications. Repetition of similar data recording was an inefficiency in carrying out routine data recording. In addition to increasing the workload, this problem also likely to cause input errors. The same problem was also found in previous studies which showed that the error rate of data recording increased and the compliance of recording staff decreased due to a large number of MCH data recording forms. This condition was not unnoticed by the data recording staff. They realized what they were doing was a repetitive job that could have been made more concise. But in reality, it returns to the recording system which did originate and was aimed at separate management.

“We at the family health directorate, have no application and manually operated. There are some apps for nutrition routine data. There is no MCH application. The problem in this city is the internet, the staffs have overlapping work, and the high workload…” (City health department informant)

The shortage of human resources that were experienced became an operational burden when the routine data recorder at the primary healthcare center was a staff who did not only have responsibility for recording routine data only. Mostly the staff responsible for recording routine MCH data were midwives who had more than one program responsibility. From the interviews conducted with a director of a primary healthcare center, one midwife bears three or more responsibilities. This burden did not include the responsibility for health services.

“Honestly we feel overburdened. The workload is too high. Imagine, 15 midwives have to handle 32 villages and our jobs are not only about MCH but also other duties not related to MCH. If we may focus on MCH only then the outcomes would be better.” (PHC C1)

Behavioral problems in recording routine MCH data at the primary healthcare center showed that the division of tasks has not been carried out with the principle of a clear separation of functions. The staff at the primary healthcare center has many functions at once. Midwives, for example, not only provide delivery services, but they are also responsible for other programs. This problem prevents staff from honing expertise in one particular area.
Organizational problems

Lack inter and intradepartmental coordination in data sharing

Routine data recording at the health center includes the MCH program (pre and post-natal care), immunization, surveillance, and several other programs. The recording is carried out by the Primary healthcare center staff, who is usually a village midwife. Each program had its data recording application.

Most of the MCH routine data recording applications were not integrated, indicating that there was a technical problem. Data recording was done repeatedly because each MCH program had different applications even though they had the same data. Regarding maternal and child health, there were at least two applications, namely “e-ppgbm” [13] and “simpus KIA”[14]. This condition showed the weak coordination between policymakers in the Ministry of Health.

Human resource shortage

Of the four research loci, the lack of human resources had always been a problem in carrying out routine data recording. This problem has become the main topic in health services in Indonesia. Even in urban areas,

The problem of lack of human resources was not only caused by a lack of quantity but also due to maldistribution. The uneven distribution contributed to the constant lack of health workers at the primary healthcare centers[15]. This phenomenon can be seen in the findings of this study, in Buru District, the number of midwives was sufficient even though they were not permanent employees. In other findings, in urban areas such as Surakarta or Purworejo, the human resources at the primary healthcare center were not excessive but they were sufficient. The problem faced was the high workload, especially for midwives who were responsible for more than one program.

Maldistribution of human resources and high workload showed a lack of efficiency. A primary healthcare center was not designed as an organization that encourages specialization in every function it carries out. According to Indonesia Minister of Health Regulation No. 43 of 2019, primary healthcare center management does not emphasize efforts to increase staff professionalism by encouraging the specialization of duties and functions. Primary healthcare centers are managed to cover a wide range of areas and are deliberately encouraged to holistically address public health problems.

The holistic approach does provide the benefits of integrative and coordinative services. However, if this approach is not implemented properly, it will reduce the efficiency of the management of the primary healthcare center. These potential problems were found in the implementation of routine data recording. The integration between the data recording process and health services meant that there was no separation of the duties and functions of the staff which results in a double burden of work. This problem is a manifestation of inefficiency in human resource management in the primary healthcare center.

Lack of infrastructure

The last problem identified was the inadequate infrastructure for MCH routine data recording. This problem arose as a result of the lack of allocation for carrying out routine data. Even regions with adequate fiscal capacity, such as Surakarta and Purworejo, also experience the lack of infrastructure, although to a different degree compared to regions with low fiscal capacity. Especially for areas such as Buru Regency and Ambon City, infrastructure became the main obstacle after human resource problems. Regional budget allocations in the health sector are strongly influenced by the subjectivity of regional mayors. Often curative efforts get more budget allocations because their impact could be immediately measured. So that success in the curative sector is expected to increase the electability of the incumbent regional head-bearing party.[16]

Routine data recording that has adopted information technology has made the adequacy of infrastructure
an obstacle. These constraints are mainly faced by regions that are still struggling with basic problems such as electricity supply and the availability of internet connections. This problem directly hinders the implementation of a routine MCH data recording and reporting system. The constraints of MCH routine data recording found in this study were formalized in the following conceptual model (figure 1).

![Diagram](image)

**Figure 1 Determinant in MCH Routine Data Obstacle in Buru, Ambon, Purworejo, and Solo – Modified from Hoxha et al. (2020).**

The organizational problems had caused the occurrence of technical problems. Whilst technical problems were the root of behavioral problems endured by the MCH routine data administrators.

**Potential Solutions**

The results of this study indicated that the problems of implementing the MCH routine data recording including behavioral and technical problems. These problems were formed as a result of the organizational culture that drives them.

Therefore, efforts to improve routine data recording require at least two keys of improvements. The first is about the simplification of data recording. This simplification is needed to reduce the workload. Second is the improvements in the management structure. Improved management of routine data recording is required for the sustainability of records.

**Routine data recording simplification**

The first solution that can be taken is to simplify the routine MCH data recording system. As discussed above, the number of recording systems both online and offline has caused a decrease in the level of compliance in filling in data and increased input errors, many of which with the same variables cause the workload of
midwives at the primary healthcare centers to increase. Not only the number of forms that must be filled in, but the village midwife also has many help books to use manually. Assistive book for midwife personal records which also means that the existing system is not yet effective and efficient.\textsuperscript{[18,19]}

**Routine data administration enhancement**

Primary healthcare center needs to have skilled and competent human resources in data recording. The success of recording and reporting routine data is largely determined by human resources that implement routine data information systems. Staffs who are in charge of data recording and reporting must have an awareness that the data generated is indispensable for policymaking\textsuperscript{[20,21]}

If the solutions-focused only on the lack of human resources, the problem of routine MCH data recording will not be resolved properly. First, in the delivery of health services, the problem of resources including human resources is a classic problem.\textsuperscript{[15,22]} Second, the complexity of the problem of inequality in health services even at the subnational level.\textsuperscript{[23]} Therefore, optimal management of human resources is a rational choice in improving services.

In the modern bureaucracy, specialization is needed to increase the efficiency of results. In the Weberian framework of thought, efficiency results from the existence of specialization in work.\textsuperscript{[24,25]} This specialization requires a supportive organizational structure. The organizational structure of the primary healthcare center in Indonesia has not been able to answer this need. Human resource management is still based on the Health program which is carried out without paying attention to the specifications and specializations of work required.

Primary healthcare centers have not implemented the separation of staffs’ functions and duties. So that health workers at the primary healthcare centers carry out very diverse duties and functions. Human resource management in such an organizational structure makes employees unable to develop job specializations. This phenomenon is because they are not required and are not encouraged to specialize in work.

**Conclusion**

Constraints on the implementation of MCH routine data recording were evident that problems that occurred were mostly due to organizational problems. The organizational context has resulted in technical issues and eventually leading to behavioral issues. Behavioral problems were derived from the issues of insufficient human resources, a lack of coordination in both inter and Intra departments, and inadequate infrastructure.

These research findings showed that the routine data administrators were trapped in a situation that did not allow them to increase the efficiency and quality of MCH routine data. Challenges regarding the diverse job function of routine data administrators were the context of problems that prevent optimum routine data recording.

**Acknowledgments:** The authors would like to thank Tety Rahmawati as research project director. Health Office in Ambon, Buru, Purworejo, Surakarta, Maluku and Central Java for the cooperation in Maternal and Health Care routine data research. Sri Handayani, Karlina, Herti Windya, Jenny Samosir, Choirum Latifah and Primasari who helped the data collection.

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**Conflict of Interest:** The authors affirm that they have no conflict of interest

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Superior Semicircular Canal Dehiscence Syndrome in a Woman: A Case Report

Arif Surgana¹, Haris Mayagung Ekorini²
¹Resident, ²Lecturer, Department of Otorhinolaringology Head and Neck Surgery, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

Abstract

Background: Superior semicircular canal dehiscence (SSCD) syndrome is a disease that occurs very rare. Clinical presentation consists of Tullio’s phenomenon and Hennebert’s sign. This case presentation is atypical and clinician should be aware. Purpose: to present the rare case of Superior semicircular canal dehiscence (SSCD) Case presentation: A 65-year-old woman with complaints of dizziness from 2 years ago and her recurrence. The audiogram showed bilateral sensorineural deafness with a mean hearing threshold of 58 decibels (dB) of the right ear and 48 dB of the left ear. There is also an air-bone gap in the right ear at low frequencies (250 Hz). The tympanogram revealed a type A result in both ears. Positive results were obtained on the examination of Romberg sharpened, positive Fukuda step to the right, and the Gans test. CT scan showed dehiscence of the right superior semicircular canal. Therapy in patients was given conservative therapy because the participants refused to undergo surgery. Conclusion: SSCD has a specific symptom of Tullio’s phenomenon and CT scan shows dehiscence of the superior semicircular canal. Keyword: Superior semicircular canal dehiscence, Tullio’s phenomenon, vertigo

Introduction

Superior semicircular canal dehiscence (SSCD) syndrome was a disorder in which symptoms of vertigo induced by loud noises (Tullio’s phenomenon) or intracranial pressure (Hennebert’s sign) due to dehiscence of the bone lining the superior semicircular canal¹. The prevalence of developing SSCD was 3% on the computed Tomography (CT) scan survey of the temporal bone². Tullio’s phenomenon and Hennebert’s sign are characteristic sign because of the formation of a third window in the superior semicircular canal. The third window appears due to dehiscence of the superior semicircular canal. Sound stimulation and intracranial pressure on this third window cause excitation or inhibition of the superior semicircular canal leads to symptoms of vertigo. Bone conduction will lead to hyperacusis, leading to symptoms of autophonia³.

Temporal CT scan of SSCD can show dehiscence of the semicircular canal. The CT scan is about 0.5 mm thick to see the structure of the inner ear. Clinical symptoms combined with appropriate CT scan images can increase SSCD specificity by 99%⁴. Audiometric examination of SSCD can give results of conduction hearing loss, especially low frequencies and tympanometry examination yields normal values⁵. Based on the description above, we are interested in reporting a case of SSCD that we had in a 65 year old woman.

Case Presentation

A 65-year-old woman with complaints of vertigo since 2 years ago has disappeared. Vertigo is not triggered

Corresponding author:
Arif Surgana
Department of Otorhinolaringology Head and Neck Surgery, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Jalan Mayjend Prof. Dr. Moestopo No. 6-8, Airlangga, Gubeng, Surabaya, East Java 60286, Indonesia
Mail: arif2603@gmail.com
by change in head position. The patient has undergone medical rehabilitation therapy but the complaints persist and when she hear loud sounds feel uncomfortable and dizzy. Hearing loss since 1 year ago in both ears. Previous medical history is hypercholesterolemia. The patient has seen several doctors and finally to a neurosurgeon, then a Magnetic Resonance Imaging (MRI) examination is recommended before being referred to a specialist in otorhinolaryngology head and neck surgery.

On both ears Rinne’s examination and Weber’s examination were no lateralization and Bing’s examination was positive in both ears. The audiogram showed bilateral sensorineural deafness with hearing threshold pure tone average of 58 decibels (dB) of the right ear and 48 dB of the left ear. There is also an air-bone gap in the right ear at low frequencies (250 Hz). The tympanogram revealed a type A result in both ears. The acoustic reflex was not continued because the patient experience vertigo during examination. When the acoustic reflex was performed, nystagmus appeared but the direction could not be determined because the patient asked to stop the examination. The valsalva test was not performed in these patients because of vertigo.

Vestibular examination was carried out including the Romberg test, the Romberg sharpened test, the Fukuda step test, the Gans test, and the Dix Hallpike test. Positive results were obtained on the examination of Romberg sharpened, positive Fukuda step to the right, and the Gans test. Negative results were obtained on the Romberg, Dix-Hallpike examination and the coordination examination in the form of dysdiadokinesia and finger nose tests. In conclusion, the results of the vestibular examination showed that Vertigo with non-Benign Paroxysmal Positional Vertigo (BPPV) type.

A CT scan of the temporal bone with suspicion of SSCD was performed on the patient. CT scan showed dehiscence of the right superior semicircular canal. The left semicircular canal appeared normal. The cochlea and vestibule appeared normal. Bilateral normal internal acoustic canal. Both patent of vestibular aqueduct and facial nerve. Osicles and scutum were normal, no erosion found. The foramen ovale and rotundum were normal bilaterally (Figures 1 & 2). Therapy in patients was given conservative therapy because the participants refused to undergo surgery.

Figure 1. CT scan of the coronal temporal cut shows dehiscence of the right superior semicircular canal (black arrow). Left superior canal appears normal (white arrow).
SSCD syndrome was a disorder due to dehiscence of the roof of the superior semicircular canal occurs. Symptoms that arise involve hearing and balance problems. In general, the symptoms that arise are divided into two, related to sound and pressure changes. Symptoms associated with sound are vertigo triggered by loud noises (Tullio’s phenomenon), autophonia (can hear sounds from one’s own body), hyperacusis, fullness in the ears, tinnitus, low frequency conduction deafness, while symptoms related to pressure changes are vertigo when the intracranial pressure increases (Hennebert’s sign) eg when coughing, valsalva, and lifting heavy objects\(^{(5)}\). The age of SSCD patients in this case is in accordance with previous studies where many SSCD patients were found to be over 60 years of age\(^{(2)}\) and other studies stated that the incidence of SSCD increases with increasing age\(^{(6)}\). In this case there was dehiscence of the superior semicircular canal in the right ear. Dehiscence often occurs unilaterally but can also be obtained bilaterally\(^{(7)}\).

The Tullio phenomenon can produce vertigo when hearing loud sounds especially at low frequencies\(^{(8)}\). The Tullio phenomenon was found in 96% of these SSCD sufferers. The symptom description is characterized by vertigo or nystagmus on hearing a loud sound\(^{(7)}\). The symptoms of Tullio’s phenomenon are not given enough attention as a result of the patients are misdiagnosed with psychiatric disorders. Vertigo in SSCD can also result from the Valsalva maneuver\(^{(9)}\).

Low frequency (250 Hz) air bone gap in the right ear corresponds to hearing loss in SSCD usually conduction hearing loss at low frequencies below 1000 Hz\(^{(3)}\). This conduction hearing loss results from the presence of a third window of the superior semicircular canal. Sound energy that enters the cochlea through the foramen ovale will partially go to the dehiscent semicircular canal, resulting in an increase in the threshold with air conduction. This results in an air-bone gap without pathology in the middle ear\(^{(10)}\). Electronistagmography (ENG) can help diagnose SSCD where nystagmus occurs when tympanometry and valsalva are performed. A 100-110 dB stimulation examination at 125-4000 Hz will show vertical and torsional nystagmus. Fistula examination revealed vertical and torsional nystagmus with a slow phase moving away from the side of lesion, but valsalva examination revealed nystagmus in the opposite direction\(^{(11)}\).

The results of temporal CT scan in this patient showed dehiscence of the right superior semicircular canal. Temporal CT scan is the main modality in diagnosing SSCD, especially if the patient is planning for surgery. Normal view of the superior semicircular canal has 3 layers of roof, namely the otic capsule, trabecular bone and bone cortex. These three layers can be clearly seen on a temporal CT scan with 0.5 mm thickness. The recommended CT scan is multi-axial with a parallel image of the coronal, sagittal and axial slices with a thickness of 0.5 mm. Superiority the picture of dehiscence when viewed from the coronal slice. Temporal reconstruction CT scan with multiple slices can increase the sensitivity and specificity in detecting the presence of SSCD. Dehiscence that appears on one slice can not confirm the presence of dehiscence in other slices\(^{(4)}\).
SSCD therapy can be both conservative and surgical. Conservative is aimed primarily at SSCD patients with mild symptoms. Conservative therapy by avoiding increased intracranial pressure such as straining, blowing hard, air travel, lifting heavy weights, or bending over. These activities can worse the vertigo. Surgical therapy has had high success in reducing vestibular symptoms and autophonia. Hearing symptoms rarely improve after surgical therapy\(^{(12)}\).

Surgical therapy may involve window reinforcement, resurfacing, and plugging / occlusion techniques. The reinforcement window has a high recurrence rate in the long term and is less effective in reducing symptoms of autophonia. This technique is usually combined with resurfacing and occlusion techniques. Resurfacing technique is to give flap over the dehiscence of the canal with a special material to prevent the transmission of intracranial pressure into the inner ear. The materials used can be bone, cartilage, fascia or ceramic implants. This technique can be effective in reducing the symptoms of vertigo and autophonia. The occlusion technique is clogging the canal thereby eliminating the function of the semicircular canal\(^{(12)}\).

The operation can be performed by trans cranial medial fossa, transmastoid, and transcanal approaches. The cranial media fossa approach is a traditional technique with first craniotomy to find arcuate eminence and areas of dehiscence. The transmastoid approach is less invasive than craniotomy. This approach also allows for plugging and resurfacing through a mastoidectomy. The success rate for this approach is also quite high at 94%. The transcanal approach is the newest technique by strengthening (reinforcement) the foramen ovale or rotundum. This technique can reduce the risk of complications of facial nerve paralysis, cerebrospinal fluid leakage, and intracranial hemorrhage\(^{(13)}\).

**Conclusion**

A 65 year old woman diagnosed with SSCD showed symptoms of the Tullio phenomenon, namely vertigo when hearing loud sounds, especially low frequencies. On audiogram examination, bilateral sensorineural deafness and air-bone gap of the right ear were found at low frequency (250 Hz). Temporal CT scan showed dehiscence of the right superior semicircular canal. Romberg’s test results, Gans test and Fukuda’s step test gave positive results to the right. Therapy in patients is carried out conservatively.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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**Ethical Approval:** All procedures performed in studies involving human participants were in accordance declaration of helsinki the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

**References**


Estimation of Stature by Measuring Ring Finger Length in Young Adults

Arijit Datta¹, Manish Kumar Singhal², Rachna Agarawal³, Preeti Tiwari⁴
¹Associate Professor, Forensic Medicine, Manipal Tata Medical College, Jamshedpur; ²Associate Professor, Pathology, Government Medical College, Bharatpur; ³Assistant Professor, Anatomy, Government Medical College, Bharatpur; ⁴Assistant Professor, Community Medicine, Manipal Tata Medical College, Jamshedpur

Abstract

Background: The “anatomical” and “mathematical” methods are the two main types of adult stature estimation methods available. Till date, most of the studies on stature estimation have used the length of bones such as femur, humerus, radius, etc. Very limited work has been done to estimate stature from finger length, as there is very little literature available for the same.

Aim: Measurement of ring finger to estimate the stature in young adults

Material and Methodology: A correlation study was conducted with the approval of institutional Ethical Committee during the period of May 2017 to October 2014 among 500 adults. All the right handed males and females in the age group of 22 to 40 years were included in the study. Individuals having congenital defects on right upper limb, congenital defects on right lower limb, pregnant woman, previous history of fracture in upper limb or lower limb or any bony malformation due to rickets, osteomalacia was excluded from the study. Data analysis was done using SPSS 17.

Results: In the study 49.8% of the participants were males with mean age of 31.18 ± 5.54 and females with mean age of 32.14 ± 6.36. Mean stature among males was 162.51 ± 7.56 cm and in females it was 153.59 ± 5.21 cm. The mean value of ring finger length in males and females were 7.02 ± 0.42 cm and 6.40 ± 0.67 cm respectively.

Conclusion: Ring finger length showed higher and better correlation coefficient values among males as compare to females.

Key words- stature, height, ring finger, young adults

Introduction

Dwight proposed in 1894 that because human populations differ in physical features, it is only natural that their bones should differ as well. Dwight’s theories and discoveries influenced the foundation and development of a new field in psychology. Dwight’s ideas and discoveries influenced the foundation and development of a new field of forensic science known as forensic anthropology, earning him the title of “Father of Anthropology”. The study of ante mortem histories of people using skeletonized remains is referred to as forensic anthropology. In suspected cases of infanticide, murder, or suicide, it is thus useful in a medicolegal context. Disaster Victim Identification is the process of determining the identity of dismembered or otherwise unidentified human remains in the cases of mass disasters such as explosions, bomb blasts, mass killings, transportation crashes, and natural calamities (DVI). The methods used to positively identify deceased victims of a multiple casualty event are referred to as disaster victim identification. The practice of estimating stature began in the late twentieth century. The “anatomical” and “mathematical” methods are the two main types of adult stature estimation methods available. Dwight first
proposed the anatomical method in 1894, which entails articulating the entire skeleton on a measuring board, correcting for the curvature of the spine, and then adding a correction factor for soft tissue and skin to estimate stature\textsuperscript{5}. Folly\textsuperscript{6} reintroduced anatomical method with minor variations in 1956, which he called Fully’s procedure. The major disadvantage of the anatomical method of estimating stature is that it requires a nearly complete skeleton. Regression formulae (or ratios) based on the correlation of individual skeletal components to living stature\textsuperscript{7} are used in the mathematical method. In 1899, Karl Pearson\textsuperscript{8} succeeded in developing the first formal stature regression formulae for estimating stature from long bone measurements. The femur and tibia are the most precise long bones to use for this analysis\textsuperscript{9, 10}. Anthropologists may measure long bones found in human remains and apply their measurements to mathematical linear regression equations, which were first proposed by Allbrook in 1961.\textsuperscript{13} These equations were created specifically to estimate the stature of an unclaimed cadaveric body or a missing individual, where long bone data from a particular population can be used to calculate the estimated stature using a linear regression equation, which is then compared to the missing individual’s reported stature. Age, sex, and race are all factors in experiments that use a mathematical method to estimate stature\textsuperscript{11, 12, 13, 14}. Trotter contributed to the development and refinement of mathematical methods for calculating stature\textsuperscript{15}. They compared the data collected from American soldiers to cadaver lengths and came to the conclusion that 0.06 cm would be multiplied from calculated body length for subsequent years after the age of 30. The estimated stature (age 30) should be used as a correction formula\textsuperscript{16}. They had the opportunity to test and assess their previously established formulae after conducting additional research on soldiers following the Korean War. They could also assess differences in growth and proportion among American soldiers. These experiments were performed on the humerus, femur, tibia, and fibula, and the researchers determined that stature estimation formulae should be recalculated within appropriate time intervals, even for the same racial population. When a full dead body is discovered, determining the individual’s stature is relatively simple; however, when only parts of the body are available, determining the individual’s stature is difficult.\textsuperscript{17} Till date, most of the studies on stature estimation have used the length of bones such as femur, humerus, radius, etc. Very limited work has been done to estimate stature from finger length, as there is very little literature available for the same. Hence this study intends to fill this lacuna and looks into the possibility of estimation of stature by measuring the length of ring finger, to estimate the stature using the mathematical method among native adult population of a village in Dakshin Kannada district, Karnataka, India, which will be of great importance to anthropologists and forensic experts.

**Aim**

Measurement of ring finger to estimate the stature in young adults

**Methodology**

**Materials**

The material used to conducted this study includes stadiometer to measure height, digital sliding calliper to measure ring finger length

**Methods**

A correlation study was conducted in Harekela village with the approval of institutional Ethical Committee during the period of May 2017 to October 2017 among 500 adults constituting 250 males and 250 females falling in the age group of 22 years to 40 years.

To calculate the sample size, correlation coefficient value (r value) was selected which was least among all the previous studies. The correlation coefficient value found was 0.546\textsuperscript{26} at 5% of level of significance with power of study as 90%. Considering the r value as 0.546, sample size was found to be 152. To increase the impact of the study correlation coefficient value was anticipated as 0.3 at 5% of level of significance at 80% of power. The sample size was found to be 438 but for the feasibility to conduct the study round figure of 500 sample size was taken where 250 were males and 250 were females.
The sample size of the present study was calculated using the following formulae

\[ n = \frac{2(Z_{\alpha} + Z_{\beta})^2}{C^2} + 3 \]

Where

\[ Z_{\alpha} = 1.96 \text{ at 5\% level of significance} \]
\[ Z_{\beta} = 0.8416 \text{ at 80\% power} \]
\[ C = 0.5 \ln \left( \frac{1+r}{1-r} \right) \]
\[ r = 0.30 \]

Required sample size = 438

Sample size taken by investigator = 500

All the right-handed males and females in the age group of 22 to 40 years (ref) were included in the study; systematic random sampling method was adopted to select the participants for the study. Individuals having congenital defects on right upper limb, congenital defects on right lower limb, pregnant woman, previous history of fracture in upper limb or lower limb or any bony malformation due to rickets, osteomalacia was excluded from the study.

The procedure and purpose of the study was thoroughly informed and explained to the study population. Information sheet was given to the study group where detail information regarding the importance of study was mentioned and a written informed consent was taken from all study subjects. Measurements of female participants were taken in presence of female colleague.

**Technique of measuring Stature**

Stature was measured accurately using stadiometer where participants were made to stand in erect posture on the board of standard stadiometer platform by keeping the foot in close contact without any footwear and arms hanging by the side, the trunk braced along the vertical board and eyes looking straight ahead and face adjusted in Frankfurt plane. The measurement was taken as maximum distance from floor to vertex of the head by bringing the horizontal sliding bar to vertex 19.

**Technique of measuring ring finger length**

Ring finger length was also calculated using the digital sliding caliper. Participants were advised to extend their hand and to keep it on flat table then the distance between the midpoints of the proximal crease of ring finger to the tip of the ring finger was measured 20.

To avoid diurnal variation all the measurements were taken during the same time period 21.

**Results**

The study population consisted of 249 males (49.8\%) with mean age of 31.18 ± 5.54 and 251 females with mean age of 32.14 ± 6.36 (fig 1). Mean stature among 500 individual was found to be 158.03 ± 7.87 cm. Mean stature among males was found to be 162.51 ± 7.56 cm and in females it was found to be 153.59 ± 5.21 cm. The mean value of ring finger length in males and females were 7.02 ± 0.42 cm and 6.40 ± 0.67 cm respectively. Males had an average height of 162.51 ± 7.56 cm while females had average height of 153.59 ± 5.21 cm. There was statistically significant difference across the group (p<0.001) (table 1). Significant correlation was found across ring finger length with stature (table 2). Males showed better correlation than females across stature and ring finger length and it was found to be statistically significant (table 3). Table no 4 provide the R square value of the predictor for RFL in case of males and females along with whole population. It is also showing the ring finger length as a predictor to determine stature is better in males than females.

Formula derived by using regression analysis to estimate stature from RFL (table 5)-

In case of males, \( \text{Stature} = 88.91 + 10.481 \times \text{RFL} \)

In case of females, \( \text{Stature} = 142.723 + 1.697 \times \text{RFL} \)

In case of whole population, \( \text{Stature} = 114 + 6.554 \times \text{RFL} \)
Figure 1: Distribution of study participants

Table 1: Sex-wise distribution of all the study variables (cm) with mean and Standard deviation

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>MEAN(CM)</th>
<th>SD</th>
<th>LOWER BOUND</th>
<th>UPPER BOUND</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n=249)</td>
<td>7.02</td>
<td>0.42</td>
<td>6.97</td>
<td>7.08</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Female (n=251)</td>
<td>6.40</td>
<td>0.67</td>
<td>6.32</td>
<td>6.48</td>
<td></td>
</tr>
<tr>
<td>Total (n=500)</td>
<td>6.71</td>
<td>0.64</td>
<td>6.65</td>
<td>6.77</td>
<td></td>
</tr>
<tr>
<td>Stature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n=249)</td>
<td>162.51</td>
<td>7.56</td>
<td>161.57</td>
<td>163.46</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Female (n=251)</td>
<td>153.59</td>
<td>5.21</td>
<td>152.94</td>
<td>154.23</td>
<td></td>
</tr>
<tr>
<td>Total (n=500)</td>
<td>158.03</td>
<td>7.87</td>
<td>157.34</td>
<td>158.72</td>
<td></td>
</tr>
</tbody>
</table>

RFL=Ring finger length, SD= standard deviation

Table 2: Correlation of RFL & Stature

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>KARL PEARSON CORRELATION COEFFICIENT (R VALUE)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFL</td>
<td>Stature</td>
<td>0.534</td>
</tr>
</tbody>
</table>

RFL= Ring finger length
Table 3: Correlation stature with RFL according to gender

<table>
<thead>
<tr>
<th>SEX &amp; STATURE</th>
<th>PEARSON CORRELATION</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stature in males</td>
<td>0.582</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>RFL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stature in females</td>
<td>0.219</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>RFL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RFL= Ring finger length

Table 4: coefficient of determination for sex and ring finger length (RFL)

<table>
<thead>
<tr>
<th>SEX</th>
<th>R</th>
<th>R SQUARE</th>
<th>ADJUSTED R SQUARE</th>
<th>ANOVA p VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEMALE</td>
<td>0.219a</td>
<td>0.048</td>
<td>0.044</td>
<td>p&lt; 0.001</td>
</tr>
<tr>
<td>MALE</td>
<td>0.582a</td>
<td>0.338</td>
<td>0.335</td>
<td>p&lt; 0.001</td>
</tr>
<tr>
<td>TOTAL</td>
<td>0.534a</td>
<td>0.285</td>
<td>0.283</td>
<td>p&lt; 0.001</td>
</tr>
</tbody>
</table>

a= Predictors: (constant, RFL), RFL= Ring finger length

Table 5: Regression analysis to estimate stature from RFL

<table>
<thead>
<tr>
<th>SEX</th>
<th>UNSTANDARDIZED COEFFICIENTS</th>
<th>STANDARDIZED COEFFICIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>STD. ERROR</td>
</tr>
<tr>
<td></td>
<td>(CONSTANT)</td>
<td>RFL</td>
</tr>
<tr>
<td>FEMALES</td>
<td>(CONSTANT)</td>
<td>RFL</td>
</tr>
<tr>
<td></td>
<td>142.723</td>
<td>1.697</td>
</tr>
<tr>
<td></td>
<td>1.6792</td>
<td>0.479</td>
</tr>
<tr>
<td>MALES</td>
<td>(CONSTANT)</td>
<td>RFL</td>
</tr>
<tr>
<td></td>
<td>88.910</td>
<td>10.481</td>
</tr>
<tr>
<td></td>
<td>10.481</td>
<td>0.933</td>
</tr>
<tr>
<td>TOTAL</td>
<td>(CONSTANT)</td>
<td>RFL</td>
</tr>
<tr>
<td></td>
<td>114.050</td>
<td>6.554</td>
</tr>
<tr>
<td></td>
<td>6.554</td>
<td>0.466</td>
</tr>
</tbody>
</table>

Dependent variable: Stature

Discussion

Mean age of study population

Present study was conducted among 500 individuals among rural Mangalore population with mean age of 31± 5 years, out of those 249 were males and 251 were females with mean age of 31±5 years and 32±6 years respectively. In the study done by Bardale R.V et al.
the mean age of male population was 21.52 years and the mean age of female population was 20.08 years. While in the study conducted by Krishan K to estimate the stature from index and ring finger length subjects were in the age group of 14 to 18 years.

**Mean stature of study population**

We found that in our study mean stature among 500 individual was found to be 158.03 ± 7.87 cm. Statistically significant stature difference between males and females was found in our study, mean stature among males was found to be 162.51 ± 7.56 cm and in females it was found to be 153.59 ± 5.21 cm. But in the study conducted by shivakumar AH in South India and Chikhalkar BG in Mumbai mean stature was found quite high in their studies as compared to the present study which was 167 ± 0.10 cm and 167.26 ± 8.494 cm respectively. Study conducted by Bardale RV et al found that the mean height of men was 171.6 cm and mean height of women was 157.3 cm his study. The mean height for Oriya males was observed to be about 162 cm and 152 cm for females respectively Studies done on international platform too showed varying results like in the study done by Habib SR et al showed mean stature in Egyptian males to be 174.61 ± 7.34 cm and among Egyptian females to be 160 ± 5.45 cm. Ahmed AA found mean stature among Sudanese males to be 174.71 ± 5.72 cm and 160.43 ± 5.49 cm among Sudanese females.

**Discussion on ring finger length**

In the present study the mean ring finger length of the study participants was 6.71 ± 0.64 cm. Significant difference was observed between ring finger length of males and females which was found to be 7.02 ± 0.42 cm in males and 6.40 ± 0.67 cm in females. In a study conducted by Krishan K the mean ring finger length was 7.26 cm in males and 6.73 cm each in females respectively. Bardale R.V et al found that the ring finger showed significant difference (P < 0.001) between stature of male and female subjects. Amongst males, correlation was higher between the ring finger length and stature (r = 0.546). Raju GM et al found that in his study there was significant difference between stature and ring finger length (p< 0.001), the correlation coefficient value in males was 0.57 and in females 0.43. Krishan K et al showed that ring finger length measurements was significantly larger in males than females (P-value < 0.001), statistically significant correlation was observed between stature and ring finger lengths.

In present study, Table no 4 provide the R square value of the predictor for RFL in case of males and females along with whole population. It is also showing the ring finger length as a predictor to determine stature is better in males than females.

Formula derived by using regression analysis to estimate stature from RFL (table 8)-

In case of males, Stature= 88.91 + 10.481 x RFL

It shows that per unit increase in RFL there is 10.481 cm increase in stature of males

In case of females, Stature= 142.723 + 1.697 x RFL

It shows that per unit increase in RFL there is 1.697 cm increase in stature of females

In case of whole population, Stature= 114 + 6.554 x RFL

**Conclusion**

- Ring finger length showed higher and better correlation coefficient values among males as compare to females

**Conflict of Interest**- Nil

**Source of Funding**- Nil

**References**


11. Meadows L. Secular change and allometry in the long limb bones of Americans from the mid 1700 through the 1970s. University of Tennessee, Knoxville;1996.


Validation of Sedative Drug Analysis Results

Aripova Nigora Baxadirxodjaevna1, Komilov Xojiasror Ma’sudovich2, Ikramova Mashhura Shuxratovna3, Mukhidinova Maxfuza Komolovna4

1PhD Pharmaceutical Sciences, (Acting as) An Associate Professor, Tashkent Pharmaceutical Institute, Tashkent 2Professor, Doctor of Pharmaceutical Sciences, Tashkent Pharmaceutical Institute, 3PhD Pharmaceutical Sciences, (Acting as) An Associate Professor, Tashkent Pharmaceutical Institute, 4Assistant, Tashkent Pharmaceutical Institute, Tashkent, Oybek

Abstract

Annotation. The article reveals validation studies of the method of quantitative analysis of high-performance liquid chromatography of a complex and multicomponent original sedative drug produced in the Republic of Uzbekistan. Criteria for repeatability, accuracy, precision, intermediate accuracy and correlation coefficients for drug validation were determined. The analytical method for determining the number of tablets covered with a film “Sedarem” from the proportion of valeric acids in the main active ingredients of the drug was determined on the basis of the 5th series of the “Accuracy” indicator. The accuracy of valeric acid is judged from at least 5 data obtained for each of the 3 levels lying within the analytical domain of the quantification method. Comparing the results of determining the amount of valeric acid, it turns out that there is no statistically significant difference between them, that the three time points for each series are mutually compatible.

The analysis procedure for determining the amount of phenolic compounds in the preparation, when comparing “Precision” with the results of determining the amount of phenolic compounds at different intervals and on different days, which characterizes repetition and “intermediate precision,” it was found that there is no statistical reliable difference between them, and three time points for each series are mutually compatible. Study of drug linearity from flavanoid fraction was carried out in the range from 80 to 120% of nominal value of analytical parameters.

Keywords: “Sedarem”, tablet, high performance liquid chromatography, repeatability, accuracy, precision, intermediate precision and correlation coefficient.

Introduction

Due to the high efficacy of sedatives and the absence of side effects, these drugs are widely used in everyday medical practice, especially in the treatment of elderly patients. According to the classification, drugs based on medicinal plant raw materials are widely used among sedatives. Progress, life growth at a rapid pace further increases the need for sedatives1,3.

An effective technology for preparing dosage forms with optimal technological indicators of the proposed original sedative has been developed, standardization methods, quality and validation standards have been established.

The validation criteria of the Sedarem sedative tablet quantitative analysis method, coated with cladding, were determined by the following indicators: accuracy, repeatability, linearity, precision, intermediate accuracy and correlation coefficient3.

Aim of the Research: Study the possibility of determining the validation criteria for Sedarem with a coating.

Materials and methods: Quantitative analysis of the sum of the main active ingredients in the coated
tablet, i.e., carboxylic acids with respect to valeric acid, flavonoids with respect to routine, as well as phenolic compounds with respect to rosmarinic acid, was carried out on an Agilent Technologies 1200 liquid chromatograph with a UV detector.

A suitable chromatographic column S-18 is Zorbax Eclipse with a particle size of 5 μm, filled with a sorbent of 4x100 m.

The validity criteria of the method of quantitative analysis of sedative tablets “Sedarem” coated with the shell were determined on the following parameters: accuracy, repeatability, linearity, precision, intermediate precision and correlation coefficient. To study the possibility of determining the criteria for validation of the drug “Sedarem” coated with the shell. Quantitative analysis of the sum of carboxylic acids relative to valerian acid, flavonoids relative to routine, and phenolic compounds relative to rosemary acid was performed on Agilent Technologies 1200 liquid chromatograph, UV detector. A suitable chromatographic column S-18 is a Zorbax Eclipse filled with 4x100 mm sorbent with a particle size of 5 mm.

The results were processed in the method of variance statistics, according to the Student’s criterion, at p = 0.05. The tables show the arithmetic mean (M), the corresponding standard error of error (m), the Student Criterion (t), the number of choices (n), and the confidence limit (lower confidence limit ÷ high confidence limit) 4.

Results and Discussion

Validation of the method of quantitative determination of the medicinal form of the tablet coated with a shell, “Sedarem” on valerian acids “Correctness”. It is known that the accuracy of the method is characterized by the deviation of the average result of its definitions from the values obtained as correct 1. The tested method is considered valid if the values obtained as true are at reliable intervals corresponding to the average results of the analyses obtained as a test for this method. After determining the quantity of the preparation by the fraction of valerian acids in different series, the following results were obtained, and the results are given in Table 1. If we compare the results of determining the quantity of valerian acids in five different series of the preparation under test, there seems to be no statistically significant difference between them, the results of exactly five series are mutually compatible. The analytical method for determining the amount of Sedarem tablets coated with valerian acid corresponds to the requirements of validation on the indicator “Accuracy”.

The accuracy of the method is explained by the dispersion of the results obtained using it relative to the average result value. The criterion of such dispersion is the value of standard deviation of the result of a specific unit obtained to select a sufficiently large volume 2,3. For any method of quantification of accuracy, the quantification method is estimated from the results of at least 5 data obtained for each of the 3 levels (low, medium and high) lying in the analytical domain of the method.

Repetition (similarity). Repeatability of the analytical method has been evaluated based on independent results obtained in a short time in the same laboratory under the same conditions. Intermediate precision in the laboratory. Intermediate accuracy in the laboratory of the approved method was evaluated in one laboratory 5. Five sets of Sedarem-coated tablets were used in the analysis. After analyzing the tablets on the indicator “Precision”, which characterizes the similarity of the repeatability of the results of quantitative determination of the drug, the following results are given in Table 1.

If we compare the results of testing valeric acids of five different series of drugs, it becomes clear that there are no statistically reliable differences between them, exactly five series are mutually compatible 6.

After checking for intermediate precision, the following results were obtained and are presented in Table 1.
Table-1.: The results on the accuracy of the quantitative determination precision analysis repeatability and intermediate precision of the results of quantitative determination of the drug on the presence of valeric acids (M±tm; p=0,05; n=5)

<table>
<thead>
<tr>
<th>Shell-coated tablets “Sedarem”</th>
<th>Repeatability</th>
<th>Accuracy of the quantitative determination</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Results of experiments on the first day</td>
<td>Results of experiments on the second day</td>
<td>Results of experiments on the third day</td>
</tr>
<tr>
<td></td>
<td>4.2651 (4.1021±4.4281)</td>
<td>4.4265 (4.2045±4.6485)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.4764 (4.2456±4.7072)</td>
<td>4.4621 (4.2501±4.6741)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.3355 (4.2347±4.4363)</td>
<td>4.4123 (4.2845±4.5401)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.4659 (4.2759±4.6559)</td>
<td>4.3489 (4.1611±4.5367)</td>
<td></td>
</tr>
</tbody>
</table>

If the results of determining the amount of ivaerian acids in different time intervals are compared, it appears that there is no statistically significant difference between them, that the three time points for each series are mutually compatible. The method of analysis for quantitative determination of valeric acid content of cedarem shell-coated tablet meets the validation requirements on the indicators of “Precision”, which characterizes the repeatability (similarity) and “Intermediate precision”. The linearity of the method is that the method of the analytical signal depends on the concentration or amount of the analyte detected in the sample under analysis, within the scope of the analysis. In method validation, its linearity in the field of analysis is verified by testing, by analytical measurements for at least five samples with complete amounts or concentrations of the analyte.

To determine the linearity of the range in the analytical field (80–120%), we calculated a correlation coefficient (R), which should be R ≥ 0,99.

Research results. Due to the requirements for the size of the analytical area of the methodology in the range of 80 to 120% of the nominal value of the analytical indicator to be determined, the following results obtained are given in Table 2.
Table 2. Results of the study of the preparation linearity by the fraction of valerian acid, (in the analysis the area is 80-120% (n = 5)

<table>
<thead>
<tr>
<th>№</th>
<th>Amount of sample taken, %</th>
<th>Series: Experimental 1</th>
<th>Series: Experimental 1</th>
<th>Series: Experimental 1</th>
<th>Series: Experimental 1</th>
<th>Series: Experimental 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>3,4556</td>
<td>3,3965</td>
<td>3,4894</td>
<td>3,4854</td>
<td>3,5622</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>3,9864</td>
<td>3,9864</td>
<td>4,0310</td>
<td>4,0054</td>
<td>3,9864</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>4,5612</td>
<td>4,3985</td>
<td>4,4895</td>
<td>4,6120</td>
<td>4,4561</td>
</tr>
<tr>
<td>4</td>
<td>110</td>
<td>5,1350</td>
<td>5,1312</td>
<td>5,0011</td>
<td>4,9984</td>
<td>5,0010</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>5,7845</td>
<td>5,6923</td>
<td>5,4759</td>
<td>5,3785</td>
<td>5,3986</td>
</tr>
</tbody>
</table>

Linear Connection Free Member (a)  
-1.22186  
-1.21542  
-0.44572  
-0.28326  
-0.20654

Linear connection angle coefficient (b)  
0.058064  
0.057364  
0.049431  
0.047792  
0.046874

Correlation coefficient (R)  
0.99936  
0.99713  
0.99971  
0.99509  
0.99894

Regression equation  
\( Y = aX + b \)  
\( Y = 1.22186X + 0.058064 \)  
\( Y = 1.21542X + 0.057364 \)  
\( Y = -0.44572X + 0.049431 \)  
\( Y = -0.28326X + 0.047792 \)  
\( Y = -0.20654X + 0.046874 \)

In accordance with the requirements of the 12 edition of GPh, in the joint venture “Remedy group” shell-coated tablets “Sedarem” meet the linearity of the drug in five series, in the field of analysis (from 80 to 120%).

The methods of analysis for determining the amount of valerian acid in a cedar-coated tablet met the validation requirements for the “Analysis Area” and “Linearity” indicators.

The validation of the method for determining the amount of phenolic compounds in shell-coated tablets “Sedarem”. After determination of the quantity of the pharmaceutical form by the fraction of phenolic compounds in different series, the following data were obtained. The obtained results are presented in Table 3.

If the results of determining the amount of phenolic compounds in five different series of the tested drug are compared, there is no statistically significant difference between them, the results of exactly five series are mutually compatible. The method of analysis for the determination of the amount of phenolic compounds in the drug “Sedarem” coated tablets meets the requirements...
of validation on the indicator “Accuracy”. Following the determination of the drug on the indicator “Precision”, which characterizes the repeatability of the results of the determination of the amount of the drug, the following data were obtained (Table 3).

If we compare the study of five different series by the

Table 3: The results on the accuracy of the quantitative determination precision analysis repeatability and intermediate precision of the results of quantitative determination of the drug on the presence of of phenolic compounds (M+tm; p=0,05; n=5)

<table>
<thead>
<tr>
<th>Shell-coated tablets “Sedarem”</th>
<th>Repeatability</th>
<th>Accuracy of the quantitative determination</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Results of experiments on the first day</td>
<td>Results of experiments on the second day</td>
</tr>
<tr>
<td>Series: Experimental 1</td>
<td>24,2311 (22,3146÷26,1476)</td>
<td>23,8954 (21,5612÷26,2296)</td>
<td>23,5896 (21,5613÷25,6179)</td>
</tr>
<tr>
<td>Series: Experimental 2</td>
<td>24,6531 (21,8956÷27,4106)</td>
<td>24,8964 (22,3456÷27,4472)</td>
<td>24,0121 (22,8952÷25,1290)</td>
</tr>
<tr>
<td>Series: Experimental 3</td>
<td>22,8964 (21,5654÷24,2274)</td>
<td>25,0012 (22,8031÷27,1993)</td>
<td>22,8961 (21,3564÷24,4358)</td>
</tr>
<tr>
<td>Series: Experimental 4</td>
<td>23,4796 (22,3131÷24,6461)</td>
<td>24,3561 (21,9864÷26,7258)</td>
<td>23,4656 (22,0121÷24,9191)</td>
</tr>
<tr>
<td>Series: Experimental 5</td>
<td>23,8564 (21,8896÷25,8232)</td>
<td>24,6551 (22,3123÷26,9979)</td>
<td>22,4512 (21,2898÷23,6126)</td>
</tr>
</tbody>
</table>

If we compare the results of determining the number of phenolic compounds at different time intervals and on different days, it turns out that there is no statistically significant difference between them, that three time points for each series are mutually compatible.

The method of analysis for determining the number of phenolic compounds in the form of shell-coated tablets “Sedarem” meets the requirements of the validation for “Accuracy”, which characterizes the reproducibility and “Intermediate Accuracy”.

In connection with requirements to the size of the analysis methodology area in the range from 80 to 120% of the nominal value of the identified analytical indicators, the following results were obtained (Table 4).
Table-4: Results of the study of linearity of the preparation in terms of the share of phenolic compounds, when the analysis area is 80-120% (n=5)

<table>
<thead>
<tr>
<th>№</th>
<th>Amount of sample taken, %</th>
<th>Series: Experimental 1</th>
<th>Series: Experimental 2</th>
<th>Series: Experimental 3</th>
<th>Series: Experimental 4</th>
<th>Series: Experimental 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>17.5641</td>
<td>16.5651</td>
<td>15.8955</td>
<td>16.0011</td>
<td>15.3256</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>20.2313</td>
<td>20.201</td>
<td>19.5621</td>
<td>20.1321</td>
<td>18.9655</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>24.3563</td>
<td>24.0203</td>
<td>23.4565</td>
<td>23.9986</td>
<td>22.3565</td>
</tr>
<tr>
<td>4</td>
<td>110</td>
<td>30.1232</td>
<td>29.8564</td>
<td>28.9564</td>
<td>28.9999</td>
<td>27.1635</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>34.2561</td>
<td>34.9856</td>
<td>33.8999</td>
<td>33.2561</td>
<td>32.5644</td>
</tr>
</tbody>
</table>

Linear Connection Free Member (a)

Linear connection angle coefficient (b)
0.432759 0.464964 0.454031 0.433778 0.426756

Correlation coefficient (R)
0.99308 0.99503 0.99644 0.99917 0.99482

Regression equation Y=aX+b
Y=-17.9697 X+0.432759 Y=-21.37072 X+0.464964 Y=-21.04902 X+0.454031 Y=-18.90024 X+0.433778 Y=-19.4005 X+0.426756

In accordance with the requirements of the 12 edition of GPh, in the joint venture “Remedy group” shell-coated tablets “Sedarem” meet the linearity of the drug in five series, in the field of analysis (from 80 to 120%). The method of analysis for determining the amount of phenolic compounds in shell-coated tablets “Sedarem” meets the requirements of validation on the indicators “Area of analysis” and “Linearity”.

Validation of the method of quantitative determination of cedarem shell-coated tablets by flavonoids

After determining the amount of flavonoids in different series of the drug, the following data were obtained (Table 5).

If the results of determining the amount of flavonoids in five different series of the tested drug are compared, there is no statistically significant difference between them, the results of exactly five series are consistent. The analytical method for determining the amount of flavonoids in the shell-coated tablets “Sedarem” met the validation requirements on the indicator “Accuracy”.

After checking the dosage form on the indicator “Precision”, which characterizes the repeatability of
the results of quantitative determination of the drug, the following results were obtained (Table 5).

Determining the proportion of flavonoids of the drug in five different series, there is no statistically significant difference between them, the results of exactly five series are mutually compatible.

The following data were obtained after the intermediate precision test analyzes (Table 5)

Table 5: The results on the accuracy of the quantitative determination precision analysis repeatability and intermediate precision of the results of quantitative determination of the drug on the presence of flavonoids (M+tm; p=0,05; n=5)

<table>
<thead>
<tr>
<th>Shell-coated tablets “Sedarem”</th>
<th>Repeatability</th>
<th>Accuracy of the quantitative determination</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Results of experiments on the first day</td>
<td>Results of experiments on the second day</td>
<td>Results of experiments on the third day</td>
</tr>
<tr>
<td>Series: Experimental 1</td>
<td>4,3231 (4,1223÷4,5239)</td>
<td>4,6356(4,3211÷4,9501)</td>
<td>4,4321 (4,2131÷4,6511)</td>
</tr>
<tr>
<td>Series: Experimental 2</td>
<td>4,4755 (4,2312÷4,7198)</td>
<td>4,4564(4,2351÷4,6777)</td>
<td>4,5312 (4,3101÷4,7523)</td>
</tr>
<tr>
<td>Series: Experimental 3</td>
<td>4,2894 (4,1356÷4,4432)</td>
<td>4,3261(4,1223÷4,5299)</td>
<td>4,6531 (4,2864÷5,0198)</td>
</tr>
<tr>
<td>Series: Experimental 4</td>
<td>4,4751 (4,1884÷4,7618)</td>
<td>4,5621(4,2311÷4,8931)</td>
<td>4,3125 (4,1754÷4,4496)</td>
</tr>
<tr>
<td>Series: Experimental 5</td>
<td>4,3241 (4,0894÷4,5588)</td>
<td>4,2313(4,0256÷4,4370)</td>
<td>4,4642 (4,2475÷4,6809)</td>
</tr>
</tbody>
</table>

If we compare the results of determining the number of phenolic compounds at different time intervals and on different days, it turns out that there is no statistically significant difference between them, that three time points for each series are mutually compatible.

The method of analysis for determining the number of phenolic compounds in the form of shell-coated tablets “Sedarem” meets the requirements of the validation for “Accuracy”, which characterizes the reproducibility and “Intermediate Accuracy”.

In connection with requirements to the size of the analysis methodology area in the range from 80 to 120% of the nominal value of the identified analytical indicators, the following results were obtained (Table 6).
Table-6: The results of the study of the linearity of the drug on the proportion of flavanoids, when the analytical area is 80-120% (n=5)

<table>
<thead>
<tr>
<th>№</th>
<th>Amount of sample taken, %</th>
<th>Series: Experimental 1</th>
<th>Series: Experimental 2</th>
<th>Series: Experimental 3</th>
<th>Series: Experimental 4</th>
<th>Series: Experimental 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>5.6894</td>
<td>5.5612</td>
<td>5.2351</td>
<td>5.3250</td>
<td>5.6122</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>6.1021</td>
<td>6.0221</td>
<td>6.0212</td>
<td>6.1223</td>
<td>6.0212</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>6.8454</td>
<td>6.5212</td>
<td>6.6231</td>
<td>6.5986</td>
<td>6.7545</td>
</tr>
<tr>
<td>4</td>
<td>110</td>
<td>7.1221</td>
<td>7.0021</td>
<td>7.1212</td>
<td>6.9851</td>
<td>7.0121</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>7.5689</td>
<td>7.4212</td>
<td>7.5655</td>
<td>7.4651</td>
<td>7.5622</td>
</tr>
</tbody>
</table>

Linear Connection Free Member (a)

<table>
<thead>
<tr>
<th>Linear connection angle coefficient (b)</th>
<th>0.047790</th>
<th>0.047000</th>
<th>0.057608</th>
<th>0.047290</th>
<th>0.048909</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient (R)</td>
<td>0.99066</td>
<td>0.99961</td>
<td>0.99314</td>
<td>0.99743</td>
<td>0.99217</td>
</tr>
</tbody>
</table>

Regression equation

| Y=1,88658X+0.04779 | Y=1.80556X+0.04700 | Y=0.75242X+0.057608 | Y=1.81162X+0.047290 | Y=1,70154X+0.048909 |

In accordance with the requirements of the 12 edition of GPh, in the joint venture “Remedy group” shell-coated tablets “Sedarem” meet the linearity of the drug in five series, in the field of analysis (from 80 to 120%). The method of analysis for determining the amount of phenolic compounds in shell-coated tablets “Sedarem” meets the requirements of validation on the indicators “Area of analysis” and “Linearity”. Validation of analytical methods for determining the amount of “Sedarem” film-coated tablets was carried out. Sedarem shell-coated tablets meet the requirements of the 12 edition of GPh of the indicator “Validation of analytical methods” for quantitative and quantitative methods of valeric acid, phenolic compounds and flavonoids.

Conclusion: Validation analysis of sedatives revealed accuracy, repeatability, linearity, precision, intermediate accuracy, and correlation coefficient.

Acknowledgements: We are grateful to the staff of the Med Standart Scientific Center for the cooperation and opportunities that they have created to conduct our research there.

Ethical Approval: The manuscript was checked by the staff of the ethical approval committee under the Ministry of health of the Republic of Uzbekistan.

Conflict of Interest: The author declare that they have no competing interests.

Source of funding: No funding sources to declare.

References
Original Article

Study to Assess the Prevalence of Mobile Phone Addiction Among Adolescents with Selected Demographic Variables

Arti1, Rahul Gusian2, Anamika Gautam2
1Assistant Professor, 2Nursing Tutor, Department of Mental Health Nursing, Shree Guru Gobind Singh Tricentenary University, Gurugram, Haryana, India

Abstract

Introduction: Adolescence is a time of moving from immaturity of childhood to maturity of adulthood. Technologies especially the mobile phone has considerably the new epidemic in century. It has become an essential part of the modern community and necessities in the people’s lifestyle over the past decade and gained unparallel fame because it facilitates communication between individual and shortening distance, a good investment of time.

Method: It was a Descriptivestudy conducted amongst 100 consented students of age 13-18yr of Gurugram schools. Data was collected through a self – structured questionnaire to assess the prevalence of mobile phone addiction among adolescents.

Result: Prevalence of smartphone addictions shows majority 65% adolescent are at high risk to develop smartphone addiction, 26% are at mild level of addiction and remaining 9% are at severe level of addiction. The chi square association of prevalence of mobile phone addiction among adolescent with selected demographic variables (0.003) was found to be statistically significant with standard only, whereas showed know no statistically significant association with other demographic variables

Conclusion: Study concludes that Prevalence of smartphone addictions shows majority 65% adolescent are at high risk to develop smartphone addiction, 26% are at mild level of addiction and remaining 9% are at severe level of addiction.

Keywords: - Adolescence, smartphone, addiction

Introduction

Adolescence is uniquely different from all other stages of human development, especially from physiological and cognitive perspectives, and it can be argued that it is the most challenging of all developmental periods. Addiction is considered by WHO (WHO Expert Committee – 1964) as dependence, as the continuous use of something for the sake of relief, comfort or stimulation which often causes cravings when it is absent.1

Technologies especially the mobile phone has considerably the new epidemic in century. It has become an essential part of the modern community and necessities in the people’s lifestyle over the past decade and gained unparallel fame because it facilitates communication between individual and shortening distance, a good investment of time.2 The mobile phone is a two-edge sword; it has both positive and negative

Corresponding Author:
Anamika Gautam
Nursing Tutor, Department of Mental health nursing, Shree Guru Gobind Singh Tricentenary University, Gurugram, Haryana, 122006
Mobile- +917011228434
gautamanamika0494@gmail.com
aspect. Excessive mobile phone use tends to lead to the certain of a non-chemical addiction, most commonly known as “mobile phone addiction”.

Smartphone offers several conveniences in our life, but we also need to be aware of the negative effects of smartphone use, the most concerning aspect being smartphone addiction. Smartphone addiction is a phenomenon that pertains to uncontrollability of smartphone use. People with this problem encounter social, psychological and health problems.

Students may disturb others through ring tones and they also misuse cell camera. They can use their cell phones to write and send text messages, take and send digital photos, and even take and send short digital video clips, in addition to making phone calls. Nearly all the user can become inappropriate and undesirable in middle and high schools classrooms.

A conducted a cross sectional observational study on 382 students to know the magnitude of Smartphone addiction among students of the medical university at Yenepoya university campus, Mangalore, Karnataka state, south India. Study concludes that 46% of students using Smartphone 4-6 hours per day and main use of Smartphone were for social networking. Based on cut off values, 36.8% of students were addicted to Smartphone.

A conducted a descriptive study to assess the risk of developing nomophobia among adolescents in selected PU colleges at Bangalore. The study revealed that there is a statistically significant association between the risk level and selected socio demographic variables. There was a moderately positive correlation between pattern of mobile phone usage and risk of nomophobes ($r = +0.83$) which was significantly associated at 0.05 levels.

A conducted a study to assess the severity score of mobile phone use and internet use among 114 Bsc. Nursing students at Manipal college of Nursing, Manipal. Result revealed that most of the subjects 50 (43.9%) belongs to 19-20 years of age. 1.8% subjects reported of having mild addiction to mobile phone use. 2.6% subjects had moderate and 30.7% had mild internet addiction.

**Methodology**

The study was done with quantitative approach and non-experimental design was adopted. The setting of the study was in Gurugram, Haryana. The sample was 100 students and convenient sampling was used for study. Self-structured questionnaire was prepared to assess the prevalence of mobile phone addiction among adolescents. The researcher had conducted a pilot study on minimum 10% of the sample and that sample was excluded from the main study. Approval from the research and ethical committee of SGT University, faculty of nursing was taken to conduct the research to assess prevalence of mobile phone addiction among adolescents. Permission for data collection was taken from Principles of various schools of Gurugram. Written consent was taken from students before asking questions and confidentiality of information was maintained.

**Statistical Analysis**

The collection of data were appropriate descriptive statistics using frequency, percentage, mean, mode, median and SD of different variables. The Chi-square test were used for the analysis of data. Value of $P< 0.05$ was considered statistically significant. The IBM statistical Package for the Social Sciences (SPSS) Version 16 was applied to analyse the data.

**Results**

The findings are summarized as follows: Majority (64%) of the samples belong to age group is 15-16. 53% of the total samples were studying in 10th standard. 59% of the total samples were male. Mostly i.e. 89% samples were living in urban area and belong to nuclear family (72%). Majority of parents of samples were graduated.

Prevalence of smartphone addictions shows majority 65% adolescent are at high to develop smartphone addiction, 26% are at mild level of addiction and remaining 9% are at severe level of addiction.
Chi square association of prevalence of mobile phone addiction among adolescent with selected demographic variables (0.028) was found to be statistically significant with standard only, whereas showed know no statistically significant association with other demographic variables.

**Discussion**

The Study concluded that the majority (64%) of the samples belongs to age group is 15 -16. 53% of the total samples were studying in 10th standard. 59% of the total samples were male. Prevalence of smartphone addictions shows majority 65% adolescent are at high to develop smartphone addiction, 26% are at mild level of addiction and remaining 9% are at severe level of addiction

The findings of the study were supported by the study done by Prekshaa Jai 1, Sachin Ratan Gedam2Pradeep S Patil3 (2019). Objectives of the study were to estimate the prevalence of smartphone addiction, to understand its pattern of use, and to determine association of smartphone addiction with pattern of use and personality dimensions among medical students. Study revealed that the prevalence of smartphone addiction was found to be 24.65% with high risk of addiction being 7.53% and 17.12% among males and females respectively.

The smartphone addiction was associated with smartphone use duration on a typical day, frequency of use, and most personally relevant smartphone function (p<0.05). The personality dimensions, such as assertive-submissive, depressive-non depressive, and emotional instability-emotional stability also had significant association with smartphone addiction (p<0.05); and these dimensions were also found to be predictors along with duration of use on multivariate analysis.7

In other study done by Ashwini dongre et al (2017) a cross sectional exploratory study to assess the prevalence of nomophobia among mobile phone users; to study the cell phone dependence pattern among adults; and to study the health effects of mobile phone usage. Study conclude that a higher proportion of males (82.91%) were dependent on mobile phone compared to females (31.25%). The most common 13 selfperceived symptom due to increase mobile phone usage was lack of sleep (70.61%) followed by eyestrain (42.46%).8
The study done by Arpita kumari et al (2013) to assess the severity score of mobile phone use and internet use among 114 Bsc. Nursing students at Manipal college of Nursing, Manipal. Result revealed that most of the subjects 50 (43.9%) belongs to 19-20 years of age. 1.8% subjects reported of having mild addiction to mobile phone use. 2.6% subjects had moderate and 30.7% had mild internet addiction. 

The present study of concluded that the chi square association of prevalence of mobile phone addiction among adolescent with selected demographic variables (0.028) was found to be statistically significant with standard only, whereas showed know no statistically significant association with other demographic variables.

The findings supported by the study done by Seonq-soo cha, Bo-kyunseoa(2018) on 1824 middle SCHOOLS student in South Korea to examine smartphone use patterns, smartphone addiction characteristics, and the predictive factors of the smartphone addiction. Result revealed that among 1824 participants, 563 (30.9%) were identified as a risk group for smartphone addiction and 1261 (69.1%) were classified as a normal user group according to their scores on the Smartphone Addiction Proneness Scale.

Our study concluded that majority of the adolescent students were prone to develop smartphone addiction. The study also concluded that there is association between smartphone addiction and standard class. It can be concluded that 9th and 10th standard students are prone to develop smartphone addiction.

There is a considerably debate on addiction and abuse to smartphone among adolescents and its consequent input on their health not only in a global context, but also specifically in the Indian population. The adolescents between 14-18 teens 96% of them have at least one mobile phone and 22% of them own multiple mobile phones.

Over usage of mobile phone leads to physiological and psychological health hazards. Apart from the various benefits of cell phones, it’s over usage leads to mobile phone addiction. It is the biggest non drug addiction in the world. The world health organization confirmed that mobile phone use may represent a long term health risk, classifying mobile phone radiation as a “carcinogenic hazard” and “possibly carcinogenic to human”.

**Conclusion**

This chapter present conclusion drawn based on the present study. The study attempts to assess the prevalence of mobile phone addiction among adolescents in selected schools of Gurugram. Study concludes that Prevalence of smartphone addictions shows majority 65% adolescent are at high risk to develop smartphone addiction, 26% are at mild level of addiction and remaining 9% are at severe level of addiction.

**Future implications**

In service and continuing education programs should be organized for schools as well as college students regarding mobile phone addiction. This aids to prepare the school going students to prevent themselves from the addiction. Schools teachers need to lay emphasis on the mobile phone addiction.

**Limitation**

The study was limited to only one setting. The time duration for the study was limited.Only prevalence was assessed no attempts was made to identify other attributes.

**Ethical Statement**

Approval from the research and ethical committee of SGT University, faculty of nursing was taken to conduct the research to assess prevalence of mobile phone addiction among adolescents. Permission for data collection was taken from Principles of various schools of Gurugram

**Declaration of participants consent**

Written consent was taken from students before asking questions and confidentiality of information was maintained.
Financial support and sponsorship: Nil

Conflicts of Interest: There are no conflicts of interest

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3. HMF. Safna. Negative Impact of Selfies on Youth. International Journal of Computer Science and Information Technology Research. September 2017; 5, (3)68-73.ISSN2348-120X (online) ISSN 2348-1196 (print).


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Comparison of Gender Prediction Accuracy between Regression Models Derived from Hand, Foot Measurements and Long Bone Measurements in a Sample of Kolhapur Population

Arun S. Karmalkar¹, Vasudha R. Nikam²

¹Associate Professor ²Professor and Head, Department of Anatomy, D.Y. Patil Medical College, D. Y. Patil Education Society (Deemed University), Kolhapur, Maharashtra, India

Abstract

Introduction: Here, we aim to compare the accuracy of the regression formula derived to predict gender using data on measurements of foot and hand with the formula derived to predict gender using the length measurements data of the long bones (tibia and ulna).

Methods: Patients attending the outpatient services at the Orthopedic Department, and between the age range 18 to 50 years were recruited (n=1000; 500 males and 500 females). Subjects suffering from any kind of bone deformity were excluded. Vallois method was used to estimate the measurements of hand, foot, tibia, and ulna. Regression formulas were obtained from the hand, foot-long bones measurements; to predict gender, using multiple logistic regression.

Results: Differences between male’s and female’s measurements of hand (P<0.001), long bones (P<0.001), and foot (P<0.001) were significant. The accuracy of the model used to predict gender, which was calculated from the dimensions of hand and foot was 81.5%. The accuracy of the model used to predict gender calculated from the long bone measurement was 78.3%.

Conclusions: Dimensions of hand and foot are a better predictor (81.5%) of gender vs the length of long bones (tibia and ulna) (78.3%) in the Kolhapur population.

Keywords: Tibia, Ulna, Foot, Hand, Foot deformities.

Introduction

Measurements of length of bones, hand, and foot vary across both sexes as is established in numerous studies. Consequently, these measurements have been successfully used for the gender estimation of a population.¹,² Sex determination from bone measurements are ofmedico-legal and forensic importance. Experts from forensic fields are called to decipher age, sex, and stature of whole or fragmented skeletal material from many different populations.³ Gender estimation has been done using the measurements of various bodyparts such astrunk, vertebral column, limbs, long and short bones, hand, foot, frontal bone, etc.⁴,⁵ However, pelvis remains the commonly used bone for gender identification, due to its accuracy.⁶,⁷ Other important bones where sexual dimorphisms are distinct and are used for gender assessment are from the postcranial and craniofacial region.⁸,⁹
The advantage of hand and foot measurements for the estimation of gender is that these are easily accessible and would be the most convenient and feasible option for the estimations. Currently, the data on estimation of gender in the population of south-western region of Maharashtra is scarce. The objective of the study was to compare the accuracy of gender prediction model derived from the measurements of hand and foot with the accuracy of a model derived from the measurements of long bones.

Material and Method:
This study was designed to be a prospective, observational study. The participants were recruited from the outpatient service of the Orthopedic Department at a Tertiary care center in Maharashtra. Sample size calculation was done on the R software (version 1.2.5001) using the formula pwr.t.test (d=0.098, power=0.85, sig.level=0.05, type= “one.sample”) where, d = effect size, power = 85%, significance level= 5%. The sample size was calculated to be 937 and therefore a total of 1000 subjects (500 males and 500 females), between the age range 18 to 50 years were recruited. The study was undertaken after the Institutional Ethics Committee approval, and after obtaining a written informed consent from the study participants. Subjects with any kind of bone deformity, injury, fracture of either foot or hand were not included. Standard anthropometric apparatus, a digital Vernier calipers (Mitutoyo, Japan), were used for the measurements. To avoid diurnal variations measurements were performed at approximately same time of the day (3:00-6:00 PM). Height of the cases were recorded as the vertical distance between vertex and floor, using a stadiometer. Foot length measurements were recorded as the distance from the tip of the hallux to the most prominent point of the back of the heel. Foot breadth measurements were recorded as the distance from the most anterior point of the skin on the middle finger to the midpoint of the distal crease of the wrist joint. Hand breadth measurements were recorded as the distance from the most medially placed point located on the head of the 5th metacarpal to the head of the 2nd metacarpal. Length of tibia was recorded as the distance from the farthest upper point of the medial part of condyle to the lowest point on the medial malleolus. Similarly, ulnar length was measured from the olecranon to the styloid process with elbow being fully flexed and palm spread over the opposite shoulder. Markings were made using a skin marking pencil. Continuous data were analysed using the Mann Whitney U-test test. Multiple logistic regression analysis was performed to derive the equation for gender prediction using R software v 1.2.5001. A P-value of <0.05 was considered as statistically significant.

Results
The Mean±SD age of male volunteers was 32.9±8.9 and the median was 32 years. The Mean±SD age of female volunteers was 33.8±9.4 and the median was 33 years. The average heights (Mean±SD) of the males and females were 166.6±5.6 cms and 157±6.4 cms, respectively. A significant difference (P<0.05) between the heights of participants of different sexes was detected. All the measurements were significantly higher in males when compared with females (P<0.001) (Table 1, 2, 3).

The gender prediction was done with the usage of logistic regression models; one model derived from the measurements of long bones and the other model derived from the data of foot and hand measurements. The odds of the gender being male increased 17.3 times with every unit increase in the right tibial length (P<0.001). The odds of the gender being male decreased 0.06 times with every unit increase in the left tibial length. The odds of the gender being male decreased 0.06 times with every unit increase in the left tibial length (Table 4).

The odds of the gender being male increases 108.21 times with unit increase in length of right foot, 8.94 times with unit increase in hand length of left side, and 53.53 times with unit increase in breadth of left hand. The odds of the gender being female increases 0.012 times with unit increase in length of left foot, 0.49 times with unit increase in length of right hand, and 0.14 times with unit increase in right hand breadth. (Table 5).
The regression equations are presented below for the estimation of gender by long bones, hand and foot measurements:

**Gender estimation by long bones:**

\[-20.8259 + 2.8507 \times \text{Length of Tibia Rt} - 2.8097 \times \text{Length of Tibia Lt} + 0.1480 \times \text{Length of Ulna Rt} + 0.5779 \times \text{Length of Ulna Lt}\]

Equation 1

**Gender estimation by hand and foot dimensions:**

\[-48.5756 + 4.684 \times \text{Foot Length Rt} - 4.4053 \times \text{Foot Length Lt} - 0.4276 \times \text{Foot Breath Rt} + 0.3341 \times \text{Foot Breath Lt} - 0.7048 \times \text{Hand Length Rt} + 2.1906 \times \text{Hand Length Lt} - 1.9575 \times \text{Hand Breath Rt} + 3.9802 \times \text{Hand Breath Lt}\]

Equation 2

The accuracy of the formulae in predicting the gender was found to be 78.5\% (equation 1) and 81.5\% (equation 2) respectively at a cut-off point of 0.5. The cut-off points of 0.5 implies that for values above 0.5, the predicted sex is male and for values below 0.5 the predicted sex is female. Results suggest that measurements of the hand and foot provides a superior prediction of gender as compared to the prediction of gender which was calculated from the lengths of tibia and ulna.

Table-1: Comparison of dimensions of tibia and ulna between genders

<table>
<thead>
<tr>
<th>Variable</th>
<th>Female Mean ± SD (Min-Max)</th>
<th>Male Mean ± SD (Min-Max)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of Tibia Right</td>
<td>34.5±2.7 (24-40)</td>
<td>36.9±3.0 (26-44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Length of Tibia Left</td>
<td>34.5±2.7 (24-40)</td>
<td>36.9±3.0 (26-44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Length of Ulna Right</td>
<td>25.7±1.7 (22-31)</td>
<td>27.7±1.6 (23-31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Length of Ulna Left</td>
<td>25.7±1.7 (22-31)</td>
<td>27.7±1.59 (23-31)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Rt-Right, Lt-Left, SD-Standard Deviation, Min-Minimum, Max-Maximum, *Mann Whitney U-test

Table-2: Comparison of foot measurements between genders

<table>
<thead>
<tr>
<th>Variable</th>
<th>Female Mean±SD (Min-Max)</th>
<th>Male Mean±SD (Min-Max)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot Length Right</td>
<td>23.1±1.1 (21-25.5)</td>
<td>36.9±3.0 (26-44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Foot Length Left</td>
<td>23.1±1.1 (20.9-25.6)</td>
<td>36.9±3.0 (26-44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Foot Breath Right</td>
<td>9.5±1.4 (7.96-14.8)</td>
<td>27.7±1.6 (23-31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Foot Breath Left</td>
<td>9.4±1.4 (7.96-14.8)</td>
<td>27.7±1.59 (23-31)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Rt-Right, Lt-Left, SD-Standard Deviation, Min-Minimum, Max-Maximum, *Mann Whitney U-test
### Table-3: Comparison of hand measurements between genders

<table>
<thead>
<tr>
<th>Variable</th>
<th>Female Mean±SD (Min-Max)</th>
<th>Male Mean±SD (Min-Max)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand Length Right</td>
<td>16.8±0.9 (15-18.8)</td>
<td>18.2±1.0 (16.6-24.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hand Length Left</td>
<td>16.8±0.9 (15-18.8)</td>
<td>18.2±0.8 (16.6-19.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hand Breath Right</td>
<td>7.7±0.5 (6.8-9.2)</td>
<td>8.4±0.5 (6.7-9.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hand Breath Left</td>
<td>7.7±0.5 (6.8-9.0)</td>
<td>8.4±0.5 (6.8-9.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Rt-Right, Lt-Left, SD-Standard Deviation, Min-Minimum, Max-Maximum, *Mann Whitney U-test*

### Table-4: Logistic regression model for estimating gender based on long bone.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval for Odds Ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.50%</td>
<td>97.50%</td>
</tr>
<tr>
<td>Intercept</td>
<td>-20.82</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Length of Tibia Right</td>
<td>2.85</td>
<td>17.30</td>
<td>5.38</td>
<td>64.64</td>
</tr>
<tr>
<td>Length of Tibia Left</td>
<td>-2.81</td>
<td>0.06</td>
<td>0.02</td>
<td>0.19</td>
</tr>
<tr>
<td>Length of Ulna Right</td>
<td>0.15</td>
<td>1.16</td>
<td>0.29</td>
<td>4.70</td>
</tr>
<tr>
<td>Length of Ulna Left</td>
<td>0.58</td>
<td>1.78</td>
<td>0.44</td>
<td>7.20</td>
</tr>
</tbody>
</table>

*statistically significant*
Table-5: Logistic regression model for estimating gender based on short bone.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval for Odds Ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.50%</td>
<td>97.50%</td>
</tr>
<tr>
<td>Intercept</td>
<td>-48.27</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Foot Length Right</td>
<td>4.68</td>
<td>108.21</td>
<td>29.95</td>
<td>437.91</td>
</tr>
<tr>
<td>Foot Length Left</td>
<td>-4.40</td>
<td>0.012</td>
<td>0.003</td>
<td>0.04</td>
</tr>
<tr>
<td>Foot Breath Right</td>
<td>-0.43</td>
<td>0.65</td>
<td>0.23</td>
<td>1.84</td>
</tr>
<tr>
<td>Foot Breath Left</td>
<td>0.33</td>
<td>1.39</td>
<td>0.49</td>
<td>3.98</td>
</tr>
<tr>
<td>Hand Length Right</td>
<td>-0.71</td>
<td>0.49</td>
<td>0.35</td>
<td>0.76</td>
</tr>
<tr>
<td>Hand Length Left</td>
<td>2.19</td>
<td>8.94</td>
<td>5.24</td>
<td>14.93</td>
</tr>
<tr>
<td>Hand Breath Right</td>
<td>-1.96</td>
<td>0.14</td>
<td>0.03</td>
<td>0.56</td>
</tr>
<tr>
<td>Hand Breath Left</td>
<td>3.98</td>
<td>53.53</td>
<td>13.32</td>
<td>238.67</td>
</tr>
</tbody>
</table>

*statistically significant

Discussion

Bone dimensions ranging from skull to hands and foot have been effectively used to predict gender in the past for themedico-legal cases and forensic applications. Measurements of hand and foot provide the most feasible options to estimate gender as they are in most cases easily accessible. Here we studied whether measurements of hand and foot could predict gender with an accuracy comparable to that of the long bone dimensions (tibia and ulna).

This study report that gender can be predicted by measurements of hand and foot; with an accuracy of 81.5% and the findings are in line with similar studies reported from other nations. Zebek et al. predicted gender by utilizing measurements of foot with an accuracy of 95.6% in the Turkish population. Similarly, Jowaheer et al., identified gender from the dimensions of hand and foot with an accuracy of 90-92% in an Indo-Mauritian sample.

This study reports the accuracy of gender prediction from the lengths of tibia and ulna was 78.5%. Other studies have already reported excellent data for gender prediction using long bones. Tibia was used to predict gender with an accuracy of 80-84%. In 2019, another study among a Ghana sample showed that gender could be predicted by height of a person with an accuracy of 80.7 %, by ulnar length with an accuracy of 82.3% and by radial length with an accuracy of 75.3 %. A derived equation from a combination of all measurements predicted gender with an accuracy measurement of 82.4%. Likewise, in 2017, among Poland population with the accuracy of gender estimation by radial length
was 84 %. Other long bone such as humerus was also reported to predict gender with the accuracy of 93.3% in an Egyptian sample. Gender prediction has also been calculated by femur dimensions with the accuracy of 72.5% in males, and 85% in females.

Surprisingly, dimensions of hand and foot seems to be a better predictor of gender, than long bones (tibia and ulna) length in the Kolhapur population. The major strength of this study is that it is one of the largest studies conducted to predict gender using data on the measurements of hand and foot. Though other accurate methods of measurements of bone such as radiographs, scanograms, computerized digital radiographs and computerized tomography (CT) are available, measurement methods adopted in the present study; is easily reproducible and cost effective as it does not require costly equipments.

This study is a first-time report on the approximate dimensions of foot and hand, and their utility in the prediction of gender in a sample of Kolhapur population.

**Conclusion**

The regression equation derived in the study can be used to predict gender in the Kolhapur population. Dimensions of hand and foot are better predictors of gender when compared to the length of bones (tibia and ulna) in Kolhapur population.

**Conflict of Interest :** Nil.

**Acknowledgement:** None.

**Funding:** Nil.

**References**


Biochemical and Histopathological effects of Acetaminophen and Protective Effects of Naringin on Liver Rats

Aryaf Mahmood Sabea1, Ahlam J. H. AlKhamas2, Bashar Sabah Sahib3, Jawad Kadhim Faris4

1Assist. Lecturer, Department of Medical Laboratory Techniques, AL-Hilla-University College, Iraq, 2Assist. Prof., Department of Anatomy and Histology, College of Veterinary Medicine, Al-Qasim Green University, Iraq, 3Assist. Lecturer, Department of Medical Basics Sciences, Faculty of Nursing, University of Al-Qadisiyah, Iraq, 4Prof. Department of Physiology, College of Veterinary Medicine, Al-Qasim Green University, Iraq

Abstract

This study was conducted to find out liver protective activity of naringin (NRG) 40-80 mg/kg body weight (b.w.) against acetaminophen (ACN) 2 g/kg induced liver damage in rats. Thirty two male rats were divided into four groups: group I: negative control, (1 ml/kg Saline orally) group II: positive control ACN (2 g/kg), orally as single dose at first day, group III: ACN (2 g/kg), orally as single dose at first day, plus NRG (40 mg/kg) orally for (8) consecutive days, group IV: ACN (2 g/kg), orally as single dose at first day, plus NRG (80 mg/kg) orally for (8) consecutive days. All the rats were anesthetized to collect blood then killed on the (9) day of the experiment to take the liver samples. ACN induced liver damage was proved by a significant (P<0.01) reduction in the body weight, total protein (TP), albumin (AB), superoxide dismutase (SOD) and catalase (CAT) enzymes and a significant (P<0.01) increased in liver weight, serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), direct bilirubin (DB), malondialdehyde enzyme (MAD) and histopathological changes. Protective liver toxicity effect and oxidative damage caused by ACN significantly (P<0.01) increasing in body weight, TP, AB, SOD and CAT and significantly (P<0.01) decreasing in liver weight, AST, ALT, ALP, TB, DB and MAD and improving tissue morphology by a meliorative in NRG 40, 80 mg/kg b.w. These results confirm that NRG antioxidant effects can protect ACN induced hepatic toxicity in rats.

Key words: Naringin, Acetaminophen, Anti-oxidants, liver damage

Introduction

Acetaminophen (N-acetyl-p-aminophenol; APAP), popularly known as paracetamol, is a most common and widely used as analgesic and antipyretic drug that is safe at therapeutic dosages for a wide range of treatments (1). Acute overdose of acetaminophen is known to cause hepatic and renal damage in both human and experimental animals. Nephrotoxicity is less common compared to hepatotoxicity in acetaminophen overdose, but renal damage and acute renal failure can occur even in the absence of liver injury and can even lead to death in humans and experimental animals (2). Paracetamol has been used as a non-narcotic analgesic and antipyretic drugs. This drug works by inhibiting the synthesis of prostaglandins in the central nervous system (3). It is widely used to treat muscle pains, arthritis and acute headaches. It is safe at therapeutic levels, but at high doses it can lead to undesirable side effects such as hepatotoxicity and nephrotoxicity (4). Naringin is a plant flavonoid of great human value. Flavonoids are ubiquitous polyphenolic secondary metabolites isolated from vascular plants (5). They have a general structure of 15-carbon skeleton that contains two phenyl rings A, B and a heterocyclic ring C. Flavonoids are the most important flavonoids participating in the stress responses of plants (6). Approximately 8000 flavonoids have been identified from various citrus fruits, vegetables and beverages (7). They behave as chemical messengers, pollinator attractants and stress regulatory
elements of plants (8). Flavonoids also exhibit human health promoting abilities like antioxidant and free radical scavenging potential (9). They act as antiviral, antibacterial, anti-inflammatory, vasodilator, anticancer and ant ischemic agents(10). Flavonoids can undergo various metabolic transformations such as methylation and sulfation to change their structures and hence their biological activities (11).

Materials and Methods

This study was conducted during November 2020 in the Physiology Department of Veterinary Medicine of AL-Qassim green university.

Drugs

Naringin (NRG) 100% Natural was purchased from BULK Supplements.com USA. Paracetamol, (Qnardol –SR 1g) from Star biomed -India. Ketamine 10% inj. from KEPRO- HOLLAND. Xylazine, XYL- M2, VMD- Belgium.

Experimental Rats

The number of laboratory animals used in the experiment are thirty two healthy male rats at aged (170-180) days and weighted (200-210) grams, obtained from the animal house of the College of Veterinary Medicine, AL-Qadisiya University, were kept for (10) days as acclimatization period before the starting of the experiment, all rats were feed on concentrated food (pellets) and were given plain water, the animals room temperature was (19-23)°C, and the humidity was (45-55), that room was washing and sterilization once a week.

Experimental design

After a quarantine period of (10) days, the animals were divided into four equal groups, each group consist of (8) rats, and they received the treatment as follows:

Group I: Negative control received normal saline (1 ml/kg b.w orally by stomach tube), daily for (8) days. Group II: Positive group were treated ACN (2g/kg) orally by stomach tube as single dose at first day, then NRG (40 and 80 mg/kg b.w) orally by stomach tube, per day, respectively, for (8) days. The doses of treatment are according to Kumar et al., (13).

Body and liver weight

All animals were weighed before and after treatment, changes in the body weights of rats were recorded, in addition to the weights of rat livers, with using digital electronic balance.

Sacrifice Animals and Serum Preparation

At the end of experimental period, rats were fasted for (10) hrs, rats were anaesthetized with ketamine (75 mg/kg) combined with xylazine (2.5 mg/kg). Blood samples were collected by heart puncture in non-heparinized tubes, centrifuged at (4000) rpm for (10) minutes. After separation the serum from the clot, using a sampler, the samples were used to measurement of serum biochemical parameters for liver function tests AST, ALT, ALP, TP, AB, TB, DB and oxidative stress parameters in liver MAD, SOD and CAT. The rats were sacrificed by cervical dislocation and the abdominal cavity was immediately opened, liver tissues were excised and weighed immediately after sacrifice. livers were processed for histopathological and immunohistochemical studies.

Histopathological techniques

Sections were taken from livers of the animals in each group immediately after sacrificed. These tissues were washed with the normal saline solution to remove blood, then fixed in 10% neutral formalin for (24) hrs, dehydrated in different concentration of alcohol, and processed for paraffin embedding. Sections of (5) µm thickness were cut using a rotary microtome. The sections were processed and passed through graded alcohol series stained with Haematoxylin and Eosin, cleared in xylene and examined microscopically according to (15).
Statistical Analysis

The statistical results of the data were analyzed according to Complete Randomized Design (C.R.D.) (16). The mean differences between the averages of the studied traits were determined at the probability level of (0.01) using the Duncan test (17).

Results

Body, liver weight after treatment

Oral administration of ACN at a dose of 2g/kg caused a significant (P<0.01) decrease in body weight and a significant (P<0.01) increase in liver weight. Treated rats with NRG 40 mg/kg and NRG 80 mg/kg showed significant (P<0.01) increase in body weight and significant (P<0.01) decrease in liver weight was observed (Table 1).

Serum biochemical analysis

The administration of ACN to rats resulted in a significant (P<0.01) reduction in serum TP, AB, and a significant (p > 0.01) increased ALT, AST, ALP, TB, DB, when compared with that in control. Preventative treatment with NRG ameliorated these altered biochemical parameters toward normal values in comparison with the ACN group in (Tables 2).

Antioxidant Enzymes

The MAD activity was significantly (P<0.01) increased and SOD and CAT were significantly (P<0.01) decreased in the ACN group when compared to the control group. In the treated groups with NRG 40-80 mg/kg, ameliorated these altered biochemical parameters toward significantly (P<0.01) decreased in the MAD and significantly (P>0.01) increased in SOD and CAT this result shown in (Table 2).

Histopathological Examination

Light microscopic of liver examination using (H&E 400X) stain in control rats, showed normal histological structure of the liver tissue was observed with the central vein and cords of hepatocytes radiating toward the periphery, bile duct were surrounded by connective tissue and normal sinusoids were observed. (Fig:1a). Histopathological section of rat liver group II which treated with ACN (2g/kg), orally by stomach tube as single dose at first day, showing severe fatty degeneration and necrosis into the surrounding cells in the central vein. (Fig:1b). Histopathological section of rat liver group III treated with ACN (2g/kg), orally by stomach tube as single dose at first day, then NRG (40 mg/kg b.w) orally by stomach tube, per day, respectively, for (8) days, showing moderate fatty degeneration arranged of hepatic cells around central vein (Fig:1c). Histopathological section of rat liver group IV, which treated with ACN (2g/kg), orally by stomach tube as single dose at first day, then NRG (80 mg/kg b.w) orally by stomach tube, per day, respectively, for (8) days, showing hepatoocyte arranged around the central vein, normal sinusoids and nuclei with no signs of necrosis (Fig:1d).

Table 1: Effect of Naringin on Acetaminophen on body weight, liver weight / gram of rats

<table>
<thead>
<tr>
<th>Traits</th>
<th>Control Mean ±SE</th>
<th>ACN Mean ±SE</th>
<th>ACN+ NRG 40 Mean ±SE</th>
<th>ACN+ NRG 80 Mean ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats :</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Weight at 1 day (g/animal) Ns</td>
<td>200.194 ± 0.04 A</td>
<td>200.358 ± 0.11 A</td>
<td>200.442 ± 0.10 A</td>
<td>200.468 ± 0.15 A</td>
</tr>
<tr>
<td>Weight at 9 day (g/animal) **</td>
<td>208.020 ± 0.91 A</td>
<td>170.488 ± 0.67 D</td>
<td>183.942 ± 1.34 C</td>
<td>192.394 ± 1.39 B</td>
</tr>
<tr>
<td>Liver weight (gm) **</td>
<td>5.824 ± 0.11 C</td>
<td>7.636 ± 0.24 A</td>
<td>7.196 ± 0.14 AB</td>
<td>6.752 ± 0.08 B</td>
</tr>
</tbody>
</table>

NS: Non-significant. Significant difference at 0.05. ** High significant difference at 0.01.
Table 2: Effect of Naringin on Acetaminophen on liver function of rats

<table>
<thead>
<tr>
<th>Traits</th>
<th>Control Mean ± SE</th>
<th>ACN Mean ± SE</th>
<th>ACN+ NRG 40 Mean ± SE</th>
<th>ACN+ NRG 80 Mean ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L) **</td>
<td>40.09 ± 1.24 D</td>
<td>98.30 ± 2.17 A</td>
<td>65.74 ± 1.21 B</td>
<td>47.29 ± 0.75 C</td>
</tr>
<tr>
<td>AST (U/L) **</td>
<td>73.61 ± 1.35 D</td>
<td>121.71 ± 1.57 A</td>
<td>92.56 ± 0.95 B</td>
<td>82.61 ± 0.09 C</td>
</tr>
<tr>
<td>ALP (U/L) **</td>
<td>20.92 ± 1.36 C</td>
<td>41.23 ± 1.32 A</td>
<td>28.43 ± 1.21 B</td>
<td>24.19 ± 0.38 C</td>
</tr>
<tr>
<td>Total Protein (mg/dl) **</td>
<td>6.87 ± 0.02 A</td>
<td>4.93 ± 0.03 D</td>
<td>5.26 ± 0.03 C</td>
<td>6.31 ± 0.03 B</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl) **</td>
<td>0.52 ± 0.01 D</td>
<td>1.10 ± 0.02 A</td>
<td>0.97 ± 0.01 B</td>
<td>0.69 ± 0.01 C</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl) **</td>
<td>0.17 ± 0.01 D</td>
<td>0.59 ± 0.01 A</td>
<td>0.48 ± 0.01 B</td>
<td>0.28 ± 0.01 C</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.49 ± 0.08 A</td>
<td>2.54 ± 0.03 C</td>
<td>3.68 ± 0.02 B</td>
<td>3.72 ± 0.02 B</td>
</tr>
<tr>
<td>Malonaldehyde (µmol/ml) **</td>
<td>1.66 ± 0.02 D</td>
<td>3.97 ± 0.04 A</td>
<td>2.55 ± 0.08 B</td>
<td>1.86 ± 0.02 C</td>
</tr>
<tr>
<td>Superoxide dismutase (U/ml) **</td>
<td>1.70 ± 0.01 A</td>
<td>0.89 ± 0.01 D</td>
<td>0.98 ± 0.03 C</td>
<td>1.36 ± 0.01 B</td>
</tr>
<tr>
<td>Catalase (U/ml) **</td>
<td>0.58 ± 0.01 A</td>
<td>0.27 ± 0.01 D</td>
<td>0.37 ± 0.01 C</td>
<td>0.49 ± 0.01 B</td>
</tr>
</tbody>
</table>

NS: Non-significant . Significant difference at 0.05. ** High significant difference at 0.01.
Discussion

This study is to show that NRG could alleviate ACN induced liver toxicity, NRG has an ameliorative effect against liver toxicity induced by 2g/kg body weight of ACN as explained by reduction in the body weight and increased in liver weight when compared to control. Our results are in acceptance with prior findings of (18). Mentioned that the oral administration of 800mg/kg of paracetamol for seven days, it causes significant loss in weight among the liver damaged rats than the control. According to (19), increased catabolism and anorexia are responsible for decreased food intake and causes body weight loss further, following loss of the tubular cells, involved in renal water reabsorption leads to dehydration and decreases body weight (20). While previous study of Ali et al., (21) that the paracetamol administration did cause significant increase in the average liver weight when compared to the normal control group. The enlargement of livers in ACN - treated rats suggested hepatic lesions and liver injury associated with the toxic effects of ACN. These significant changes in the liver weights may be attributed to the accumulation of extracellular matrix protein and collagen in liver tissue. Treatment of ACN causes histopathological and immunohistochemical changes, sever fatty degeneration and necrosis into the surrounding cells in the central vein and significant increase in the serum level of liver function markers such as ALT, AST, ALP, TB, DB levels in addition to a significant reduction TP and AB levels. These results are in acceptance with those obtained by other investigators (22). These enzymes are originally present in the cytoplasm, when there is hepatopathy, these enzymes and molecules leak into the bloodstream and serve as an indicator for the liver damage (23). ACN is a widely used drug, produce N-acetyl-Pbenzoquinone-imine at overdoses and covalently binds to sulphhydryl groups causing cell necrosis and lipid peroxidation by depleting reduced glutathione in the liver, subsequently leading to liver toxicity. Oxidative stress is considered an important contributing factor to the development of liver and kidney diseases. Oxidative stress mainly develops due to increased generation of Reactive Oxygen Species (ROS) and a decrease in ROS scavenging capacity (24). The significant increase in MAD and significant reduction SOD and CAT are
attributed to increased production of ROS. Excessive lipid peroxidation causes lipid degradation that impairs cell membrane function, resulting in tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals in paracetamol-intoxicated rats (25). The results of the present study suggest that, the accumulation of free radicals, and that increased oxidative stress is a basis for cellular damage. Through the aforementioned findings, we conclude that there is liver damage due to the use of ACN. Treatment with NRG for (8) days reduced the ACN injured liver produces a significant increased in body weight, TP, AB, SOD and CAT and significant decrease in weight of liver, ALT, AST, ALP, TB, DB and MAD. The results of this study are in agreement with (26). Flavonoids are an important group of secondary metabolites and a source of bioactive compounds in plants (27). Our results demonstrate the protective effect NRG on ACN for (8) days induced liver toxicity in the rats. This can be demonstrating on the anti-inflammatory effects, such as pathogens, damaged cells or irritants (28). NRG, Phenolic phytochemicals are thought promote health partly via antioxidant activity and free radical scavenging effects (29). Also has antioxidant properties and confers protection against H2O2-induced chromosome breakage and loss and DNA damage. NRG may avoid H2O2-induced oxidative damage not only by decreasing DNA damage but also by increasing the DNA repair capacity (30). NRG an anti-inflammatory, antioxidant and anti-apoptotic potential effect at colorectal sites as it modulates the production and expression of oxidative mediators by reducing DNA damage (13). Also has beneficial effects on many CNS diseases, including Alzheimer’s disease, Parkinson’s disease and epilepsy (31). Based on the advance findings, it can be conclude that, ACN had adverse effects on the liver. NRG administration showed a marked hepatic protective activity. The protective effects of NRG may be due to the its anti-inflammatory effects or antioxidant effects or ant apoptotic effects individually or synergistically.

**Conclusion**

This study explained that NRG declines ACN induced hepatotoxicity. The effect of NRG against ACN induced hepatotoxicity could be mediated through its antioxidant, antiinflammatory, antimicrobial and antiapoptosis action. However, NRG treatment was able to alleviate liver damage associated with ACN treatment and this is assign to its antioxidant activity and its ability to prevent inflammation.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** Obtained from Institutional ethical committee

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Investigation the Role of IL-17 rs2275913 Gene Polymorphism with the Risk of Osteoporosis in a Sample of Iraqi Pre and Postmenopausal Women

Asmaa Mahmoud Salman¹, Da’ad A. Hussain¹

¹Scholar Researcher, Institute of Genetic Engineering and Biotechnology, University of Baghdad, Iraq

Abstract

Objective: This study aimed to investigate the relationship between the genetic polymorphism and alleles frequencies of IL-17 rs2275913 G/A with the risk of low bone mineral density (osteoporosis and osteopenia). Furthermore studying the relation between some immunological and biochemical parameters with this disease in a sample of Iraqi pre and postmenopausal women. Methodology: In this study, we investigated about the IL-17 rs2275913 G/A polymorphisms and the risk of low bone mineral density (BMD) among 30 patients with osteopenia, 30 patients with osteoporosis and 30 healthy controls. Serum IL-17 level and its correlation with the IL-17 rs2275913 G/A genotypes were analyzed. The study was carried out from November 2020- January 2021 in Baghdad Teaching Hospital/ Bone density examination unit/ in Baghdad-Iraq. Conclusion: It is concluded that the IL-17 rs2275913 G/A genotype was not associated with increased risk for development of low (BMD) in Iraqi pre and postmenopausal women. Recommendation: Based on study conclusion, during menopause, it is important for women to have a DEXA scan to detect low BMD and receive appropriate care to prevent degradation of the micro-architecture of bone tissue, which increases the risk of bone fractures.

Keywords: Low Bone Mineral Density, IL-17, Single-nucleotide polymorphism Susceptibility, Baghdad Teaching Hospital.

Introduction

Bone mineral density (BMD) is a measure of the inorganic mineral content in bone, and is one of the more informative assessments of bone quality in both clinical studies and forensic investigations. Several factors, such as age, sex, disease, genetics, and lifestyle, affect BMD measurements, and normative standards must be applied for specific groups and individuals. One of the most common disorders associated with low BMD is osteoporosis and increased fracture risk, due to a decrease in bone strength and an increase in bone fragility (1).

Osteopenia is a clinical term used to describe a decrease in BMD below normal reference values, yet not low enough to meet the diagnostic criteria to be considered osteoporotic. It is, as defined by the World Health Organization (WHO), is a t-score between -1 to -2.5 (2). This condition happens when the body disposes of more bone than it is making. It is transform into osteoporosis so far as that is concerned isn’t unavoidable. Diet, work out, and in some cases prescription can help keep the bones thick and solid for quite a long time. Furthermore Osteopenia as a rule doesn’t have any side effects. This makes it difficult to analyze except by a bone mineral thickness test (3).

Postmenopausal osteoporosis is a chronic disease associated with age-related declines in bone mass, changes in bone microarchitecture, and skeletal fragility. These changes place postmenopausal women at increased risk of fragility fractures, which are linked to significant morbidity, economic cost, and negative impact on health-related quality of life (4).
Interleukin-17 (IL-17) is characteristic cytokines of CD4+ cells and the group of Th17 cells secrete, showed that IL-17 plays an important role in the formation of osteoclast. IL-17 is secrete factors that can work together with tumor necrosis factor alpha (TNF-α) enhance the process of the development of inflammation and bone transformation (5). IL-17 is a recently discovered family of cytokines composed of six members. Additional isoforms homologous to IL-17A designated as IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F were discovered afterwards (6).

Material and Method

Study population: This study was conducted with 30 osteopenia patients and 30 osteoporosis patients with pre and postmenopausal Iraqi women who attended Baghdad Teaching hospital in Baghdad/Iraq between November 2020- January 2021. Osteopenia and Osteoporosis was defined according to the conventional World Health Organization (WHO) definition. Subjects with a history of bone disease, metabolic or endocrine disorders such as diabetes mellitus, hyperthyroidism, hyperparathyroidism, renal disease, liver disease, medications known to affect bone metabolism (e.g. anticonvulsants, corticosteroids, heparin sodium) were excluded. The control group comprised 30 healthy volunteers for the general health checkup in our hospital during the same period. After obtaining written informed consent, 5 mL of peripheral blood was collected for DNA extraction and ELSA test. Each participant was interviewed using a standard questionnaire by a trained nurse, to collect demographic characteristics and medical histories. All the specimens we recruited were of Arabic/ Iraq ethnicity and were filtered based on their clinical characteristics. Before the assay, we obtained a written informed consent from each participant in our study.

Bone mineral density measurements: Area BMD (g/cm²) was measured by dual energy X-ray absorptiometry (DEXA). Densitometers were calibrated daily. Left hip and posterior–anterior lumbar spine (L2–L3–L4) scans were performed with the patient lying supine on the imaging table using the protocols recommended by the manufacturer.

DNA extraction and genotyping: Genomic DNA was isolated from EDTA anticoagulated peripheral blood with a commercially available extraction kit (Geneaid/ Taiwan) according to the manufacturer’s instructions. Genotype determination for one SNP in the IL-17 gene (rs2275913 G/A) was performed by real-time polymerase chain reaction (RT-PCR). The polymorphisms within IL17 rs2275913 G/A were genotyped with TaqMan genotyping assays using the Roto-Gene Q apparatus Real-Time (Roto-Gene Q, Italy). Probes and primers designed for RT-PCR shown in Table 1.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Primer sequence</th>
<th>Product</th>
<th>RT-PCR conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2275913 G/A</td>
<td>F: 5’-CGTGTGCAGTGGGTGTTCA-3’&lt;br&gt;R: 5’-TTCTGCCCCTCCCATTTTCC-3’</td>
<td>18 mer</td>
<td>40 cycles: 95°C for 10s, 95°C for 15s, 60°C for 1m.</td>
</tr>
<tr>
<td></td>
<td>Probe sequence</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P/G: 5’-AGAATCTCTCTCTCTCTGAA-3’&lt;br&gt;P/A: 5’-AGAATCTCTCTCTCTGTGAA-3’</td>
<td>22 mer</td>
<td></td>
</tr>
</tbody>
</table>
Serum level measurement of IL-17: The interleukin-17 (IL-17) level were determined by enzyme-linked immunosorbent assay (ELISA) using a commercially available ELISA quantitative kit (Shanghai Yehua/ China) according to manufacturer’s instructions.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) (7).

Result

This study included 30 osteopenia, 30 osteoporosis patients and 30 healthy controls, the mean ages of osteopenia, osteoporosis patients and healthy controls were 36.46 ±1.36, 55.80 ±1.48 and 41.33 ±1.81 years, respectively. The genotype and allele frequencies of the IL-17 rs2275913 G/A polymorphisms for all the studied variations are shown in Table2.

<table>
<thead>
<tr>
<th>genotype</th>
<th>Osteoporosis No (%)</th>
<th>Osteopenia No (%)</th>
<th>Control No (%)</th>
<th>P –value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>15 (50.00%)</td>
<td>10 (33.33%)</td>
<td>10 (33.33%)</td>
<td>00092 **</td>
</tr>
<tr>
<td>GA</td>
<td>11 (36.67%)</td>
<td>10 (33.33%)</td>
<td>13 (43.33%)</td>
<td>0.0452 *</td>
</tr>
<tr>
<td>AA</td>
<td>4 (13.33%)</td>
<td>10 (33.33%)</td>
<td>7 (23.33%)</td>
<td>0.0076 **</td>
</tr>
</tbody>
</table>

Allele frequency

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>0.68</td>
<td>0.50</td>
<td>0.55</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>0.32</td>
<td>0.50</td>
<td>0.45</td>
<td>-</td>
</tr>
</tbody>
</table>

* (P≤0.05), ** (P≤0.01).

There were no association in the genotypes between patients and controls, and the allele frequencies of IL-17 rs2275913 G/A for the three study groups appeared that there were no significant different between patients and controls. Compared GA genotype between control and patients, heterozygous GA genotype was associated with significantly increased in control (43.33%) and decreased in osteoporosis (36.67%) and osteopenia (33.33%). AA genotype was higher in osteopenia (33.33%) and control (23.33%) then decreased in osteoporosis (13.33%). In addition, GG genotype was higher in osteoporosis (50.00%) and less in osteopenia (33.33%) and control (33.33%). While in the allele frequencies G and A there were no significant different between patients and controls.

Serum IL-17 level and its correlation with the IL-17 rs2275913 G/A genotypes. There were no statistically significant associations between IL-17A gene polymorphisms and Serum IL-17 Level in osteoporosis and osteopenia, while there were statistically significant differences between IL-17A gene polymorphisms and Serum IL-17 Level in control group p-value (0.038). In
contrast IL-17A plasma levels were significantly higher in patients (31.72-16.37)) comparatively to controls (15.05), and this result due to our study and another studies that the serum IL-17 level related to osteoporosis but the IL-17 G/A (rs2275913) genotypes has no related to osteoporosis (Table 3).

Table 3: Serum IL-17 level and its association with the IL-17 G/A (rs2275913) genotypes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>Mean ± SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IL-17</td>
<td></td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>GG</td>
<td>25.53 ±1.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>42.69 ±12.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>24.81 ±1.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.238 NS</td>
<td></td>
</tr>
<tr>
<td>Osteopenia</td>
<td>GG</td>
<td>18.32 ±1.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>15.59 ±1.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>14.99 ±0.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.364 NS</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>GG</td>
<td>16.82 ±1.62 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>15.88 ±1.40 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>10.98 ±0.79 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.038 *</td>
<td></td>
</tr>
</tbody>
</table>

Means having with the different letters in same column differed significantly. * (P≤0.05).

Discussion

In the present study, we selected, one SNP IL-17 rs2275913 G/A to evaluate their association in patients with Iraqi pre and postmenopausal women with osteopenia, osteoporosis and healthy controls. Inflammatory processes and cytokines play essential roles in the pathogenesis of women osteoporosis. Variations in cytokine levels among individuals are a plausible explanation for differences in disease susceptibility and severity, and are principally attributable to single nucleotide polymorphisms (SNPs) in cytokine-encoding genes (8).

Interleukin-17 (IL-17) is a proinflammatory cytokine produced by the memory CD4 + T cells after activation and has been shown to be involved in amplifying inflammatory response by recruiting immune
cells such as neutrophils and monocytes and inducing other proinflammatory molecules (9). IL-17 is essential to both the adaptive and innate immune systems. It has five confirmed receptors (IL-17RA-RD and SEF) and six members (IL-17A-F). Moreover, IL-17, as a pro-inflammatory cytokine, can trigger the release of chemokines and cytokines (10).

**Conclusion**

It is concluded that there were no significant differences in the allele frequencies of IL-17 rs2275913 G/A for the three study groups osteopenia patients, osteoporosis patients, and controls. So IL-17 rs2275913 G/A genotype and A allele, was not associated with increased risk for development of low BMD in Iraqi pre and postmenopausal women and there were no association between Serums calcium, alkaline phosphatase (ALP), BMD and IL-17 rs2275913 G/A genotype. Also it is concluded increase IL-17 level play an important role in the development risk of osteoporosis and osteopenia in pre and postmenopausal women (11) (12).

**Recommendations**

During menopause, it is important for women to have a DEXA scan to detect low bone mineral density and receive appropriate care to prevent degradation of the micro-architecture of bone tissue, which increases the risk of bone fractures. a.To increase bone density and reduce the risk of osteoporosis, it is critical to consume calcium and vitamin D3-rich foods, as well as get enough sunlight for a sufficient period of time to promote production of vitamin D3. It is also critical to exercise regularly to maintain bone density and reduce the risk of low bone mineral density.

**Conflict of Interest:** None

**Funding:** Self

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

**References**


Treatment of Lupus Nephritic with Severe Preeclampsia: A Case Report

Asri Insanur Rahma¹, Lita Diah Rahmawati²
¹Resident, ²Rheumatologist, Department of Internal Medicine, Universitas Airlangga/Dr. Soetomo General Hospital, Surabaya, Jawa Timur, Indonesia

Abstract

A 25 years-old pregnant woman with history of Systemic Lupus Erythematosus (SLE) complained shortness of breathing and swelling legs. Physical examination showed hypertension, tachycardia, tachypnea, anemic conjunctiva, and pitting edema in both legs. Workup examination found anemia, hypoglycemia, hypoalbuminemia, proteinuria, metabolic acidosis, strong positive dsDNA, trivial MR, mild TR, PR, dilated LV, decrease of LV systolic function, hypokinetic global LV, eccentric LVH, and severe oligohydramnios. The patient was diagnosed with severe preeclampsia, severe oligohydramnios, SLE, Lupus Nephritic (LN), stage II JNC VII hypertension, and peripartum cardiomyopathy.

An emergency caesarean section was planned as the main management to prevent infant and maternal morbidity and mortality. MgSO₄ was given to prevent eclampsia. Methylprednisolone and azathioprine were given to control SLE and LN activity. Furosemide, spironolactone, ramipril, methyldopa and nifedipine were given to control blood pressure, help normalizing heart function, and prevent organ failure. The patient was suggested to postpone next pregnancy until 1 year after LV function turns to normal.

Keywords: Lupus Nephritic, Pregnancy, Systemic Lupus Erythematosus, Severe Preeclampsia

Introduction

LN developed during conception or pregnancy increases the risk of preterm birth (34%), pre-eclampsia (10-35%), maternal mortality, fetal death, neonatal death (12-38%), intrauterine growth delay (30%), chronic kidney disease (CKD) and end-stage renal disease (ESRD).¹

Pre-eclampsia causes risk of stroke, premature birth and even death. Severe pre-eclampsia can develop into eclampsia with grand mal seizures.²

Differentiating active lupus nephritis from pre-eclampsia becomes one of the biggest challenges in managing SLE patients with pregnancy. They both present with proteinuria, hypertension, leg edema and systemic effect, yet the treatment of these two diseases is different. Pre-eclampsia will improve once the pregnancy terminated and active SLE requires immunosuppression as treatment.³

Case Report

A pregnant woman, 25 years old housewife living in Gresik, Jawa Timur, Indonesia, visited Dr. Soetomo General Hospital with a chief complaint of shortness of breathing.

The patient suffered from shortness of breath starting from early pregnancy worsened 1 week before admission with exertion or while walking long distances and improved with rest. The patient also complained of
swelling in both legs since early pregnancy, worsened in the last week before admission.

It was her third pregnancy aged 39 weeks. There was no history of hypertension in the previous pregnancies. During the third pregnancy, the blood pressure was always normal, except 1 month before admission.

The patient was diagnosed with SLE in 2017 in other hospital due to malar rash, photosensitivity, joint pain, hair loss, mouth sores and positive result of ANA test and routinely consumed methylprednisolone 4 mg once per day, azathioprine 50 mg twice per day, and calcium lactate 1 tablet once per day. The therapy was continued during this pregnancy.

Physical examination found hypertension (170/100 mmHg), tachycardia (104 beats per minute), tachypnea (24 times per minute). The conjunctiva was anemic. There was pitting edema in both legs.

Laboratory tests resulted anemia (Hb 9.3 g/dL, Hct 28.1%). Complete urine examination showed proteinuria (3+). ANA profile test showed strong positive dsDNA. Echocardiography examination found trivial mitral regurgitation (MR), mild tricuspid regurgitation (TR), mild pulmonal regurgitation (PR), dilated left ventricle (LV), decrease of LV systolic function (EF by Teich 44%), hypokinetic global LV, eccentric LV hypertrophy (LVH). Obstetric USG showed severe oligohydramnios.

According to the history taking and examination, the patient was diagnosed with severe preeclampsia, severe oligohydranmios, SLE, LN, stage II JNC VII hypertension, and peripartum cardiomyopathy (PPCM).

A CITO caesarean section was planned due to severe preeclampsia and severe oligohydramnios. The patient was given MgSO4 40% intravenously 1 gram every hour up to 24 hours postpartum, nifedipine 10 mg every 8 hours orally if blood pressure ≥ 160/110 mmHg, methyldopa 500 mg every 8 hours orally, furosemide 20 mg every 8 hours intravenously, isosorbide dinitrate 5 mg every 8 hours orally, methylprednisolone 62.5 mg once 3 hours before C-section continued by methylprednisolone 62.5 mg 3-6 hours post C-section and calcium 500 mg per day. The patient was also given furosemide injection 20mg intravenously every 8 hours, spironolactone 25mg every 24 hours orally, and ramipril 2.5mg every 24 hours orally to help normalizing heart function. The dose of methylprednisolone was tapered down to 16 mg every 8 hours and azathioprine 50 mg twice per day was given.

The patient was discharged on the eight day of care and suggested to postpone next pregnancy until 1 year after LV function turns to normal. The patient was also planned to visit out-patient clinic after discharged, but she did not.

**Discussion**

Pregnancy and SLE influence each other. Hormonal and immunological changes in pregnancy can affect SLE activity in general, causing both short-term and long-term adverse effects on renal function. On the other hand, SLE leads to pregnancy complications such as preeclampsia, premature birth and fetal loss. Women with SLE are able to control their pregnancy if they choose the optimal time for conception and have proper management during their pregnancy.1,4

Distinguishing LN flare and pre-eclampsia is challenging due to similar symptoms and possibly simultaneous occurrence.5

Diagnosis criteria of SLE according to ACR are: (1) malar rash: (2) discoid rash (3) Photosensitivity (4) inflammation of the mouth or nose (5) non-erosive arthritis in two or more joints accompanied by tenderness, swelling or effusion, (6) heart-lung involvement (7) neurological disorders (8) kidney disorders (9) hematological disorders (10) immunological disorders (11) nuclear antibody (ANA) positive test if no known drugs can trigger.6

The patient had been diagnosed with SLE in 2017 in other hospital due to malar rash, photosensitivity, joint pain, hair loss, mouth sores and positive result of ANA test before admission.
Criteria of LN based on ACR are persistent proteinuria more than 0.5 mg per day or more than 3+ by dipstick, and/or cellular casts including red blood cells, white blood cells, hemoglobin, granular casts, tubular casts, or mixed casts. According to the Italian Nephrology Society, renal flare-ups are defined as an increase in proteinuria of at least 2 g per 24 hours if the basal proteinuria is less than 3.5 g every 24 hours, or doubled if the basal proteinuria is more than 3.5 g per 24 hours, accompanied with concurrent microhematuria and cell cylinders in the urine sediment. Active LN is defined as the appearance of active urine sediment and/or proteinuria more than 0.5 g per day with or without an increase in serum creatinine. Other results found are increase in ds-DNA antibodies and decrease in complement levels. Meanwhile, inactive LN lupus was defined as proteinuria less than 0.5 g per day without urine sediment.

Complete urine examination in this patient resulted proteinuria (protein 3+). C3 and C4 examinations showed low values (C3 27.4 mg/L C4 7 mg/dl). And obtained high ds-DNA antibody results on the ANA profile examination. Therefore, this patient was categorized into active LN based on criteria mentioned.

SLE disease activeness in pregnancy is assessed by modifying the SELENA-SLEDAI score. The score of 3 or less is not counted as a flare, 2-12 is counted as mild to moderate flare, and more than 12 is counted as severe flare. The score of this was 2, so that the patient did not undergo flare.

Diagnostic criteria of preeclampsia are persistent systolic blood pressure of 140 mmHg or higher, or a diastolic blood pressure of 90 mmHg or higher after 20 weeks of gestation in women with previously normal blood pressure, may accompanied by proteinuria 300 mg or more in a 24-hour urine collection. Proteinuria is not absolute criteria for preeclampsia. According to the criteria of the American College of Obstetricians and Gynecologists (ACOG), if preeclampsia is accompanied by systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg while resting, thrombocytopenia, elevated liver enzymes, severe right upper quadrant pain or epigastric pain that does not respond to treatment, progressive renal insufficiency, pulmonary edema, visual disturbances or brain disorders, it is classified into severe preeclampsia. In this case, he patient was classified into severe preeclampsia due to blood pressure of 170/100 mmHg and edema in both legs.

Termination is the main therapy for preeclampsia. Those who have not reached term and have no severe manifestations are still evaluated with a non-stress test 2 times a week. Therapy for severe preeclampsia includes corticosteroids for fetal lung maturation, MgSO4 for eclampsia prophylaxis, and anti-hypertension. The systolic blood pressure target is less than 160mmHg and diastolic less than 110mmHg. The first-line treatment for anti-hypertension in preeclampsia is nifedipine, hydralazine, and labetalol with alternative nitroglycerin and methylldopa.

Patients with severe preeclampsia should be treated with expectative care. If there are contraindications to expectative care, delivery of the pregnancy should be prepared and carried out in stable condition. Contraindications for expansive treatment include eclampsia, pulmonary edema, dissemination of intravascular coagulation (DIC), severe and uncontrolled hypertension, fetal emergency, placental solution, and intrauterine fetal death. Complications of expected treatment include persistent symptoms, HELLP syndrome, IUGR, severe oligohydramnios, premature rupture of membranes, and severe kidney problems. If there are complications, corticosteroids should be given for lung maturation and delivery must be prepared. The patient was then planned for a caesarean section with indications of severe pre-eclampsia and severe oligohydramnios. Patients were also given injection therapy of 40% MgSO4 intravenously 1gram every hour up to 24 hours postpartum, nifedipine 10mg every 8 hours orally (if blood pressure ≥ 160/110 mmHg), methylldopa 500mg every 8 hours orally.

The treatment of LN consists of two phases: induction and maintenance. Induction therapy refers
is purposed to cure active disease while maintenance therapy to continuously maintain the remission and prevent organ damage.

Corticosteroids the main treatment of LN. They are effective in controlling kidney flares, but not to improve long-term results alone. However, steroid exposure should be limited to a minimum during pregnancy. High doses during pregnancy are associated with an increased risk of diabetes, hypertension, pre-eclampsia, premature rupture of membranes and congenital fetal abnormalities. Nevertheless, short doses and/or high doses of intravenous methylprednisolone can be used in the case of flares.\textsuperscript{12}

Azathioprine may be the safest immunosuppressant drug to take during pregnancy. This drug is recommended especially if it has been consumed before pregnancy.\textsuperscript{2} Apart from azathioprine, other immunosuppressants that can be used during pregnancy are cyclosporin A and hydroxychloroquine.\textsuperscript{3}

The patient had taken 3x8mg Methylprednisolone orally, Azathioprine 2x50mg orally, 1x1 calcium lactate before admission. Then, the patient was then given methylprednisolone 1x62.5mg intravenous therapy 3 hours before cesarean section then followed by injection of methylprednisolone 62.5 mg intravenously every 3-6 hours postoperatively. Methylprednisolone therapy was continued and tapered down on the third day. Azathioprine 2x50mg was continued.

SLE in pregnancy should be monitored by an obstetrician and rheumatologist.\textsuperscript{5} Pregnancy should be postponed until the disease is said to be inactive, at least 6 months before conception.\textsuperscript{2} Basic laboratory tests should be repeated at the first prenatal visit and must be checked if no preconception counseling has been done. Monthly prenatal visits are necessary. The frequency of laboratory examinations depends on SLE or LN activity. Serial fetal ultrasound examination is planned to monitor fetal growth.\textsuperscript{1}

A kidney biopsy is only indicated to rule out or classify LN if the results will affect management, due to risk of bleeding after biopsy in pregnancy.\textsuperscript{5} A kidney biopsy is not recommended after 32 weeks of gestation.\textsuperscript{1}

**Conclusion**

LN is often superimposed severe preeclampsia, which could end up with maternal and fetal mortality. This case showed early diagnosis and proper treatment. This kind of case commonly happens in pregnant women with LN.

**Conflict of Interest:** The authors report no conflict of interest.

**Funding:** None.

**Ethical Clearance:** Not required for a case report.

**Acknowledgment:** The authors would like to thank to Medical faculty of Universitas Airlangga, Surabaya, Indonesia.

**References**


Knee Osteoarthritis Severity in Relation to Neutrophil-Lymphocyte Ratio

Auday Hussain Hassan Al-Janaby
Orthopedic Surgeon, Orthopedic Department, Al-Zahrawi Hospital, Misan Health Directorate, Ministry of Health/ Environment, Misan, Iraq

Abstract

Background: Osteoarthritis is a disease with a significant inflammatory component. Neutrophil-lymphocyte ratio (NLR) level is a marker to determine inflammation. The study aimed to evaluate the association between severity of knee osteoarthritis and NLR.

Methods: A case-control study conducted in Al-Zahrawi during June 2017 to June 2018. A total of 100 knee osteoarthritis were recruited and 50 healthy subjects as a control group.

Results: NLR was significantly higher in knee osteoarthritis than controls, the mean value was (2.19±0.72) and (1.97±0.88), respectively. Older age, chronic pain, prolong onset of diagnosis and high NLR were significantly associated with severe osteoarthritis.

Conclusions: Knee osteoarthritis had higher NLR compared to healthy people. Higher NLR is associated with severity of knee osteoarthritis and it is a poor indicator.

Key Words: Neutrophil-lymphocyte ratio; osteoarthritis; bone sclerosis; osteophytes

Introduction

Osteoarthritis (OA) is the most common form of arthritis, and it is a leading musculoskeletal cause of disability in elderly persons\(^1\). OA is chronic multifactorial disease with progressive joint degeneration accompanied by sub-chondral bone sclerosis, bone cysts formation, marginal osteophytes, knee flexibility decrease, arthralgia, joint effusion, crepitus, and deformities\(^2\). It is likely to increase due to the aging and obesity\(^3\). The diagnosis confirm by history, physical examination, imaging studies and laboratory testing\(^4\). X-rays reveal marginal osteophytes, narrowing of the space, increased density of the subchondral bone, subchondral cyst formation, bony remodeling and joint effusions\(^5\). NLR is the ratio between the absolute neutrophil and lymphocyte counts\(^6-8\).

Methods

Study design and Setting

This was a case-control study conducted in Al-zahrawi during June 2017 to June 2018. A total of 100 knee osteoarthritis were recruited and 50 healthy subjects as a control group.

Participants

A total of 100 patients with OA according to the American College of Rheumatology clinical criteria \(^9\), who attended the outpatients clinic. A total of 50 healthy subjects who agreed to participate in the study.
Exclusion criteria

1. History of knee trauma.
2. Post infection arthropathy.
3. Tumor.
4. Rheumatoid arthritis.
5. Systemic lupus erythematosus (SLE).

Data collection

Data were collected including age, gender, BMI, smoking, drug history, and medical history. The clinical and laboratory data including the radiological findings and NLR. The OA grade was assessed according to the Kellgren-Lawrence (KL) grading system\(^{10}\).

Statistical Analysis

Data entered and analyzed using the statistical package for social sciences (SPSS) version 24, International Business Machines Corporation (IBM), United states (US), 2015. Descriptive statistics presented as frequencies, percentage (%), mean and standard deviation. Analytic statistics performed using Chi-square to compare frequencies for student’s t test and ANOVA test were used to compare means. Level of significance was set at < 0.05.

Results

No statistically significant differences had been found between the groups in demographic variables, (Table 1). The value of NLR; patients with OA had significantly higher NLR than controls, the mean NLR was \((3.02\pm0.87)\) and \((1.86\pm0.9)\), respectively, \((P=0.001)\), (Table 2). Regarding the distribution of the knee osteoarthritis patients according to their KL grading scale, \((18\%)\) grade I, \((32\%)\) grade II, \((21\%)\) grade III and \((29\%)\) grade IV, (Table 3). Regarding to the severity, among these variables, age, and NLR were significantly different, (Table 4).

<table>
<thead>
<tr>
<th>Table 1. Demographic of the studied groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA (No.= 100)</td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Age (year)</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>BMI</td>
</tr>
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</table>
Table 2. NLR of the studied groups.

<table>
<thead>
<tr>
<th></th>
<th>OA(No.= 100)</th>
<th>Control(No.= 50)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLR</td>
<td>3.02±0.87</td>
<td>1.86±0.9</td>
<td>0.001</td>
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</tbody>
</table>

Table 3. Osteoarthritis patients according to the Kellgren-Lawrence grading system.

<table>
<thead>
<tr>
<th>Grade</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>II</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>III</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>IV</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Association of age and NLR with severity of OA.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mild (No.= 18)</th>
<th>Moderate(No.= 53)</th>
<th>Severe (No.= 29)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>60.1 ± 8.2</td>
<td>62.4 ± 7.7</td>
<td>64.1 ± 9.1</td>
<td>0.001</td>
</tr>
<tr>
<td>NLR</td>
<td>2.76 ± 0.55</td>
<td>3.11 ± 0.6</td>
<td>3.81 ± 1.21</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Discussion

The demographic data are similar to the clinical picture of OA documented previously, where majority of the patients were old age and females were the dominant gender\(^{(11, 12)}\). There is no statistically significant differences in all hematological parameters except NLR. Despite that there were very few studies available about the association between NLR and OA. The findings were consistent with Tasoglu, et al.\(^{(13)}\).

Recently, Hira and Tamam\(^{(14)}\), reported the mean NLR was significantly higher in OA group than controls. There was inconsistency about the predictive role of NLR in diagnosis, prognosis or as a marker for disease activity for other diseases\(^{(15-17)}\).

The correlation between severity of OA and the variables that showed significant association on univariate analysis, older age and higher NLR were still significantly associated with severe OA. Previous studies mentioned that old age is a significant risk factor of OA and associated with severe OA\(^{(11-13, 18)}\). The explanation beyond could be attributed to the general incidence and prevalence of OA has been reported to increase by ten times in older age than younger, particularly > 65 years who are commonly affected by OA. Also, there is a change in behavior, lifestyle, diet and other factors. In addition, the cells and extracellular matrix of joint
tissues increase the susceptibility of older adults to OA (18, 19).

Conclusions

Osteoarthritis had higher NLR compared to healthy subjects. NLR is a poor predictor of OS severity. Age was a stronger risk factor of severity.

Declaration of Competing Interest-Nil

Source of Funding- Self.

Ethical Clearance- Taken from Orthopedic Department, Al-Zahrawi Hospital committee

References


Bronchodilator Effect of *Crataegus azarolus* var *aronia* Unripped Fruit Extracts on Rat’s Tracheal Smooth Muscle

Aveen A. Mohammed¹, Omar A. M. Al-Habib²

¹Lecturer, Department of Biology, Faculty of Science, University of Zakho, Zakho, ²Professor, Department of Biology, College of Science, Nawroz University, Duhok, Kurdistan Region, Iraq

Abstract

**Objectives:** The present study aimed to investigate the bronchodilator effect of different *Crataegus azarolus* var *aronia* unripped fruit extracts on tracheal smooth muscle of rats and to study the roles of K channels and EDHFs in produced responses.

**Background:** *Crataegus* is widely used in Kurdistan Region for the prevention of several diseases such as respiratory, cardiovascular, hypertension …. etc. due to its content of several bioactive compounds like aromatic amines, phenolic acid, flavonoids, and proanthocyanidins.

**Method:** The bronchodilator effects of various *Crataegus* fruit’s extracts on rat’s tracheal rings and the roles of K⁺, Ca²⁺ channels and EDHFs in bronchodilation using specific blockers.

**Results:** Most of *Crataegus* fruit’s extracts used showed a potent bronchodilator effect on trachea, in which the HME produced a highly significant dilation, followed by methanol and ethyl acetate extracts which showed a considerable relaxant effect. The results confirmed that the induced dilation was NO and PGI₂ dependent, along with activations of K<sub>ATP</sub> and Kir and Kca while Kv plays a minor and limited role.

**Conclusion:** The novel results indicate that HM, M, EA extracts prepared from *Crataegus* unripped fruit produce a potent bronchodilator effect, which was NO and PGl₂ dependent along with the activation of K<sub>ATP</sub>, Kir and Kca<sup>2⁺</sup> channels.

**Key Words:** *Crataegus*, trachea, bronchodilator, methanol, hydromethanol, ethylacetate, extracts.

Introduction

A widespread use of herbal formulations throughout the world has increased due to their cultural acceptability, availability, affordability, efficacy, and safety claims<sup>(1,2)</sup>. Surveys on the therapeutic uses of *Crataegus aronia*, the dominant species which populate the Mediterranean Basin Mountains, has been used in the traditional medicine to treat diabetes, cancer, as well as cardiovascular and respiratory diseases<sup>(3)</sup>. During the past two decades, herbal medicine have received a great concern as novel options of therapy for human disease treatment<sup>(4)</sup>. It is widely agreeable that the presence of various bioactive compounds such as aromatic amines, essential oils, phenolic acids, flavonoids, proanthocyanidins and triterpenes<sup>(5,6,7)</sup>. These Components are responsible for most of the pharmacological effects of the medicinal plants<sup>(8)</sup>. The most promising among which are edible plants, due to their negligible adverse effects<sup>(9,10)</sup>. Medicinal plants have been used as primary sources in treatment and prevention of several diseases such as congestive heart failure<sup>(11)</sup>, angina pectoris, hypertension, and arrhythmias<sup>(12)</sup>, blood thinning effect<sup>(13)</sup>, lower the total cholesterol in plasma<sup>(11)</sup>. In addition, it also acts as antioxidant<sup>(14)</sup> and in cardio-myogenesis and angiogenesis from stem cells<sup>(15)</sup>. However, due to the availability of limited studies on its effects on pulmonary diseases, especially obstructive pulmonary diseases, (<sup>(16)</sup>) and since most of the studies related to the use of *Crataegus* in the treatments of various diseases
focused on the use of leaf and root extracts, whereas very little is known about the effect of *Crataegus* fruits. Thus, the current research project is adopted to study the bronchodilator effect of *Crataegus aronia* unripped fruit extracts to explore the possibility of its use in the treatment of respiratory diseases and its mechanism of action.

**Materials and Methods**

**Preparation of *Crataegus aronia* unripped fruit Extracts**

The shade-dried powder (500g) prepared from *C. aronia* was soaked in 2.5 L of 99.98% ethylacetate, methanol and hydromethanol (60% methanol) repeatedly 5 times, each for 10 days with occasional shaking. The extract was filtered through muslin clothes and then through Whatmann No.1 filter paper. The filtrates of each extract were combined and concentrated by thin film rotary evaporator at 40 °C under reduced pressure. Thick crude extracts of *C. aronia* fruits were stored at −4 °C for future use. For the preparation of desired serial dilutions, a needed amount of the extract was dissolved in deionized water from which serial dilutions were prepared and warmed up at 37°C.

**Animals**

Wister male Albino rats, weighing 210–310 g used in this study were obtained from Animal House (Department of Biology, College of Science, University of Zakho). Animals were reared in PCV cages kept in a well-ventilated animal house room. During the experimental period, four to six animals were kept in each cage, kept under controlled laboratory conditions at 25 °C and photoperiod of 12 hours’ light–dark cycle. Rats had free access to dechlorinated tap water and standard rodent pellets.

**Isolated Tracheal Preparation and Experimental Protocol**

After anesthetization of the rat, the trachea was removed, cleaned, and transferred into a petri-jar containing aerated physiological solution. The trachea cut into small rings (each consists of 3-4 cartilaginous ring). The ring was fixed in a 10 ml glass chamber containing Krebs solution at 37°C and the two stainless-steel wires were used to connect the ring to the bottom of glass chamber from one side and to force transducer from the other side and subjected to 1 gm tension. The preparation allowed to equilibrate for 1 h before the addition of any drug. Meanwhile, it was washed with buffer solution every 15 min during the equilibration period. The pH of the buffer was maintained at 7.4 by continuous aeration of the physiological solution with a carbogen gas (consists of 5% CO<sub>2</sub> and 95% O<sub>2</sub>). To study the effect of *Crataegus* unripped fruits extracts, a dose response curve was established for each extract by cumulative addition of different concentrations of each extract and the response was recorded. To find out the role of various ion channels and hyperpolarizing agents, the tissues were pre-incubated either with desired blocker or inhibitor such as TEA, BaCl<sub>2</sub> and 4-AP (1×10<sup>-3</sup>M), GLIB (1×10<sup>-5</sup>M), nifedipine (1x10<sup>-5</sup>M) for 20 minutes or L-NAME (3×10<sup>-4</sup>M), methylene blue (1x10<sup>−5</sup>M) and indomethacin (3x10<sup>-3</sup> M) for 30 minutes prior to the application of ACh.

**Statistical Analysis**

Data are presented as Means ± SEM. The IC50s values (the concentration of the agonist or extract that produced 50% reduction in the maximal relaxant responses) were determined from the concentration–response curves by non-linear regression analysis using GraphPad Prism™ software, version 6.0 (GraphPad Software, USA).

**Results**

**Effect of Ethyl Acetate Extract on Isolated Rat’s Trachea**

1. Effect of Pre-Treatment of Tracheal Rings with L-Name, Methylene blue and Indomethacin on EAE-Induced Relaxation

Both L-NAME and Indomethacin produced a mild and non-significant inhibition in the in the DRCs
of EAE induced relaxation in tracheal ring, which reflect the limited participation of NO and PGI\textsubscript{2} in the induced relaxation (Table 1); while methylene blue produced a reverse response in the induced relaxation since the DRC was significantly enhanced, especially at the last three doses used.

Table 1: The LogIC\textsubscript{50}, (LogIC\textsubscript{50} of 95% CI) and Emax ± SEM induced by EAE in trachea pretreated with L-NAME, methylene blue and indomethacin.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Ethylacetate</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>Control</td>
<td>Indomethacin</td>
<td>Methylene Blue</td>
</tr>
<tr>
<td>LogIC\textsubscript{50}</td>
<td>-0.6959</td>
<td>-0.6369</td>
<td>-0.8056</td>
</tr>
<tr>
<td>LogIC\textsubscript{50} of 95%</td>
<td>-1.183 -0.2092</td>
<td>-1.204 -0.07001</td>
<td>-0.9657 -0.6456</td>
</tr>
<tr>
<td>Emax ± SEM%</td>
<td>44.10 ± 0.24</td>
<td>41.118 ± 0.28</td>
<td>72.24 ± 24.34</td>
</tr>
</tbody>
</table>

2. Effect of Pre-Treatment of Tracheal Rings with K\textsuperscript{+} and L-type Ca\textsuperscript{2+} channel blockers on EAE-Induced Relaxation

The DRCs induced by EAE in tracheal rings were inhibited to a variable extent in the presence of K channel blockers since TEA, GLIB and BaCl\textsubscript{2} caused inhibition in induced relaxant responses. Thus, the inhibition was significant (P<0.05-0.1) in the presence of TEA at high doses, whereas both GLIB and BaCl\textsubscript{2} produced non-significant inhibition (Table 2). In contrary, 4-AP produced non-significant enhancement in the induced relaxation since the Emax was increased from 44.1 in the control to 55.17 %. This indicate that Kca\textsuperscript{2+} plays a significant role in the EAE induced relaxation, while both Kir and K\textsubscript{ATP} channels played a limited role in the induced responses. However, Kv channel showed no role in this relaxation at all. The presence of Nifedipine also showed non-significant inhibition in the relaxant response, which also reflects the limited and indirect role of L-type Ca\textsuperscript{2+} channel in the induced response.

Table 2: The LogIC\textsubscript{50}, (LogIC\textsubscript{50} of 95% CI) and Emax ± SEM induced by EAE in trachea pretreated with TEA, GLIB, BaCl\textsubscript{2} and 4-AP.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Ethylacetate</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>Control</td>
<td>TEA</td>
<td>BaCl\textsubscript{2}</td>
</tr>
<tr>
<td>LogIC\textsubscript{50}</td>
<td>-0.6959</td>
<td>-0.6048</td>
<td>-0.6978</td>
</tr>
<tr>
<td>LogIC\textsubscript{50} of 95%</td>
<td>-1.183 -0.2092</td>
<td>-1.457 0.2475</td>
<td>-0.7791 -0.6164</td>
</tr>
<tr>
<td>Emax ± SEM%</td>
<td>44.10 ± 0.24</td>
<td>15.65 ± 0.42</td>
<td>44.10 ± 6.93</td>
</tr>
</tbody>
</table>
Effect of *Crataegus* Fruit's Methanol Extract on Isolated Rat's Trachea

1. Effect of Pre-Treatment of Tracheal Rings with L-Name, Methylene blue and Indomethacin on ME-Induced Relaxation

Pretreatment of tracheal rings with Indomethacin and L-Name inhibited the relaxant effect induced by ME, but not to the same extent since the inhibition produced by high doses of ME was highly significant, while L-Name produced a non-significant inhibition (Table 3). In contrast, the DRC in presence of methylene blue was slightly and non-significantly enhanced as indicated by changing Emax from 71.40 ± 0.13 in the control to 74.48% in MB treated rings. These results indicate that the induced relaxation by ME is partially produced by PGI2 through COX pathway (P<0.001), but the role of NO pathway was limited. On other hand, cGMP inhibitor (MB), slightly and non-significantly enhanced the induced relaxant response.

Table 3: The LogIC50, (LogIC50 of 95% CI) and Emax ± SEM induced by ME in trachea pretreated with L-NAME, methylene blue and indomethacin.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>LogIC50</td>
<td>0.6078</td>
</tr>
<tr>
<td>LogIC50 of 95%</td>
<td>0.3476</td>
</tr>
<tr>
<td></td>
<td>To</td>
</tr>
<tr>
<td></td>
<td>0.8681</td>
</tr>
<tr>
<td>Emax ± SEM%</td>
<td>71.40 ± 0.13</td>
</tr>
</tbody>
</table>

2. Effect of Pre-Treatment of Tracheal Rings with K+ and L-type Ca2+ channel blockers on ME-Induced Relaxation

The results of the effect of preincubation with K+ channels blockers on ACh precontracted tracheal ring revealed that K_{ATP} channels played a highly significant (P<0.001) role in the induced relaxation whereas Kir played a limited role in the relaxation as indicated by the inhibitory effects of GLIB and BaCl2 on DRCs. In contrary, both Kca^{2+} and Kv played no role at all in the induced relaxation responses since their blockers enhanced rather than inhibiting the relaxant responses (Table4). Nifedipine (L-type Ca^{2+} channel blocker) produced no effect on the relaxant response produced by ME, this indicate that L-type Ca^{2+} channels plays no role in the induced relaxation.
Table 4: The LogIC50, (LogIC50 of 95% CI) and Emax ± SEM induced by ME in tracheal Rings pretreated with TEA, GLIB, BaCl2 and 4-AP.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Methanol</th>
<th>Treatments</th>
<th>Control</th>
<th>TEA</th>
<th>GLIB</th>
<th>BaCl2</th>
<th>4-AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LogIC50</td>
<td></td>
<td></td>
<td>0.6078</td>
<td>0.4656</td>
<td>0.4974</td>
<td>0.5160</td>
<td>0.4465</td>
</tr>
<tr>
<td>LogIC50 of 95%</td>
<td></td>
<td></td>
<td>0.3476 to 0.8681</td>
<td>0.3328 to 0.5984</td>
<td>0.4588 to 0.5360</td>
<td>0.4225 to 0.6095</td>
<td>0.2074 to 0.6857</td>
</tr>
<tr>
<td>Emax ± SEM%</td>
<td></td>
<td></td>
<td>71.40 ± 0.13</td>
<td>74.96 ± 22.86</td>
<td>44.48 ± 5.26</td>
<td>57.92 ± 20.39</td>
<td>126.13 ± 0.12</td>
</tr>
</tbody>
</table>

Effect of *C. aronia* unripped fruits HME on Isolated Rat’s Tracheal Rings

1. Effect of Pre-Treatment of Tracheal Rings with L-Name, Methylene blue and Indomethacin on HME-Induced Relaxation

   Typical DRCs from representative experiments on the effect of L-Name, methylene blue and indomethacin on the relaxation induced by HME in tracheal smooth muscle precontracted against ACh are shown in figure (Table 5). The results showed that all the DRCs are shifted to the right hand but to variable extents. The relaxation response for ACh precontracted tracheal rings showed a highly significant (P<0.01) inhibition in the presence of L-NAME at a concentration 0.42 mg/ml with Emax inhibition from 91.63 ± 0.09 in the control to 67.46% (Table 5). However, both Indomethacin and methylene blue caused a mild and non-significant inhibition in their DRCs. The mild inhibitory effect of methylene blue was diminished at high doses of ME. This indicates that NO pathway playing an important role in the induced relaxation, whereas the roles Indomethacin and MB were limited.

Table 5: The LogIC50, (LogIC50 of 95% CI) and Emax ± SEM induced by HME in trachea pretreated with L-NAME, methylene blue and indomethacin.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Hydromethanol</th>
<th>Treatments</th>
<th>Control</th>
<th>L-NAME</th>
<th>Methylene Blue</th>
<th>Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>LogIC50</td>
<td></td>
<td></td>
<td>0.3965</td>
<td>0.4546</td>
<td>0.4713</td>
<td>0.4395</td>
</tr>
<tr>
<td>LogIC50 of 95%</td>
<td></td>
<td></td>
<td>0.2121 To 0.5809</td>
<td>0.4223 To 0.4869</td>
<td>0.3747 To 0.5679</td>
<td>0.3467 To 0.5324</td>
</tr>
<tr>
<td>Emax ± SEM%</td>
<td></td>
<td></td>
<td>91.63 ± 0.09</td>
<td>67.46 ± 2.96</td>
<td>91.27 ± 0.05</td>
<td>67.21 ± 0.05</td>
</tr>
</tbody>
</table>
2. Effect of Pre-Treatment of Tracheal Rings with K⁺ and L-type Ca²⁺ channel blockers on HME-Induced Relaxation

Preincubation of ACh precontracted tracheal rings with both BaCl₂ and GLIB caused significant (P <0.01 to 0.05) inhibition in DRCs at high doses (0.54 & 0.58mg/ml) of HME (Table 6). This reflecting the major role of K<sub>ATP</sub> and Kir channels in the relaxant effect produced by HME. In addition, Kv channels also offered a limited participation in the induced relaxation, while Kca2+ played no role at all in this relaxation since the presence Kv blocker (TEA) did not affect the DRC which was very close to that of the control. Preincubation of the tracheal rings with L-Type Ca²⁺ channels blocker (Nifedipine) caused a mild and non-significant inhibition in DRC, which reflect the limited role of L-Type Ca²⁺ channels in the relaxation induced by HME.

Table 6: The LogIC50, (LogIC50 of 95% CI) and Emax ± SEM induced by HME in trachea pretreated with TEA, GLIB, Bacl2 and 4-AP.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Hydromethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>Control</td>
</tr>
<tr>
<td>LogIC50</td>
<td>0.3965</td>
</tr>
<tr>
<td>LogIC50 of 95%</td>
<td>0.2121</td>
</tr>
<tr>
<td>Emax ± SEM%</td>
<td>91.63 ± 0.09</td>
</tr>
</tbody>
</table>

**Discussion**

Pretreatment of the tracheal rings with L-NAME, (NO synthase inhibitor), reduced the relaxant effects induced by HME, EAE and ME against ACh precontracted tracheal rings, indicating that this relaxant effect involves the synthesis and release of NO by nNOS which has beneficial effects in asthma, causing bronchodilation by relaxing airway smooth muscle via NANC nerves activation (17). Nitric oxide causes relaxation in smooth muscle via both cGMP dependent and -independent mechanisms depending on the levels of NO. At intermediate NO level, it activates Kca channel whereas at higher NO levels is accomplished by a cGMP – dependent mechanism (18). This pathway also includes the activation of cGMP-dependent protein kinase (PKG) by cGMP, which in turn, regulates numerous target proteins, e.g., Kca channel (IKCa), L-type Ca²⁺ channel (ICaL), sarcolemma Ca²⁺-ATPase pump (ICaP) currents, and myosin light chain phosphatase (MLCP), that lead to SMC relaxation. Prakash et al., confirmed that NO donors inhibit Ca²⁺ ion liberations by blocking sarcoplasmic reticulum Ca²⁺ release and promoting Ca²⁺ extrusion in porcine tracheal rings causing tracheal smooth muscle cells relaxation and subsequent bronchodilation (19). While cGMP did not participate in HME induced relaxation in tracheal SMCs; which was confirmed by using methylene blue (cGMP inhibitor) that did not alter the HME induced relaxation. This indicates that the relaxant effect of NO in rat tracheal smooth muscle is due to the activation of guanylate cyclase and production of the second messenger cGMP which reduces intracellular Ca²⁺ in smooth muscle.
cells. This mechanism was confirmed by (20), who concluded that cGMP activates intracellular effectors, such as PKG, which decreasing the intracellular Ca$^{2+}$ and dissociation of actin and myosin filaments and finally leading to SMCs relaxation.

The relaxant effect of ME, HME and EAE in association with production of the prostaglandin (PGI$_2$) was confirmed in trachea by inhibition of cyclooxygenase on its preincubation with indomethacin, which highly inhibited the relaxation responses. This clearly reflects the important role of PGI$_2$ in the induced relaxation which diminished in the presence of PGI$_2$ blocker Indomethacin. A similar effect of PGI$_2$ was exhibited by rat’s aorta in response to quercetin (21) and euscaphic acid (6) which are the important constituents C. aronia extracts (22).

Voltage-gated potassium (Kv) channel had an important role in relaxation induced by HME, because it decreased maximum relaxation response in tracheal rings pre-incubated with 4-AP (Kv channel blocker). This suggesting that the transient increase of cytosolic Ca$^{2+}$, activate calcium channels in the plasma membrane to influx Ca$^{2+}$ from extracellular fluid and to produces depolarization which in turn activate Kv channels to hyperpolarize membrane and facilitate smooth muscle relaxation (22, 23).

Calcium activated potassium (Kca) channel also plays an important role in the relaxation mediated by EAE and HME since KCA channel blocker) that significantly inhibited the induced relaxation. This clearly indicate that Kca channels playing an important role in the relaxation induced by both EAE and HME, whereas in ME-mediated relaxation, plays a limited role in the induced relaxation which was mildly inhibited. Depending on K$^+$ influx through the activation (Kca) channel because TEA (Kca channel blocker) highly inhibited EAE-induced relaxation and non-significantly inhibited ME-induced relaxation effects. A similar trend of response was reported for C aronia active ingredients such as quercetin induced relaxation of coronary arteries (24) and euscaphic acid induced relaxation (6) since both studies reflecting the important role of Kca channel in inducing smooth muscle relaxation.

The results of the current research indicated that HME, ME, and EAE -mediated relaxation partially dependent on the activation of K$_{ATP}$ sensitive channels since in the presence of GLIB (K$_{ATP}$ sensitive channel), the HME and ME induced relaxant responses in tracheal rings were strongly inhibited. However, EAE induced relaxation was non-significantly inhibited. K$_{ATP}$ channels are stimulated by its openers, such as pinacidil and cromakalim, so the opening of these channels causing K$^+$ efflux, hyperpolarization in the smooth muscle cell membrane and subsequent vasodilation and drop in blood pressure (25).

Inward-rectifier potassium (Kir) channel also involved in relaxation induced by HME. This was confirmed by pre-incubated of tracheal rings with BaCl$_2$ (Kir channel blocker), which strongly inhibited HME-mediated relaxation. The importance of this type of channel is confirmed by (26) who found that Kir channel has an important role in the organizing the resting membrane potential and resting tone in smooth muscle. Similarly, (27) reported that Kir channels have an important role in determining the resting potential of the cell.

Pre-incubation of trachea with nifedipine (L-type Ca$^{2+}$ channel blocker) non-significantly decreased the relaxant effects of HME, EAE extracts or not changed at all on preincubation with ME. However, (28) found in mouse that the relaxation induced by NO in lung’s smooth muscle cells occurs through cGMP-PKG pathway, decreasing the frequency of agonist-induced Ca$^{2+}$ oscillation and inhibiting the release of Ca$^{2+}$ via IP3 receptors, leading to the activation of K$^+$ channels and relaxation of smooth muscle cells.

**Conclusions**

Ethyl acetate, methanol and hydromethanol extracts Crataegus unripped fruit’s produced a potent bronchodilator effect in rat’s trachea in which hydromethanol extract produced a highly significant
dilation followed by methanol and ethyl acetate extracts which produced considerable relaxant effects. This potent relaxant effect on the bronchial smooth muscle cells was depending on NO and PGI2 along with the activation of dependent on $K_{\text{ATP}}$ and Kir and Kca channels.

**Conflict of Interest:** The authors declare that there is no conflicts of interest regarding the publication of this manuscript. Ethics Approval: Obtained from Animal Ethics Committee of the University of Zakho- Duhok- Kurdistan Region Iraq

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Effect of Murotal Sound Stimulation during Pregnancy on the Number of Neuron Cells of Cerebrum and Cerebellum of the Newborn Rattus norvegicus

Ayu Putri Yani1, Hermanto Tri Joewono2, Widjiati3

1 Postgraduate Student of Reproductive Health Science, Airlangga University, Surabaya, Indonesia, 2 Lecturer at Department of Obstetrics and Gynecology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia, 3 Professor at Department of Embryology, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia

Abstract

Background: The human development index (HDI) illustrates how superior human resources are. The stimulation of Murotal during growth and development has been shown to increase cell growth in plants. The aim of the study was to analyze the effect of the differences against the number of neuron cell’s in the cerebrum and cerebellum Rattus norvegicus offspring between those who received Al-quran Murottal surah Ar-Rahman stimulation, Qori and Qoriah sounds voice in pregnancy day of 6th.

Methods: An Experimental study using 30 pregnant (Rattus norvegicus). Subjects were randomized into three groups, control group (Q1), voice of Qori group (Q2), and voice of Qoriah group (Q3). sound stimulation was given on 6th-17th days of pregnancy. From each mother, two Newborn of Rattus norvegicus were taken to make preparations from brain tissue. Eosin Hematoxylin was performed to assess number of neuron cells.

Results: The study shows that the mean and standard deviation of the number neuron cells cerebrum and cerebellum in the control group is 9.88 ± 1.71 and 21.10 ± 2.11, the voice qori group is 10.16 ± 1.01 and 22.46 ± 1.71, and the voice qoriah group 11.12 ± 1.52 and 22.50 ± 1.76. ANOVAs statistical test results show there is no significant differences among groups with a value of p = 0.152 in the cerebrum and p=0.183 in the cerebellum.

Conclusion: Murotal sound stimulation during pregnancy increased the number of neuron cells in the cerebrum and cerebellum of newborn rats.

Keywords: neurons; cerebrum; cerebellum; murotal; sound

Introduction

Global challenge in all countries is competition for human resource development, not only related to material functions, but directly related to the intelligence function of the brain intelligence to intelligence competitive, brain to brain competition. preparing the next generation needs to be done, especially creating quality human resources, has adequate intelengensi prepare for life in an increasingly competitive[1].

Integrated management of brain intelligence will produce human resources who are intelligent, have high competence, ability, skills, and competitiveness. Intelligence is related to the efficiency of the information transfer process in the brain that can be attempted since the prenatal period. Indonesian Child Doctors Association, the first day of life is called the golden age, the period from early pregnancy until the child is born and 2 years of age is an important period, determining the quality of health in life next. At that time the brain development has reached 80% of the brain[2].

Corresponding Author:
Prof. Widjiati, PhD
Associate Professor, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya 60115, Indonesia.
E mail : widjiati@fkh.unair.ac.id
The development and growth of the brain as well as the maintenance of intellectual health in the fetus affect cognitive, social and emotional abilities for the foundation of development and development of future generations. The cerebral cortex is a part of the brain that has cognitive function, but recent research has shown that the cerebrum (cerebrum) and cerebellum (cerebellum) contain more neuron cells than the cerebral cortex which allows them to be involved in cognitive function\[^3\].

Demonstrated that the cerebrum and cerebellum are interconnected via polysynaptics, forming a system associated with cognitive function and neuropsychiatric disorders. So it can be ascertained that the cerebrum and cerebellum play an important role in motor, cognitive, emotional and behavioral development\[^4,5\]. Therefore, stimulus and nutrition provided during pregnancy play an important role in fetal brain function. The fetal brain at that time undergoes proliferation, migration, differentiation, myelination, synaptogenesis, and apoptosis which are influenced by environmental and genetic factors. environment such as nutrition and stimulation\[^6,7\].

Efforts that can be made during pregnancy are to provide adequate nutrition and stimulation. Stimulation that is easily accepted by the fetus is stimulation in the form of sound. Experts claim that sound stimulation in the prenatal period is an environmental factor that can affect the growth of the fetus in the womb and that there are certain parts of the brain that are affected by familiar music rather than music unknown to listeners\[^3,4,8,9\]. In Indonesia, the majority of people are Muslim so that the murotal voice is often heard. Listening to murotal has a calming and relaxing effect on a person, so that it will also contribute to lowering blood pressure\[^10,11\]. Murotal both sung by men and women can be distinguished by type frequency and sound intensity. The accuracy of the voice in women reaches 100% while in men 95.47% this happens because the male voice is detected to be similar to the female voice so that the accuracy of the male voice is reduced\[^12–14\].

This study identifies differences the effect number neuron cells of stimulating the murotal quran in the cerebrum and cerebellum of newborn mice (\textit{Rattus norvegicus}) whose mothers to murotal qori voice stimulation and murotal qoriah voice stimulation during pregnancy.

**Materials and Methods**

This research is an experimental study on mice (\textit{Rattus norvegicus}) which was conducted from February to April 2021 at the Laboratory of the Faculty of Veterinary Medicine, UNAIR, Surabaya. This study used 30 adult female mice (\textit{Rattus norvegicus}) aged 2-3 months of pregnancy which were exposed to stress during pregnancy on the 6th until 17th days of pregnancy. The research subjects were divided into 3 groups which were randomly selected (Q1, Q2, and Q3) with 10 mice in each group.

The first group: the group was not given any treatment, the rats were only pregnant and were allowed to eat and drink like pregnant rats in general.

Second group: qori sound stimulation group, pregnant rats on days 6-17 in a soundproof room for 1 hour playing ar rahman letters with sound intensity close to 65 dB at night.

The third group: the qoriah sound stimulation group, pregnant rats on days 6-17 in a soundproof room for 1 hour playing ar rahman letters with a qoriah sound with a sound intensity close to 65 dB at night Fourth group: Control group with standard treatment without stress exposure.

**Sampling Inspection**

\textit{Rattus norvegicus} mothers were anesthetized then the pups were born by sectio caesarea (SC) on the 17th day of pregnancy. The pups of \textit{Rattus norvegicus} which were to be sacrificed were anesthetized first, and then the cranium was cut in the sagittal direction from caudal (occipital) to rostral (frontal), right between the two hamisters of their brain. Furthermore, the brain was released. The separated brain was weighed, and then put in a 10% formalin solution for organ preservation; the cerebrum and cerebellum were taken. Furthermore,
Hematoxylin-Eosin (HE) staining were made.

**Data Analysis**

To see the normality of the data, the Shapiro-Wilk test was used. If the data obtained are normally distributed, then the ANOVA test is used followed by LSD (Least Significant Difference) to see the differences in all groups. If the data obtained are not normally distributed, the Kruskall Wallis test and the Mann Whitney test are used. This study uses a significance level of P<0.05. To simplify statistical calculations, researchers used the SPPS tool version 21.

**Results and Discussion**

**Results**

The results showed the highest number of neurons in the cerebrum and cerebellum on murotal sound stimulation compared to the control group, the qori sound murotal stimulation group, and the qoriah sound murotal stimulation group (Table-1).

The results of the normality test using the Shapiro-Wilk test in the treatment group obtained a significance value (p-value)> 0.05, which means that the data distribution was normally distributed, so the Analysis of Variance (ANOVA) test was used. Tested whether there were differences in the treatment group in the number of neuron cells in the cerebrum and cerebellum.

Based on the results of the ANOVA test in table-2, it is known that there is no significant difference between groups in the number of neurons in the cerebellum and cerebellum in the newborn *Rattus norvegicus*.

**Table 1: Mean and standard deviation of neuron cell counts in the cerebellum and cerebellum of *Rattus norvegicus* newborns.**

<table>
<thead>
<tr>
<th>Group of Treatment</th>
<th>Mean ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cerebrum</td>
</tr>
<tr>
<td>Q.1</td>
<td>9.88 ± 1.71</td>
</tr>
<tr>
<td>Q.2</td>
<td>10.16 ± 1.01</td>
</tr>
<tr>
<td>Q.3</td>
<td>11.12 ± 1.52</td>
</tr>
</tbody>
</table>

**Table 2: Anova test results on neuron cell counts in Cerebrum and Cerebellum of newborn *Rattus norvegicus*.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of neuron Cells in Cerebrum</td>
<td>0.152</td>
</tr>
<tr>
<td>Number of neuron Cells in Cerebellum</td>
<td>0.183</td>
</tr>
</tbody>
</table>

* Significantly different <0.05
Figure 1: The difference in the description of the number of neuron cells in the cerebrum tissue of newborn rats. The blue arrow indicates the presence of neuron cells which is indicated by the presence of blackish gray chromogen. The combination of the murotal sound qoriah stimulation group (Q3) occurred most often between the murotal sound qori stimulation group (Q2), and the control group (Q1), the control group the least. hematoxyline-eosin with a magnification of 5 fields of view as much as 400 times.

Figure 2: The difference in the description of the number of neuron cells in the cerebellum tissue of newborn rats. The blue arrow indicates the presence of neuron cells which is indicated by the presence of blackish gray chromogen. The combination of the murotal sound qoriah stimulation group (Q3) occurred most often between the murotal sound qori stimulation group (Q2), and the control group (Q1), the control group the least. hematoxyline-eosin with a magnification of 5 fields of view as much as 400 times.
Discussion

The highest mean number of neuron cells cerebrum and cerebellum was in the qoriah sound stimulation group followed by qori sound stimulation and the control group. Female voices tend to have better frequency intensity and are familiar to fetal ears so they are most easily captured by the fetal brain. However, there was no significant difference between the treatment group and the control group on the number of neuron cells in the Cerebrum and Cerebellum Rattus norvegicus offspring then continued in the test. post-Hoc LSD which showed that there was no significant difference in the number of Cerebrum and Cerebellum neuron cells between the treatment group and the control group.

Sound can resonate with cell organelles, one of the cells is brain nerve cells, where the brain tissue has main cells, namely neurons that function to convey signals from one cell to another\cite{15,16}. Sound containing the tone - the tone harmonious and orderly able to generate rhythmic stimulation to the central nervous system because the performance of the brain that can coordinate and control all activities of the body into homeostasis\cite{14}. In contrast to murottal, music will sound beautiful as created by the tool or the human voice sounds carefully structuring can form a specific pattern\cite{17}.

In pregnancy nuclei in the brain stem have formed at the beginning of the second trimester. At 20-22 weeks of gestation, some neurons in the hearing center have formed dendrites and axons, with the physiological and behavioral responses of the fetus to sound, including brainstem reflex activity, indicating that the auditory stimulus has reached the center of the auditory system in the brain\cite{9,18,19}. The growth of children has been started since I was in the womb it is not surprising that Islam says education has started since the baby still in the womb. The fetus begins to hear clearly at the age of 6 months in the womb so that the fetus can move its body according to the rhythm of the mother’s tone of voice\cite{20}. Factors the environment in the form of sound on fetal brain development in the form of sound affects the growth of neural connections and causes the process of neurons to adapt to the stimulation given\cite{21}.

There was a difference in the results of the study, but it was not significant because of the fetus being able to hear sounds from outside apart from treatment, sounds from outside and from within. Sounds from the inside are hidden and can be heard more in low frequencies such as the mother’s heart sound, the sound of the mother’s intestinal peristaltic movements and the sounds around the mother, and the similarity of the voice of women/qoriah to the voice owned by the mother.

Stimulation for fetal hearing is the easiest form to do because it is automatic more often hear sounds in the mother’s body such as the sound of the heartbeat, body fluids and digestion. A report found similarities in the accuracy of women’s voices and men’s voices tested with a sound frequency detection system with a backpropagation neural network, for the type of voice it was known that the accuracy of women’s voices reached a value of 100% while in men 95, 47% of this happens because male voices are detected to be similar to women’s voices so that the accuracy of men’s voices is reduced\cite{12}. The research conducted which compared the mother’s voice with the recorded voice of the father and played in the fetus showed no significant difference in fetal heart rate, number and duration of fetal movement\cite{22}.

Sounds from the outside and the inner environment that the fetus can hear can activate neurotransmitters in neuron cells that have ionic ions such as glutamate and Ca\textsuperscript{2+} influx which will bind to CAMK then phosphorylate with CREB which will then stimulate BDNF mRNA to become BDNF, then BDNF will bind to its receptors, namely TrKB which will affect the process of proliferation, differentiation, migration, synaptogenesis, apoptosis and myelination which will increase the number of neuron cells.

Conclusion

In conclusion, our data show that there is a difference in the mean number of neuron cells in the cerebrum and cerebellum. However the it is not significant. The highest
mean number of neuron cells was in the qoriah voice stimulation group followed by qori voice stimulation and the control group. Female voices tend to have better frequency intensity and are familiar to fetal ears so they are most easily captured by the fetal brain.

**Conflict of Interest:** There is no conflict of interest in this study.

**Sources of funding:** This research was self-funded by the author.

**Ethical Approval:** This study has obtained ethical eligibility permit based on the Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University No: 2.KE.004.01.2020

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Acute Aerobic Exercise Decreased Tumor Necrosis Factor Alpha (TNF-α) Levels in Obese Adolescent Females

Bana Yusrian Hawari¹, Sugiharto², Desiana Merawati², Adi Pranoto³

¹Student, Department of Sport Science, Faculty of Sport Science State University of Malang, Indonesia, ²Lecturer, Department of Sport Science, Faculty of Sport Science State University of Malang, Indonesia, ³Doctoral Student, Medical Science, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia

Abstract

Introduction – Systematically, obesity was associated with low-grade inflammation indicated by abnormal adiponectin secretion, thereby activating inflammatory signaling pathways that could impact chronic inflammation and increased the risk for causing cancer cells. The exercise was an effective and efficient strategy in maintaining pro and anti-inflammatory homeostasis. Exercise performed regularly could increase adiponectin levels as anti-inflammatory markers and decreased pro-inflammatory cytokines, such as Tumor Necrosis Factor-alpha (TNF-α).

Objective – This study aimed to prove the effect of acute aerobic exercise in the decrease of TNF-α levels on obese adolescent females.

Material and Methods – A total of 14 obese adolescent females were participated in this study and were randomly divided into two groups, namely CG (n=7, control group), AEG (n=7, acute aerobic exercise group). ELISA was used to measure TNF-α levels in all samples. Statistical analysis was performed using the Paired Samples T-Test and Independent Samples T-Test.

Results – The results of the Paired Samples T-Test on CG showed that there was no significant difference between the mean of TNF-α levels in pre-exercise and 10 min post-exercise (p>0.05). However, AEG showed a significant difference between the mean of TNF-α levels in pre-exercise and 10 min post-exercise (p<0.05). The results of the Independent Samples T-Test showed that there was no significant difference between the mean levels of TNF-α pre-exercise in CG and AEG (p>0.05), while the mean TNF-α level of 10 min post-exercise among CG and AEG showed a significant difference (p<0.05).

Conclusion – Our data showed that TNF-α levels were decreased by 10 min post-acute aerobic exercise. Therefore, acute aerobic exercise could be used as a method to decrease the level of inflammation in obese adolescent females.

Keywords: Acute aerobic exercise, TNF-α levels, obese adolescent females

Introduction

Obesity was a world health problem that must be noticed¹, because obesity increased the risk of various chronic diseases such as type 2 diabetes (T2D), hypertension, and cardiovascular disease (CVD)²-³, and several types of cancer⁴. Obesity was also associated with bad impact on health quality including metabolic complications which involved many cytokines and hormones¹. One of the cytokines that played a role in obesity was Tumor Necrosis Factor alpha (TNF-α)⁵. TNF-α was a pro-inflammatory cytokine that found increased in obese condition⁶-⁷. Research conducted by
Dahlman et al.\textsuperscript{8} reported that there was a correlation between TNF-\(\alpha\) polymorphism and obesity-related phenotypes. The increased TNF-\(\alpha\) could cause hyperlipidemia and insulin resistance, especially in the presence of single nucleotide polymorphisms (SNP) on the promoter (-308 G/A and -238 G/A)\textsuperscript{9}. In addition, TNF-\(\alpha\) was also an insulin receptor antagonist, so that increased TNF-\(\alpha\) production could interfere with the signaling of insulin and inhibited glucose absorption\textsuperscript{10}, which could lead to insulin resistance\textsuperscript{11}.

Systematically, obesity was associated with low-grade inflammation indicated by abnormal secretion of adiponectin, so that could activate inflammatory signaling pathways\textsuperscript{12} which could impact chronic inflammation\textsuperscript{13} and increased the risk for causing cancer cells\textsuperscript{14}. That was proved by an increase in inflammatory markers, such as TNF-\(\alpha\) in obese individuals compared to normal and lean individuals\textsuperscript{5-6}. The high level of TNF-\(\alpha\) was the characteristic of many malignant cancers, including breast cancer, and was often associated with cancer cell aggressiveness and poor prognosis\textsuperscript{15}. Therefore, a specific strategy was needed to prevent the increase of inflammation in obese individuals through non-pharmacological therapy based on exercise. The exercise was an effective and efficient strategy in maintaining pro and anti-inflammatory homeostasis in the body\textsuperscript{16}. Exercise performed regularly could increase adiponectin levels as a marker of anti-inflammatory\textsuperscript{17-18} and decreased pro-inflammatory cytokines, such as TNF-\(\alpha\)\textsuperscript{19}, so that exercise could be used as a strategy in maintaining the balance of inflammation level. However, several previous studies reported controversial results. Such as research conducted by Dos Santos et al.\textsuperscript{20} reported that moderate-intensity continuous exercise (55% \(\text{VO}_2\text{max}\)) did not significantly change TNF-\(\alpha\) levels in obese adolescents aged 15-18 years. Study conducted by Gerosa-Neto et al.\textsuperscript{21} found a decrease in TNF-\(\alpha\) levels in obese subjects who were exercised with 70% \(\text{HR}_\text{max}\) intensity for 16 weeks and increased in 90% \(\text{HR}_\text{max}\) intensity. Meanwhile, research conducted by Mokhtarzade et al.\textsuperscript{22} also reported that aerobic interval exercise significantly decreased TNF-\(\alpha\) levels. Research conducted by Salamat et al.\textsuperscript{5} reported that there were no significant decrease in TNF-\(\alpha\) level after endurance, resistance and concurrent (endurance–resistance) exercises.

Based on those exposures, this study aimed to prove the effect of acute aerobic exercise on decreasing TNF-\(\alpha\) levels in obese adolescent females. We hypothesize that acute aerobic exercise could decrease TNF-\(\alpha\) levels in obese adolescent females.

**Materials and Methods**

**Study design**

This study was true experiment with the pretest-posttest control group design. The total subjects were 14 obese adolescent females, aged 20-23 years, body mass index 27.5-35 kg/m\textsuperscript{2}, normal blood pressure, normal resting heart rate, fasting blood glucose <100 mg/dL, hemoglobin 13-17 g/dL and randomly divided into two groups, namely CG (\(n\)=7, control group), AEG (\(n\)=7, acute aerobic exercise group). All subjects received information verbally and written about this research. Subjects filled out and signed informed agreements before participating in the study. All procedures in this study have approved by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Brawijaya Malang by number 26/EC/KEPK–S1/02/2020.

**Exercise protocol**

The intervention was given at the Malang City Health Office Fitness Center. The intervention of aerobic exercise was performed by running on a treadmill with an intensity of 60-70% \(\text{HR}_\text{max}\) for 40 minutes with details of 5 minutes for warming-up (50-60% \(\text{HR}_\text{max}\)), 30 minutes of the core performed continuously (60-70% \(\text{HR}_\text{max}\)) and 5 minutes for cooling down (50-60% \(\text{HR}_\text{max}\))\textsuperscript{23-25}. The intervention was performed at 07.00-09.00 A.M. using a treadmill (Pulsar 4.0 HP Cosmos Sports & Medical, Nussdorf-Traunstein, Germany). The heart rates were monitored during exercise using a polar heart rate monitor (Polar H10 Heart Rate Sensor, Inc., USA). The research environment had a room temperature of 26±1 °C and a humidity level of 50-70%\textsuperscript{26-27}. 
Anthropometric measurements and physical fitness

Measurement of body height used stadiometer (SECA, Chino, CA, USA). Measurement of body weight used electronic scale (Tech 05®, China). Body mass index (BMI) was measured by calculating body weight (kg) divided by body height in meter quadrat ($m^2$). Blood pressure was measured using an OMRON automated device (OMRON, HEM-7130 L Model, Omron Co., Osaka, Japan). Maximum measurement of oxygen volume ($VO_2\text{max}$) used Astrand 6-minute cycle test method using Monark 828 E tools Version 1010 ergo cycle (Monark, Vansbro, Sweden).

Blood collection and analysis

The blood sample was taken 3 ml from cubital veins. At the time of blood taking, the subject was in a sleeping position. Blood was taken two times, before exercise and 10 minutes after exercise. The blood was centrifuged for 15 minutes at a speed of 3000 rpm. Measurement of TNF-α levels used Enzyme-Linked Immunosorbent Assay (ELISA) kit (Catalog No. E-EL-H0109; Elabscience, Inc., China) by standard curve range 7.81–500 pg/mL and sensitivity 4.69 pg/mL. Blood was taken to check FBG and Hb levels performed on the capillaries located at the tip of the middle finger. FBG was measured in mg/dL using an Accu-Chek Performa (Roche, Mannheim, Germany), while Hb was measured in g/dL by Easy Touch GCHb (Easy Touch, Hsinchu, Taiwan).

Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 17 (SPSS Inc., Chicago, IL, USA). The normality of data was tested using Shapiro-Wilk. To compare the results, we used Paired Samples T-Test and Independent Samples T-Test. All data were presented as mean ± standard error of the mean (SEM), and $p<0.05$ was considered significant.

Results

The basic profiles of the samples, including age, body height, body weight, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), resting heart rate (RHR), maximal oxygen volume ($VO_2\text{max}$), hemoglobin (Hb), fasting blood glucose (FBG), were displayed in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CG (n=7)</th>
<th>AEG (n=7)</th>
<th>Independent Samples T-Test p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20.43±0.57</td>
<td>20.71±0.52</td>
<td>0.718</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>1.58±0.02</td>
<td>1.59±0.02</td>
<td>0.746</td>
</tr>
<tr>
<td>Body height (m)</td>
<td>75.21±2.32</td>
<td>74.26±2.86</td>
<td>0.800</td>
</tr>
<tr>
<td>Body mass index (kg/m2)</td>
<td>29.94±0.56</td>
<td>29.30±0.46</td>
<td>0.395</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>115.14±1.81</td>
<td>115.00±1.69</td>
<td>0.955</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.14±1.26</td>
<td>76.43±1.51</td>
<td>0.526</td>
</tr>
<tr>
<td>Resting heart rate (bpm)</td>
<td>71.00±2.34</td>
<td>72.00±2.00</td>
<td>0.751</td>
</tr>
<tr>
<td>Maximal oxygen volume (mL/kg/min)</td>
<td>26.66±0.49</td>
<td>27.94±0.66</td>
<td>0.148</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>16.09±0.67</td>
<td>15.21±0.38</td>
<td>0.286</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>92.71±1.89</td>
<td>88.43±2.06</td>
<td>0.151</td>
</tr>
</tbody>
</table>
Based on Table 1, the results of the Independent Samples T-Test showed that there was no significant difference in the mean data of the research subject’s characteristics between CG and AEG ($p>0.05$). The analysis results of TNF-α levels between pre-exercise and 10 minutes post-exercise could be seen in Figure 1.

**Figure 1. TNF-α level pre-exercise vs. 10 min post-exercise.** CG: Control group; AEG: Acute aerobic exercise group. Data were presented as mean±SEM. $p$-values was obtained using Paired Samples T-Test to compare 10 min post-exercise and pre-exercise TNF-α level.

Based on Figure 1, the results of Paired Samples T-Test on CG showed that there was no significant difference between the mean TNF-α levels in pre-exercise and 10 min post-exercise (19.30±1.10 vs. 19.41±0.77 pg/mL, ($p$-values=0.940). However, the results of AEG showed a significant difference between the mean of TNF-α levels in pre-exercise dan 10 min post-exercise (19.50±1.31 vs. 16.35±0.29 pg/mL, ($p$-values=0.038)). The analysis results about the mean TNF-α levels in pre-exercise (CG*AEG) dan 10 min post-exercise (CG*AEG) could be seen in Figure 2.

**Figure 2. TNF-α level CG vs. AEG.** CG: Control group; AEG: Acute aerobic exercise group. Data were presented as mean±SEM. $p$-values was obtained using Independent Samples T-Test to compare AEG and CG TNF-α level.
Based on Figure 2, the results of the Independent Samples T-Test showed that there was no significant difference between the mean of TNF-α levels in pre-exercise of CG and AEG (19.30±1.10 vs. 19.50±1.31 pg/mL, (p-values=0.909). Meanwhile, the mean between TNF-α level in 10 min post-exercise of CG and AEG showed a significant difference (19.41±0.77 vs. 16.35±0.29 pg/mL, (p-values=0.003).

**Discussion**

Based on the results of the study, it showed that the acute aerobic exercise which was performed for 40 minutes/exercise session significantly decreased TNF-α levels. These results were in line with the results of research conducted by Jahromi et al.19 reported that resistance exercise significantly decreased pro-inflammatory cytokines, such as TNF-α. Likewise, exercise with an intensity of 70% VO2 max significantly decreased TNF-α levels28. However, these results were opposite with the research conducted by Bernecker et al.29 which reported that heavy-intensity exercises exactly increased pro-inflammatory markers while circulating, such as TNF-α.

These different results might be due to the differences in the intensity of the exercises performed. In our research, exercise was performed in moderate-intensity (60-70% HRmax) meanwhile the previous studies used heavy-intensity. Exercise with an intensity of 70% VO2 max could significantly decrease TNF-α21. Based on a review article conducted by Gonzalez-Gil et al.16 also reported that moderate-intensity exercise could increase the anti-inflammatory environment, so that could decrease systemic inflammation indicated by the decreased pro-inflammatory cytokines such as TNF-α and the increase of adiponectin as anti-inflammatory markers. Moderate-intensity exercise could be an effective strategy in maintaining the anti-inflammatory environment16, because moderate-intensity exercise could decrease pro-inflammatory cytokines such as TNF-α30 and increased the level of adiponectin as the anti-inflammatory marker31, so the moderate-intensity exercise could be used to maintain the balance of inflammatory level.

Exercise played a role in regulating the level of systemic inflammation because exercise could increase muscle contraction so that it could suppress pro-inflammatory activity through the release of myokines and cytokines30. The increase of muscle contraction regularly could produce and released cytokines to the circulation and other body areas, including the immune system30. Interleukin-6 (IL-6) was a cytokine produced and released by binding the skeletal muscle fibers to affect the other organs32. The production and the release of IL-6 was believed that had a correlation with the decrease of TNF-α level induced by exercise, so it could activate the anti-inflammatory response, which was affected by the increase of interleukin-10 (IL-10), interleukin-1 receptor antagonist (IL-1RA), and the level of tumor necrosis factor-soluble receptor (sTNFr), so that caused pro-inflammatory cytokines, such as TNF-α decreased33-36. When inflammation occurred in the human body, IL-6 could limit the gen expression which coded pro-inflammatory cytokines, such as TNF-α, Interleukin 1 beta (IL1β), Nitric Oxide Synthase 2 (NOS2), and activated terminal c-Jun N kinase (JNK), so that could add the responsiveness of macrophages on interleukin-4 (IL-4) to decrease the inflammation level37. Besides, exercise could also increase adiponectin levels17-18. When adiponectin levels increased and binded to adiponectin receptor 1 (ADIPOR1) or adiponectin receptor 2 (ADIPOR2) there would be activation of AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor alpha (PPARα)16, caused a decrease in liver gluconeogenesis and lipogenesis, and increased glucose uptake in skeletal muscle (SKM) and white adipose tissue (WAT). This directly suppressed the secretion of TNF-α and monocyte chemoattractant protein-1 (MCP-1), and also increased interleukin 10 (IL-10) and macrophage polarization (M2)16,28-29.

**Conclusion**

Based on the results, it could be concluded that acute aerobic performed for 40 minutes/session exercise significantly decreased TNF-α level compared with the control group. Acute aerobic exercise could be one of the effective non-pharmacological methods to decrease
inflammation level indicated by the decrease of pro-inflammatory cytokines, such as TNF-α.

Acknowledgments

We would like to express our gratitude to the Faculty of Sport Science State University of Malang that has provided facilities in the screening process of a prospective research subject and the Fitness Center of the Health Ministry of Malang that has provided facilities well. Also, we greatly appreciate and wish to thank Palang Merah Indonesia (PMI) Blood Transfusion Unit (UTD) Malang that has assisted the blood sampling and blood centrifuge processes. This includes but is not limited to all the parties of to Physiology Laboratory Faculty of Medicine Universitas Brawijaya Malang who has helped the analysis process of TNF-alpha level and all-volunteer who have participated in this study.

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Author Contributions: Conceived and designed the experiments: BYH S. Performed the experiments: DM AP. Analyzed the data: AP. Contributed reagents/materials/analysis tools: BYH S. Wrote the paper: BYH S DM AP.

Conflict of Interest: The authors declared that there was no conflict of interest.

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Hand Hygiene Compliance Behavior and Glove Use in the Pediatric Intensive Care Unit During COVID-19 Pandemic

Bangkit Putrawan¹, Dominicus Husada², Parwati Setiono Basuki³, Risa Etika³, Ismoedijanto³, Dwiyanti Puspitasari³, Leny Kartina³

¹Resident in Department of Child Health, Faculty of Medicine, ²Lecturer and Consultant in Paediatric Infection and Tropical Disease Division Department of Child Health, Faculty of Medicine, ³Consultant in Paediatric Infection and Tropical Disease Division Dr. Soetomo General Hospital in Department of Child Health/ Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

Background: The condition of Covid-19 pandemic potentially influences hand hygiene compliance as a result of workload changes, increased awareness of healthcare workers (HCWs) and personal protective equipment procedures, especially in terms of hand glove use.

Objective to know the adherence of healthcare workers at the pediatric intensive care unit (PICU) to comply with hand hygiene and glove use during the pandemic.

Methods An observational prospective study was carried out including all HCWs stationed at the PICU Dr Soetomo General Hospital during January 2021. All participants were to sign an informed consent before the study took place. A target of 500 opportunities was estimated during the observation, and recorded using infra-red cameras placed at ten points. Hand hygiene compliances were evaluated according to the video surveillance records by an independent auditor. Compliance was measured by dividing total number of observed appropriate hand hygiene by the sum of opportunities. Data were analysed using Chi Square test at a significance of p<0.05.

Results: A total of 28 HCWs were eligible for the study; 9 were excluded. The majority were female (21; 75%), the mean age was 37.9 (SD 5.2) years. During 72 hours’ observation among 526 glove-use opportunities 104 (19.7%) actual glove-use episodes were evident. The hand hygiene compliance was lower (41.3%) when wearing gloves as compared to those with no glove use (68.2%) (p<0.001).

Keywords: hand-hygiene compliance, hand-gloves, pediatric intensive care unit, Covid-19 pandemic

Introduction

Healthcare workers have been facing increased risk of Covid-19 occurring in hospitals during the pandemic condition, consequently leading to increased awareness to comply with hand hygiene due to concerns for Covid-19 transmission. Moreover, it was also apparent that there was an increased workload related to higher number of patients and responsibility for personal protective equipment procedures at a higher rate of duration and quality. On the other hand, the working comfort while wearing high-level personal protective equipment would certainly be low, which could in turn lead to bad influence on hand hygiene compliance.¹,²

Reports on the prevalence of nosocomial infection especially in the pediatric ward globally varied, ranging
from 4.8% in developed countries to 48% in developing countries, where the mortality rate also varied from 5% - 33.8%. Many cases hospital-acquired Covid-19 infection have been detected, where approximately 12.5% has been reported among the total cases in Europe. There has been no distinct data on total Covid-19 nosocomial infection in Indonesia.

The latest recommendation during a pandemic condition related to hand hygiene protocols and covid-19 infection control for HCWs has been issued. Local China guidelines accommodated additional hand hygiene moments to the process of putting on and removal of personal protective equipment. Use of gloves as part of personal protective equipment also increased along with the contact risk level.

Some experts expressed the hypothesis that there would be an increase in hand hygiene compliance during the pandemic. However, evidences measuring compliance have been scarcely published so far, globally as well as domestically. Although there has been a lot of research which concerned the relationship between hand hygiene compliance and glove use, the level of hand hygiene compliance and glove use during the pandemic has never been studied. Accordingly, this study was conducted to explore the profile of hand hygiene compliance related to glove use at the pediatric intensive care unit during the pandemic.

**Material and methods**

This observational prospective study, conducted during January 2021, took place at the pediatric intensive care unit (PICU) Dr Soetomo General Hospital, a third level teaching hospital of the Airlangga University. The PICU of this hospital consists of three parts, namely PICU 1, PICU 2, and the nurse station, bearing a capacity of 8 beds. Patients admitted were all negatively confirmed for Covid-19 by PCR swab. Health workers wore a level 1 protective equipment. Most of the patients were of respiratory and hematology emergencies. The bed occupancy ratio during the study was 50%, which did not significantly differ from the previous months.

Eleven doctors (1 consultant, 10 residents) and 16 nurses were occupied in the PICU. The study was performed only by doing an observation using video surveillance without any intervention. The cameras were of the eyeball type infra-red camera (Daihua technology, made in the People Republic of China) DH-HAC- T1A21P series, along with a size of 3.6 mm, which were placed at 10 points. The devices provided a high definition video picture of 1080 pixels and 30 frames per second, subsequently able to show the details of hand hygiene movements performed. One sink was provided in each part of the PICU, along with standard operational procedures (SOP) about hand hygiene in the PICU.

A 24 hours’ recording was obtained without the need for an operator. Records were sorted and edited to produce a short video clip and further evaluated for hand hygiene compliance by an auditor of the Infection Prevention and Control (IPC) committee at a certified hospital, and entitled as an Infection Prevention control Nurse (IPCN), certified by the Indonesian Association of Infection Prevention Control Nurses (HIPPII). Evaluation of hand hygiene compliance was carried out by referring to the 5 moments for hand hygiene concept as well as adherence with proper hand hygiene procedures.

A minimum number of 500 opportunities was targeted. Audit was performed using an observation sheet similar to that used by the hospital team for Infection Prevention and Control, adapted from the WHO module.

All health professionals (doctors, nurses, physiotherapists) stationed at the PICU served as study samples, and were to sign an informed consent before the study commenced. Statistical analysis applied to judge differences in hand hygiene compliances related to glove use was the Chi-Square’s test at a significance level of P<0.05.

**Operational Definition**

Hand Hygiene is a practical management of
handwashing as outlined by the 2009 WHO Guidelines, consisting of hand rub (alcohol-based) and hand wash (with water and common soap or antimicrobial soap).

Hand hygiene related to glove use is handwash or hand rub before wearing gloves, hand rub while wearing the same gloves, handwash or hand rub after glove removal.

Hand hygiene compliance is the accuracy of the hand hygiene procedures performed when indicated as outlined by the WHO. The number is measured using the formula: \((\text{the action of proper hand hygiene/oppotunity}) \times 100\), and stated as percentage.

Opportunity is the moment of requiring the action of hand hygiene. Example: A doctor intends to do physical examination on a patient, which will be counted as 2 opportunities, before and after touching the patient.

Missed is failure to perform proper hand hygiene in the form of hand rub or handwash when there is an opportunity. Repeated hand rub of a glove-wearing hand is considered as missed.

**Ethical Clearance:** This study was approved by the medical researched ethical Health Research Ethics Committee, Dr. Soetomo Hospital Surabaya No. 0271/LOE/301.4.2/1/2021. Access to acquire CCTV records has also been gained from the Director of the Dr Soetomo Hospital.

**Results**

During the study period video recording in the PICU Dr Soetomo General Hospital took place using a total time of 72 hours, where 526 opportunities for hand hygiene were identified. A total of 37 subjects were recorded doing the activity necessitating hand hygiene in the PICU. However, 9 were excluded due to missing informed consent. Subjects studied consisted of 7 males (25%) and 21 females. Mean age of the studied subjects was 35.7 (SD 5.2, 95% CI) years. Studied subjects who were a doctor by profession comprised of residents and a consultant, who did the rounds and activities in the PICU. Among the 16 nurses considered as study subjects the majority have had a period of employment for more than 10 years. One physiotherapist stationed at the PICU was also a candidate for this study subject. Characteristics of the study subjects are shown in table 1. Total compliance was 62.9%. Among the total opportunities 104 (19.7%) of glove use opportunities was evident. Hand hygiene compliance when wearing gloves was 41.3%, which was significantly lower as compared with a 68.2% adherence to hand hygiene not using gloves. (p<0.001). (Table 2).

**Table 1. Characteristics of study subjects.**

<table>
<thead>
<tr>
<th>Total subjects</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>35.7 years (5.2, 95% CI)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7 (25%)</td>
</tr>
<tr>
<td>Female</td>
<td>21 (75%)</td>
</tr>
<tr>
<td>Profession</td>
<td></td>
</tr>
<tr>
<td>Doctor</td>
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<td>Consultant</td>
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<tr>
<td>Resident</td>
<td>10</td>
</tr>
<tr>
<td>Nurse</td>
<td>16</td>
</tr>
<tr>
<td>Physiotherapist</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 2. Hand hygiene compliance related to glove use

<table>
<thead>
<tr>
<th>Hand hygiene</th>
<th>Yes, n (%)</th>
<th>No n (%)</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes, n (%)</td>
<td>43 (41.3)</td>
<td>288 (68.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Missed, n(%)</td>
<td>61 (58.6)</td>
<td>134 (32.8)</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

This study involved health workers as subjects consisting of 28 study subjects. The majority were nurses with a year-service for more than 10 years. Bin Ibrahim in his study found that seniority, profession being a nurse, and female gender were positively associated with good hand hygiene compliance, and that seniors, nurses, and women were more likely to perceive a need for improvement. They more likely have the ability to influence other healthcare workers to achieve a better hand hygiene behavior.\(^{11}\)

This study showed a 19.7 % frequency of glove use. This result was certainly lower than the majority of other studies where this action could reach 26% to 40% in a ward with high risk setting.\(^{12}\) Glove usage refers to the wearing of gloves to either prevent the hands becoming contaminated with organic matter or microorganisms, or to prevent the transfer of microorganisms to both patients and healthcare workers.\(^{13}\) Hand glove use as a component of personal protective equipment increased during a pandemic, and this was related to a personal protective equipment policy. Universal mandatory gloving in relation with the level of personal protective equipment (PPE) was not considered necessary as a policy at our research sites. Nor were we able to evaluate the increase of hand glove use during the pandemic condition due to lack of data before the pandemic.

We did not analyze the nosocomial infection rate in this study. A systematic review by Picheansanthian collected evidences of successful hand glove use to decrease contamination of health care workers’ hands.\(^{13}\) A decrease in contamination rate does not necessarily mean a decrease in nosocomial infection. The effect on decrease of nosocomial infection would only be achieved by high adherence to proper glove use. This fact has been proved by the implementation of a tight protocol as practiced by Janota in his study where participants of glove use were obliged to perform hand rub using alcohol. It was evident that this action resulted in a decrease of late sepsis in the neonatal intensive care unit (NICU).\(^{14}\) Universal mandatory gloving during RSV season was associated with significantly lower rates of bloodstream infection (BSI), central line associated blood stream infection (CLABSI) and Hospital Acquired pneumonia (HAP) in the pediatric care unit.\(^{15}\)

Our study indicates that compliance with hand hygiene at the moment of hand glove use was only 41.3%, which was lower than when gloves were not worn, where the adherence was 68.2%. Data obtained from a study before the pandemic also revealed an inversely proportional relationship between the use of hand gloves and the compliance of hand hygiene,\(^{16}\) a finding that was consistent with the present study. Cusini proved that removal of the mandatory glove program was followed by an increase in hand hygiene compliance.\(^{17}\) This previous study also showed that although the overall compliance rate for glove use was high, gloves were also overused. Gloves were worn inappropriately for tasks that did not necessitate the use of gloves, and
healthcare workers did not always remove gloves and decontaminate hands between different patients and tasks, besides non-compliance with hand hygiene which could have occurred at the moment of shifting from dirty to clean body sites of the same patient. A small increase in hand hygiene compliance especially during the moment after patient’s contact has also been reported. A converse result where hand hygiene compliance was better during glove use has been shown by a more previous study. However, this study was dealing with a far smaller number of opportunities as compared with our observation.

Another issue concerning glove use during a pandemic is glove wearing hand disinfection using alcohol-based hand rub. This protocol has been recommended in light of problems and lack of logistics at the beginning of the pandemic. Our study did not necessarily use this protocol; routine use of hand gloves was not needed because all patients have been negatively confirmed for Covid-19. The latest WHO recommendation prohibited this practice, and required hand hygiene at the moment of gloving and ungloving. At the moment this research was undertaken the scarcity has been handled by the government authorities.

This study is the first research on hand hygiene compliance in Surabaya and also East Java Regional Area. We have controlled the Hawthorne effect, high false positive compliance as a result of health workers’ awareness of being audited, by indirect evaluation using video records. Time limits and the study location were the weakness of this observation so that the overall level of long-term compliance could not be depicted.

Conclusion

Healthcare workers’ hand hygiene compliance at the moment of wearing gloves was significantly lower compared with adherence to glove use in the Pediatric Intensive Care Unit during Covid-19 pandemic.

Conflict of Interest: None declared.

Acknowledgements: The study team thank and appreciate all the other health care workers (residents, nurses, physiotherapist) involved, who willingly served as study samples, for their effort in improving hand hygiene during daily patient care. Our special thanks to Tri Budi as the independent auditor for spending the time to carry out surveillance and evaluation of the hand hygiene compliance.

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References


Assessment of Self-care Among Elderly Residents at Nursing Homes in Middle Euphrates Cities in Iraq

Bashaer Mohsen Ali¹, Ali Kareem Al-Juboori²

¹Research Scholar, Ministry of Health, Kerbala, Iraq, ²Professor, University of Kerbala, College of Nursing, Karbala, Iraq

Abstract

Self-care is known to be a health resource in later life because it enables the elderly to improve their health and live at home as long as possible⁴. It is therefore essential to resolve the question of self-care in the elderly⁵. The aim of the current study was to assess the level of self-care among elderly residents at nursing homes and to find out the difference between level of self-care with regard to socio-demographic and clinical characteristics. Descriptive study design carried out among 60 elderly persons at nursing homes in middle Euphrates, they are selected by using non probability sampling. The study instrument composed of 2 parts. 1st Elderly’s Demographic Characteristics, 2nd self-care scale. The results of study reveal the overall assessment of self-care domains was moderate. In addition, there is a high significant in difference in self-care level of the elderly with regard to their age and their marital status.

Key words: Self-care, elderly, nursing homes, assessment

Introduction

Self-care is known to be a health resource in later life because it enables the elderly to improve their health and live at home as long as possible. Personal, interpersonal and external conditions influence self-care. For instance, advanced age has negative impact on self-care, while the functional capacities to perform daily activities is positively correlated to self-care. It is therefore essential to resolve the question of self-care in the elderly. Self-care is a subjective care which people use to control their activities every day and it is a key to wellness and illness care. The behaviors of self-care are learned that the individual conducts “self-care activities” to maintain life, wellbeing and health status. Self-care has emerged as a comprehensive concept constructed from a broad collection of perspectives. Alongside such developments the World Health Organization (WHO) has formed a wide notion of self-care including “what people do for oneself to establish and maintain health, prevent and deal with illness”⁶.

It is important to underline that most seniors have a positive vision of life. The elderly may take part in or conduct various activities which give a sense of independence. Moreover, life in a very residential care homes was attributed with a restricted ability for activities and elevated inactivity, resulting in deterioration of self-care activities among residents. The aim of the current study was to assess the level of self-care among elderly residents at nursing homes and to find out the difference between level of self-care with regard to socio-demographic and clinical characteristics.

Methodology

Descriptive study design carried out among 60 elderly persons at nursing homes in middle Euphrates, they are selected by using non probability sampling. The study instrument is a questionnaire designed according to the study purpose. The study instrument composed of 2 parts. 1st Elderly’s Demographic Characteristics, 2nd self-care scale. The self-care questionnaire consists of three domains which participants rate themselves on how often and how well they take care of themselves.
in recent days. Those domains include: Physical Self-Care domain, Psychological Self-care domain, Social domain (Social Life /Family /Relationships). Self-care questionnaire had been scored and rated on five levels Likert scale. The reliability of the questionnaire determined by Cronbach’s alpha for the internal consistency reliability. The data is collected by using structured interview technique. The data is analyzed by using descriptive and inferential statistical analysis.

Results

Table (1) shows descriptive statistics (frequency and percentage) of self care assessment (physical self care) for the study subjects; it shows Assessment (mean and ratio of scores) of self care assessment (physical self care) for the study subjects, it shows that the assessment of elderly residents is (moderate) for the items (1, 4, 8, 9, 10), and it is considered (high) for the items (2, 5, 6) and (low) for the rest.

Table (2) shows descriptive statistics (frequency and percentage) of self care assessment (psychological self care) for the study subjects, it shows that the assessment of elderly residents is (moderate) for the items (1, 2, 5, 6, 7, 8), while it is considered (high) for the items (3, 4, 9).

Table (3) shows descriptive statistics (frequency and percentage) of self care assessment (social self care) for the study subjects, it shows that the assessment of elderly residents is (moderate) for the items (1-7, 9), while it is considered (low) for the item numbered (8).

Table (4, 5) shows differences in overall mean of scores in self care assessment among study subgroups according to demographic data, it reveals that there is a significant increase (P<0.05) in self care assessment in subjects with optional entry to nursing home (MS=3.19) compared to those with compulsory entry (MS=2.69). The same table demonstrate that there is a high significant increase (P<0.01) in self care assessment scores in subjects that can’t perform daily activities (MS=3.56) compared to those that can perform (MS=2.62).

| Table (1): The level of self-care (physical self-care) for the sample |
|-------------------|---|---|
| Items                                    | MS | Assessment |
| 1. Eat a whole foods-based diet rich in colorful fruits and vegetables? | 3.47 | M. |
| 2. Drink enough water?                   | 3.82 | H. |
| 3. Exercise for more than 20 minutes?    | 1.78 | L. |
| 4. Wake feeling refreshed from sleep?    | 3.62 | M. |
| 5. Sleep at least 7 hours per night?     | 4.18 | H. |
| 6. Make time to relax or nap?            | 4.48 | H. |
| 7. Take time to breathe deeply throughout the day? | 1.8 | L. |
| 8. Engage in stress-reducing activities (excluding TV or screen time)? | 2.9 | M. |
| 9. Spend time outdoors?                  | 3.43 | M. |
| 10. Feel nourished, healthy, and strong?  | 2.88 | M. |
Table (2): The level of self-care (psychological self-care) for the sample

<table>
<thead>
<tr>
<th>Items</th>
<th>MS</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Find time to participate in things you enjoy?</td>
<td>3.23</td>
<td>M.</td>
</tr>
<tr>
<td>2. Give and receive affection regularly?</td>
<td>3.27</td>
<td>M.</td>
</tr>
<tr>
<td>3. Feel understood and valued by those who are close to you?</td>
<td>3.68</td>
<td>H.</td>
</tr>
<tr>
<td>4. Feel gratitude on a daily basis?</td>
<td>3.83</td>
<td>H.</td>
</tr>
<tr>
<td>5. Find meaning in life even during difficult times?</td>
<td>3.42</td>
<td>M.</td>
</tr>
<tr>
<td>6. Take an interest in or find joy in the world around you?</td>
<td>3.03</td>
<td>M.</td>
</tr>
<tr>
<td>7. Have hope that things will get better?</td>
<td>2.83</td>
<td>M.</td>
</tr>
<tr>
<td>8. Express yourself creatively?</td>
<td>3.48</td>
<td>M.</td>
</tr>
<tr>
<td>9. Treat yourself with kindness?</td>
<td>3.85</td>
<td>H.</td>
</tr>
<tr>
<td>10. Remember to make your dreams and goals a priority?</td>
<td>2.07</td>
<td>L.</td>
</tr>
</tbody>
</table>

Table (3): The level of self-care (Social self-care) for the sample

<table>
<thead>
<tr>
<th>Items</th>
<th>MS</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Have a dependable person who listens to you?</td>
<td>2.72</td>
<td>M.</td>
</tr>
<tr>
<td>2. Have supportive and closer friends?</td>
<td>2.72</td>
<td>M.</td>
</tr>
<tr>
<td>3. Get enough social time with people who make you happy?</td>
<td>3.08</td>
<td>M.</td>
</tr>
<tr>
<td>4. Participate in group activities with people who share a common interest?</td>
<td>2.52</td>
<td>M.</td>
</tr>
<tr>
<td>5. Spend time with people who make you laugh?</td>
<td>3.37</td>
<td>M.</td>
</tr>
<tr>
<td>6. Feel like your close relationships are loving and supportive?</td>
<td>3.6</td>
<td>M.</td>
</tr>
<tr>
<td>7. Have the ability to comfortably say no?</td>
<td>3.33</td>
<td>M.</td>
</tr>
<tr>
<td>8. Do something fun with family or friends at least once a week?</td>
<td>1.95</td>
<td>L.</td>
</tr>
<tr>
<td>9. Feel comfortable about asking for help when you need it?</td>
<td>3.17</td>
<td>M.</td>
</tr>
</tbody>
</table>
MS : Mean of Scores, M: moderate, L:low, H: high

Table (4): Differences in overall mean of scores in self-care for the sample according to some of demographic and clinical data

<table>
<thead>
<tr>
<th>Items</th>
<th>Elderly’s groups</th>
<th>Overall Mean of Scores</th>
<th>SD</th>
<th>df</th>
<th>T test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>3.27</td>
<td>0.63</td>
<td>58</td>
<td>1.83</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>2.96</td>
<td>0.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Satisfaction with the services</td>
<td>Yes</td>
<td>3.22</td>
<td>0.62</td>
<td>58</td>
<td>1.37</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>2.98</td>
<td>0.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reasons of residence in nursing home</td>
<td>Optional Entry</td>
<td>3.19</td>
<td>0.64</td>
<td>58</td>
<td>2.74</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Compulsory Entry</td>
<td>2.69</td>
<td>0.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Financial source</td>
<td>Yes</td>
<td>3.23</td>
<td>0.65</td>
<td>58</td>
<td>1.27</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3.00</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical visits to the nursing home</td>
<td>Yes</td>
<td>3.24</td>
<td>0.66</td>
<td>58</td>
<td>1.81</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>2.91</td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family and/or relatives visits</td>
<td>Yes</td>
<td>3.18</td>
<td>0.61</td>
<td>58</td>
<td>0.27</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3.13</td>
<td>0.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>3.12</td>
<td>0.49</td>
<td>58</td>
<td>0.18</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3.16</td>
<td>0.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic disease</td>
<td>Yes</td>
<td>3.10</td>
<td>0.61</td>
<td>58</td>
<td>1.58</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3.51</td>
<td>0.76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difficulties in performing Activities</td>
<td>Yes</td>
<td>2.62</td>
<td>0.29</td>
<td>58</td>
<td>8.18</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3.56</td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD : Standard Deviation
Table (5): ANOVA in self-care for the sample according to some demographic data

<table>
<thead>
<tr>
<th>Items</th>
<th>Elderly’s groups</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F test</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age / Years</strong></td>
<td>Between Groups</td>
<td>4.98</td>
<td>2</td>
<td>2.49</td>
<td>7.36</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>19.28</td>
<td>57</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24.26</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Children</td>
<td>Between Groups</td>
<td>2.30</td>
<td>4</td>
<td>0.58</td>
<td>1.8</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>21.96</td>
<td>55</td>
<td>0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24.26</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of stay in nursing home/Years</td>
<td>Between Groups</td>
<td>3.46</td>
<td>4</td>
<td>0.87</td>
<td>2.28</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>20.81</td>
<td>55</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24.26</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levels of Education</td>
<td>Between Groups</td>
<td>1.14</td>
<td>5</td>
<td>0.23</td>
<td>0.53</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>23.12</td>
<td>54</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24.26</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital Status</td>
<td>Between Groups</td>
<td>6.29</td>
<td>3</td>
<td>2.10</td>
<td>6.53</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>17.97</td>
<td>56</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24.26</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

With regard to physical self-care, the results reveal that about 43.33% of the subjects never had exercise for more than 20 minutes. Many physical, social, and psychological factors affect the ability of the elderly to perform exercises. The physical factors involve normal age-related changes, especially decrease in bone mass, muscle mass and muscle strength. The prevalence of chronic diseases also impact the ability to practice the exercise. Psychological status of residents, loss of home and relocation to nursing home, daily institutional routine, lack of motivations, absence of resource; all these factors affect the desire to perform the exercise.

This result comes along with a study\(^6\) whose findings indicate that the majority of elderly people (81.7%) did not practice any type of exercise. The findings reveal that most of elderly people (43.33%) are never take time to breathe deeply. Also, the results indicates that about 5.00% of them never engage in stress-reducing activities (excluding TV or screen time). This result reinforced by a study\(^7\) about “How residents spend their time in nursing homes”. They studies seven nursing homes in New Brunswick and found that the elderly people spend an average of (2/3) of their time in their rooms and a small percentage tend to exercise whereas watching (TV or screen time) and sitting takes up nearly 40% of their time. The findings of study appears that about 3.33% of
them never spend time outdoors. These result disagree with a study\(^{(8)}\) about the favorite activities of older adults. In addition, the percentage 3.33\% of elderly are never feel nourished, healthy, and strong. The reason may be that the homes provide for their basic needs only, while their hobbies are only supported by donations.

With regarding to psychological self-care, the results reveal that about 43.33\% of the subjects never remember to make dreams and goals a priority. This results disagree with a study\(^{(9)}\) regarding resources of life and personal goals in old age. They mentioned that the personal goals extend from simple everyday activities to grand, lifelong endeavors. Also, their results determined that about half of elderly people reported at least (one) goal in the dimension of health maintenance whereas the highest percentage of them (33\%) reported social goals. Moreover, the results of study determined the percentage (13.33\%) of elderly people are never have hope that things will get better. The reason for this result is due to their separation from their children and grandchildren and their status in the institution instead of their presence in their homes. In addition, the nursing homes does not have a good reputation, but rather is considered as the individual’s failure to “secure” his old age.

Regarding the social self-care, the results reveal that about 43.33\% of the subjects never do fun with family or friends at least once a week. This finding was in agreement with a previous study which indicates that only 31.9 percent of respondents reported regular participation in trips outside the nursing home, and 25.8 percent reported regular visits to friends or family; while, 89.9 percent claimed that entertainment with other elderly residents was a regular practice\(^{(10)}\). Also, the results of study appears that the total level of social self-care was moderate, this may be due to the elderly people in nursing home live in shared rooms, and this probably increased the chance of social interaction. Additionally, the results of study reveal the overall assessment of self-care domains was moderate. This result is reinforced by a study\(^{(11)}\) conducted in Iran which mentioned that the level of self-care among elderly people was a moderate level.

Tables (4)\&(5) reveals that there is a significant increase in self-care level (MS=3.19) in subjects with optional entry compared to those with compulsory entry (MS=2.69). According to a study\(^{(12)}\) mentioned that the elderly people who voluntarily transition into residential environments in line with their needs and goals remain in the mastery and comfort zones. They continued personal growth and a sense of meaning in life, and they experience pleasurable feelings. Also, The results demonstrate that there is a high significant increase in self-care level (MS=3.56) in subjects that can perform daily activities. This result agree with a study\(^{(1)}\) mentioned that factors of self-care ability (weather to perform activities of daily life or to achieve wellbeing) showed a positive and strong correlation with ADL (r=0.83, r > 0.50, p < 0.001). Additionally, the results of study reveals that there is a high significant in difference in self-care level of the elderly and their age. This result coincides with a study\(^{(11)}\) indicated that the level of self-care decreased significantly with age (p=0.01, r = -0.212). Finally, the results of study reveals that there is a high significant in difference in self-care level of elderly people with regard to their marital status. This finding is in agreement with a study\(^{(11)}\) about the self-care and disability of elderly Iranians people. They indicated that married women and men (P= 0.027) had significantly better self-care.

**Conclusions**

Most of the elderly people are independent in performing daily activities. The level of self-care moderate level for the elderly residents in nursing homes. Among the recommendations in this research is that the competent authorities must be encouraged to improve services in the Geriatric home especially regarding the environment, entertainment activities, safety measures and transportation services.

**Ethical Clearance:** Taken from University of Kufa ethical committee

**Source of Funding:** Self

**Conflict of Interest:** Nil
References


The Relationship between Psychological Wellbeing and Self-Care among Elderly Residents in Iraq

Bashaer Mohsen Ali1, Ali Kareem Al-Juboori2
1Research Scholar, Ministry of Health, Kerbala, Iraq, 2Professor, University of Kerbala, College of Nursing, Karbala, Iraq

Abstract

Aging is described as progressive change in psychological, biological and social structure of persons. The advanced age contributed negatively to self-care. The relocation to care homes considered as stressful event for the elderly people. The purpose of this study to find the relationship between psychological well-being and self-care among the elderly residents at nursing homes in middle Euphrates in Iraq. A descriptive-correlational design was used to gather the information from the sample of 60 participants in nursing homes. The study has been carried out from November, 1st, 2019 to August, 2020. Purposive sample used to collect the data according to some criteria. The data collected by means of Ryff scales of psychological well-being (shorten version) and self-care questionnaire as well as the socio-demographical of study sample. The data collected by using structured interview technique with the elderly residents in nursing homes. The results of study indicate that a relationship exists between psychological well-being and self-care among the elderly residents in nursing homes. As the relationship between the two variables is a high significant positive relationship (r = 0.721). The recommendations focus on managing the problems existing among the elderly and monitoring their psychological state. Establishing educational programs on how to focus on the psychological and physical aspects. In addition, increasing leisure trips to various places.

Keywords: Psychological wellbeing, self-care, elderly, nursing homes.

Introduction

The various sociocultural, biological, and genetic factors as well as personal experience determine ageing process and quality of life of elderly. Besides, it encourages the elderly to experience positive emotions and attitudes, enhancing mechanisms of self-control and self-regulation and optimizing the ability to adapt and coping with problems at this vital stage(1).

Mental health affects each part of life, from job success to relations with others, friends and family members. These can affect the ability of an individual to sleep and less tangible things, such as the likelihood that an individual takes risks, push the self intellectually, and feel emotions such as fulfillment, gratitude and happiness. Thus, caring for mental health is such a crucial part of living a healthy, happy and well-rounded life(2). According to some authors, later-life research requires to focus more than just understanding the elderly as being sick and old. The mental health of older people is affected by life experiences that can be positive as well as negative(3).
The self-care affects directly on wellbeing (4), and self-care is a matter for people to support their own health and well-being on their own account (5). A study (6) indicated self-care as behaviors that promote physical and emotional wellbeing. Research on wellness has too recorded the beneficial effects of self-care activities on physical and psychological health (7). Abilities of Self-management to maintain and achieve well-being based on who the elderly have sufficient level of cognitive, physical and social activity to meet their goals and needs for well-being. Since these, lower functioning level is predicted to contribute to weaker skills of self-management. For example, lower level of emotional, physical and cognitive functioning may adversely affect the ability to self-manage ‘to have a constructive frame of mind,’ that corresponds to the capacity to adopt and sustain a positive frame of mind or positive expectation. It is believed that the willingness to maintain a positive frame of mind can lead to wellbeing, as it increases the time span and improves motivation, that in effect allows persons to participate in activities and not easily quit (8). The level of self-care engagement may lead to increased well-being. A study (4) observed an important, positive association between the level or value of self-care and well-being. Other research shows that improved self-care practice increases well-being. Based on what was mentioned above, we aim to find out whether there is a relationship between psychological wellbeing and self-care among elderly residents at nursing home in Middle Euphrates in Iraq.

Methods

A quantitative descriptive correlation design has been utilized in this study as a way to examine the relationships among the main study variables including the predictor variables, which include the psychological wellbeing variables (9), and the dependent variable, which was self-care (3). The study has been carried out from November, 1st, 2019 to August, 2020. The study covers the elderly residents at nursing home in Middle Euphrates (Karbala, Najaf, Hilla, Diwaniya) (10). A non-probability purposive sample consist of (60) elderly persons that are selected from the total population of (100) elderly residents at nursing homes. In addition to that, a pilot study has chosen from the total number. The study sample consists of 37 men and 23 women ranging in age from 65 (National Health and Aging Trends Study) to 87 years. The purposive Sample is used in order to obtain the representative sample according to some criteria. The assessment tool has been reconstructed in a form of a questionnaire that has been adopted and modified by the researcher. The questionnaire consists of 3 parts, which are socio-demographic and clinical characteristics, RYFF psychological wellbeing scale (18 items version), and self-care scale (29 items). The instrument face validity was determining through a panel of experts. The reliability can be determined by using Pearson’s Correlation Formula (r). The result of the reliability coefficient for domains of psychological wellbeing is 0.71 and Self-care is 0.93, which is considered statistically acceptable matching with the lower bound of reliability coefficient.

Results

Table (1): Distribution of the sample according to their total score of psychological wellbeing

<table>
<thead>
<tr>
<th>Elderly’s groups</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>0</td>
<td>49</td>
<td>11</td>
</tr>
<tr>
<td>Percentage</td>
<td>0.00</td>
<td>81.67</td>
<td>18.33</td>
</tr>
</tbody>
</table>
Table (2): Distribution of the sample according to their total score of self-care

<table>
<thead>
<tr>
<th>Elderly’s groups</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>4</td>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td>Percentage</td>
<td>6.67</td>
<td>68.33</td>
<td>25.00</td>
</tr>
</tbody>
</table>

Tables (1) and (2) show Distribution of the sample according to their total score of psychological wellbeing and self-care, they show the highest percentage for psychological wellbeing is moderate (81.67%), it also shows the highest percentage for psychological wellbeing is moderate (68.33%).

Table (3): Overall Assessment of level of Psychological well-being Dimensions for the study sample

<table>
<thead>
<tr>
<th>Dimensions of Psychological well-being</th>
<th>Items</th>
<th>MS.</th>
<th>Assess.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autonomy dimension</td>
<td>Q14</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q16</td>
<td>3.93</td>
<td>moderate</td>
</tr>
<tr>
<td></td>
<td>Q17</td>
<td>3.97</td>
<td></td>
</tr>
<tr>
<td>Personal growth dimension</td>
<td>Q10</td>
<td>4.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q11</td>
<td>4.4</td>
<td>high</td>
</tr>
<tr>
<td></td>
<td>Q13</td>
<td>3.07</td>
<td></td>
</tr>
<tr>
<td>Environmental mastery dimension</td>
<td>Q3</td>
<td>2.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q7</td>
<td>3.33</td>
<td>moderate</td>
</tr>
<tr>
<td></td>
<td>Q8</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Positive relations with others dimension</td>
<td>Q5</td>
<td>3.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q12</td>
<td>3.55</td>
<td>moderate</td>
</tr>
<tr>
<td></td>
<td>Q15</td>
<td>2.95</td>
<td></td>
</tr>
<tr>
<td>Self- acceptance dimension</td>
<td>Q1</td>
<td>2.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q4</td>
<td>2.88</td>
<td>moderate</td>
</tr>
<tr>
<td>Purpose in life dimension</td>
<td>Q2</td>
<td>3.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q6</td>
<td>3.05</td>
<td>moderate</td>
</tr>
<tr>
<td></td>
<td>Q9</td>
<td>2.93</td>
<td></td>
</tr>
<tr>
<td>Overall Assessment</td>
<td></td>
<td>3.29</td>
<td>moderate</td>
</tr>
</tbody>
</table>

MS : Mean of Scores
Table (4): Overall Assessment (mean and ratio of scores) of self-care domains for the sample

<table>
<thead>
<tr>
<th>Items</th>
<th>MS</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Self care</td>
<td>3.24</td>
<td>Moderate</td>
</tr>
<tr>
<td>Psychological Self care</td>
<td>3.27</td>
<td>Moderate</td>
</tr>
<tr>
<td>Social Self care</td>
<td>2.94</td>
<td>Moderate</td>
</tr>
<tr>
<td>Overall Assessment</td>
<td>3.15</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

**MS : Mean of Scores**

Table (4) reveals overall assessment (mean and ratio of scores) of self care domains for the study subjects, they show that the assessment of elderly residents is (moderate) for the social domain (mean of scores = 2.94) , while it is considered (moderate) for the physical and psychological ones (3.24 and 3.27 respectively).

Table (5): The relationship between psychological wellbeing and self-care of the sample

<table>
<thead>
<tr>
<th>Pearson’s Coefficients</th>
<th>Self-care Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychological wellbeing</td>
<td>$r = 0.721$ **</td>
</tr>
</tbody>
</table>

*Significant at $P<0.05$; ** High significant at $P<0.01$

Table (5) shows that there is a high significant positive correlation ($P<0.01$) between psychological wellbeing and self-care of elderly residents ($r = 0.721$). The same table reveals that there is a significant positive correlation ($P<0.05$) between psychological wellbeing and Spiritual health scale residents ($r = 0.322$).

**Discussion**

Tables (1,3) indicate that the mean total score of the Ryff scale was 3.29 as well as the highest percentage of the elderly’s group have moderate level of psychological wellbeing. This indicates that the level of psychological well-being of elderly residents at nursing homes was moderate, despite the circumstances associated with the relocation to residential care and the changes inherent in aging that show a shift in the main sources that contribute to their well-being. This result is supported by a study(11) indicates that the elderly have high level of psychological well-being in social welfare institutions in Jordan. Another study indicated that the majority of elderly (59%) have moderate level of psychological wellbeing in India(12). Regarding the personal growth, which mean the process of continuous development of oneself and preparation for new challenges across life span. The results of study indicates that personal growth of elderly was high level (mean of score=3.84). This result may be due to that most of elderly feel better over the time and try to enjoy their life at nursing home, as well as, the attempt to acquire new positive behaviors and attitudes. This result is supported by a study indicates that the majority of the elderly (61%) have high level of personal growth(12).

Tables (2,4) reveal that the overall assessment of self-care domains for the study sample was moderate (mean of scores = 3.15) and the high percentage of the elderly have moderate level of self-care (68.33%). It can be suggested that the process of aging raises the level of dependence on others and, as a result, it may lead to a decline in quality of life as well as the highest percentage of chronic diseases among the elderly group.
(88.3%) and those have difficulties in walking (40%). All of these reasons may lead to a mediating level of self-care. This result is supported by a study stated that the self-care performance of elderly people was at a moderate level. When analyzing personal variables, the elderly participants had a confirmed mean age of 68.3 years old. It can be described that they are getting older and their self-care behavior performance decreases as age progresses.

Table (5) indicated that there is a high significant positive relationship (r = 0.721) between psychological wellbeing and self-care of elderly residents at P<0.01, which means when the psychological well-being of elderly people increases that will lead to increase their self-care and vice versa. This result coinciding with a study observed that an important, positive association between the level or value of self-care and well-being “(r = .228, p = .014)”. In particular, an significant positive association between self-care and wellbeing suggests that greater self-care activity is correlated with an improvement in general well-being. This result supported by a study indicates that There is a positive and significant relationship between self-care and the psychological well-being of the elderly and direct effect of self-care on the psychological wellbeing of the elderly people (standard coefficient= 0.14). Also, The result of study supported by a study found that the self-management abilities significantly affected the well-being (r =0.56; p ≤ 0.001). The present finding is reinforced by a study found that the relationship between psychological wellbeing and daily life activities was statistically significant and the ‘r’ value was 0.375.

Conclusions

There is an impact of psychological wellbeing upon the level of self-care. The relationship between psychological wellbeing and the level of self-care is confounded by the variables of age, marital status, and the health status.

Ethical Clearance: Taken from University of Kufa ethical committee

Source of Funding: Self

Conflict of Interest: Nil

References


Molecular Evaluation of Efficacy of Freshwater and Marine Acellular Fish Skin Matrixes in Reconstruction of Ventro-Lateral Hernia in Bucks

Bassam H. Gumaa¹, Ahmed H. F. AL-Bayati¹

¹Scholar Researcher, Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Abstract

The objective of present study is to compare between the effectiveness of freshwater and marine acellular fish skin (AFS) matrices on the reconstruction of large abdominal wall hernias in Iraqi bucks using of molecular evaluation depending on Real Time-quantification Polymerase Chain Reaction (RT-qPCR) technique to investigate the levels of basic-fibroblast growth factor (b-FGF) and vascular endothelial growth factor (VEGF) genes during the healing process of the abdominal wall hernias. Ventral-lateral abdominal wall hernias (7X7cm) were induced experimentally in 18 bucks. Thirty days post-inducing of hernias, the animals were divided randomly into two main groups (9 Bucks/group). The hernias in animals of group (A) were treated with onlay implantation of freshwater AFS sheet. While, the hernias in animals of group (B) were treated with onlay implantation of marine AFS sheet. Molecular evaluation was depended at 2, 8, and 12 weeks post-treatment of hernias. The results revealed that the significant elevation of the level of b-FGF between treatment groups was at 8th week in group B and at 12th week in group A. While, the significant elevation of level of VEGF between treatment groups was recorded in group A, 12 week post-treatment. Keywords: Hernia, Bucks, Growth factors, Biological implants, Fish skin.

Introduction

Hernia is a protrusion of the contents of a body cavity through a weak spot of the body wall. This may be from accidental or a normal anatomical opening which does not completely fulfill its physiological function (1). Repairing of large hernia is still regards as a clinical challenge for veterinary surgeons (2). The extensive hernia opening (between 7-10cm in diameter) may require repair by mesh (hernioplasty) (3). There are two types of mesh, synthetic and biologic mesh. The synthetic mesh appears more likely to develop postoperative complications, such as, mesh extrusion, bowel adherence and wound infection (4). To avoid the potential squeal of synthetic mesh, biological materials are being developed and used for abdominal wall defect repairs (5). These biomaterials are derived from varies tissues like; dermis, small intestine submucosa (SIS) and pericardium of human, porcine and bovine (6). These materials allow neovascularization and regeneration, and have ability to become remodeled into the tissue and resist infection (7). Recently, AFS has been used in a variety of clinical applications due to the physicochemical properties and amino acid compositions (8). Due to the absence of studies about the using of AFS in the reconstruction of tissues rather than cutaneous tissue, the current study designed to compare between the efficacy of freshwater and marine AFS in the reconstruction of large abdominal wall hernia through evaluation the tissue level of b-FGF and VEGF genes.

Materials and Methods

In the present study, 18 adult local apparently healthy bucks, weighing (25-30kg) and aged (1-1.5 years) were used. All animals were examined clinically and housed under the same management conditions...
in farm animals of College of Veterinary Medicine/University of Baghdad, for two weeks before starting of the experiment. The animals were numbered and classified according to the experimental design. Under the effect of sedation by intramuscular injection of 2% xylazine hydrochloride (Interchemie, Holland), in a dose of 0.2mg/kg B.W., with lateral recumbency, the right lower flank of all animals were prepared for aseptic surgical technique. Ventro-lateral hernias (7X7cm) were created in the right abdominal wall of experimental animals by using of sedation and local anesthesia using 2% lidocaine hydrochloride (Johnlee pharmaceuticals, India), in a dose of 10mg/kg, B.W. through inverted (L) shape infiltration. Thirty days post-inducing of hernia, the animals were divided randomly into two equal groups (9 Bucks/group). The hernias in group (A) were treated with onlay implantation of sheet of freshwater AFS. While, in group (B), the hernias were treated with onlay implantation of sheet of marine AFS. The animals in each group were divided into three subgroups (3 animals/subgroup) to evaluate the level of b-FGF and VEGF genes during the healing process of hernias at 2, 8 and 12 weeks post-treatment of hernias.

Molecular Evaluation

Two biopsies of (50-100mg) of native tissue were collected from each animal of the study. One of these biopsies was taken from implanted area and the second was taken from the normal abdominal muscle tissue of the same animal which considered as a control sample for each animal. The biopsies were immersed in Trizol Regent (Thermo Scientific, U.S.A.), and kept in freezing, then evaluated using RT-qPCR technique for detection the levels of b-FGF and VEGF gene expression. Analysis and calculation of gene expression levels of one or more genes depend on RNA /mRNA concentration after conversion it to cDNA. All processes including total RNA purification, qPCR amplification and data analysis. Each step of RT-qPCR for each gene of study was contained the following data as mentioned in table (1).

<table>
<thead>
<tr>
<th>Gene: FGF, VEGF and GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Reaction</td>
</tr>
<tr>
<td>Reaction Volume /run</td>
</tr>
<tr>
<td>Safety Margin</td>
</tr>
</tbody>
</table>

Primers

In this study, three primers were used including the specific gene of muscle tissue in goats according to the method described by some researchers (9), which include forward and reverse glyceraldehydes-3-phosphate dehydrogenase (FR-GAPDH) gene primer that was used as Housekeeping gene, forward and reverse b-FGF (FR-FGF-2) and forward and reverse VEGF (FR-VEGF) gene primers that were used as target genes (Table, 2). The primers were used in quantification of gene expression by using RT-qPCR techniques based BRYT Green DNA binding dye (Promega, USA).
Table 2: Shows the details of primers used in the study.

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Seq.</th>
<th>Base pair</th>
<th>Annealing Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGF 2-F</td>
<td>5'-AGTGTTGGCAACCCGTTACCTTGCA-3'</td>
<td>107 (bp)</td>
<td>60</td>
</tr>
<tr>
<td>FGF-2-R</td>
<td>5'-ATACTGCCCAGTTCTGGCTGACTGACACC-3'</td>
<td>100 (bp)</td>
<td>60</td>
</tr>
<tr>
<td>VEGF-F</td>
<td>5'-GTGCGGCAAGCTGATGAATGA-3'</td>
<td>109 (bp)</td>
<td>65</td>
</tr>
<tr>
<td>VEGF-R</td>
<td>5'-TCAGACGGAAAGACTGACACA-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH-F</td>
<td>5'-TGGGCGTGGCAAGTGGTCAAT-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH-R</td>
<td>5'-ATGGGCGTGGCAACATGGTCAAA-3'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Relative Quantification (The DD Ct Method)**

The data of outcome of RT-qPCR for target and housekeeping gene were analyzed by the DD Ct method, also referred to as the comparative Ct method, is a means of measuring relative quantification gene expression levels (fold change), which first described by some studies (10). It determines the relative change in gene expression between a target gene under investigation, the calibrator (reference) gene, mostly, the untreated control is used as the calibrator, and the housekeeping gene (GAPDH), which considered as an endogenous control.

**Results and Discussion**

Estimation the levels of b-FGF and VEGF genes in current study was revealed the presence of differences in means values of these growth factors (GFs) among the different periods of each treatment groups and at the same period between both treatment groups. Table (3) and figure (1A) showed that the mean values of level of b-FGF gene in group A at week 2nd (1.32±0.17), then decreased at week 8th (0.35±0.08) without presence of significant differences between these two periods, while, it increased again at week 12th (6.86±2.92) post-treatment with presence of significant differences (P<0.05) with the values at weeks 2nd and 8th. Whereas, in group B, the mean values of level of b-FGF gene at week 2nd was (0.51±0.14), then, increased at week 8th (6.69±2.37) and returned to decreased at week 12th (3.82±1.87) post-treatment with presence of significant differences (P<0.05) between them. The same table and same figure showed there were differences in mean values of b-FGF gene between both treated groups at each period of following-up along the study. It was higher in group A at week 2nd without presence of significant differences between them, while, at week 8th, it was higher in group B with presence of significant differences (P<0.05) between them. At week 12th, it was higher in group A with presence of significant differences (P<0.05) between them. Table (3) and figure (1B) showed that the mean values of level of VEGF gene in group A at week 2nd was (2.65±1.09), then decreased at week 8th (1.02±0.03) without presence of significant differences between these two periods, while, it increased again at week 12th (6.08±2.53) post-treatment with presence of significant differences (P<0.05) with the values at weeks 2nd and 8th. Whereas, in group B, the mean value of level of VEGF gene at week 2nd was (0.94±0.06), then it increased at week 8th (2.07±0.30) and returned to decreased at week 12th (1.51±0.16) post-treatment without presence of significant differences between the three periods with each other. The same table and same figure showed the presence of differences in mean values of level of VEGF gene between both treatment groups at each period of study. At week 2nd, it was higher in group
A. While, at week 8th, it was higher in group B without presence of significant differences between the mean values at these two periods. At week 12th, it was higher in group A with the presence of significant differences (P<0.05) between them.

Table 3: The means ± SE values of b-FGF and VEGF in group A and group B at different periods post-treatment.

<table>
<thead>
<tr>
<th>GFS</th>
<th>Group</th>
<th>Week 2 Post-treatment</th>
<th>Week 8 Post-treatment</th>
<th>Week 12 Post-treatment</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>b-FGF</td>
<td>Group A</td>
<td>B 1.32±0.17a</td>
<td>B 0.35±0.08b</td>
<td>A 6.86±2.92a</td>
<td>2.8919</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>C 0.51±0.14a</td>
<td>A 6.69±2.37a</td>
<td>B 3.82±1.87b</td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>Group A</td>
<td>B 2.65±1.09a</td>
<td>B 1.02±0.03a</td>
<td>A 6.08±2.53a</td>
<td>2.4517</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>A 0.94±0.06a</td>
<td>A 2.07±0.30a</td>
<td>A 1.51±0.16b</td>
<td></td>
</tr>
</tbody>
</table>

Means with a different small letter in the same column significantly different (P<0.05). Means with a different capital letter in the same row significantly different (P<0.05).

Fig. 1: Shows; the mean values of b-FGF among different periods in two groups (A). The mean values of VEGF among different periods in two groups (B).

The elevation in levels of b-FGF and VEGF genes in a tissues from the hernia sites, two weeks post-implantation in both groups with superiority in group A, may be attributed to the inflammatory response and progression of the healing process after implantation, which characterized by infiltration of inflammatory cells according to the effect of each type of AFS on the host tissue and liberation of b-FGF and VEGF (11). This process was explained by study which states that the acute host response is uniformly to all biologic
materials, which characterized by an intense MNCs infiltration (12). The liberation of b-FGF by inflammatory cells that infiltrate during wound healing was explained by many studies referred that the inflammatory cells which involved in wound healing process release cytokines and GFs like b-FGF. They indicated that the significant expression of b-FGF in wound tissue begins approximately eight day after wounding and peaks at 12–14 day (13, 14). Other studies were reported that surgical wounds are characterized by a rapid and early repair and angiogenic that is mediated in part by b-FGF, which can be selectively released by cellular injury (15, 11). Whereas, other authors observed that the gradual increase of the level of b-FGF in the tendon tissue healing from 5-30 days post operation due to the presence of inflammatory cells (16).

The liberation of VEGF by inflammatory cells that infiltrate during wound healing was explained by many studies, they indicated that the VEGF released through a various cellular responses in wound site which stimulates multiple components of the angiogenic cascade; it is up-regulated during the early days of healing when the growth of capillary is maximal associated with the releasing of VEGF by activated platelets (14, 17). Some researchers observed that level of the VEGF in wound healing of patients undergoing incisional hernia repair increasing progressively (13). While, other researchers indicated that VEGF is one of the major GFs involved in angiogenesis and produced by effector cells in the wound, and the activity of VEGF rises after the inflammatory phase, especially during the proliferative and remodeling phases (18).

At week 8th post-implantation, the levels of b-FGF and VEGF genes in group A decreased clearly than week 2nd. This fact was explained by a studies which referred that the GFs are retained in the bioimplants and their liberation will occur during gradually degradation of bioimplants post-implantation and by infiltration of inflammatory cells which bind to ECM proteins (20). In addition, some authors stated that the continuous releasing of GFs during and until complete degradation of implant, will exert their biologic effects as they are dissociated from their binding proteins and activated (21). After that, initially granulation tissue will be formed to replace the degraded part of the implant, which required a high levels of VEGF for angiogenesis to enhance granulation tissue formation, as well as, liberation of b-FGF for fibroblast proliferation and collagen deposition. While, the remodeling process required less GFs due to tissue maturity (19). The researchers indicated that the differences in the level of GFs may be related to the exact compositions and substructures of biomaterials which vary depending on many factors including; species from which materials were harvested, tissue source, method of manufacture,
efficiency of decellularization and post-processing modification (22). Many studies were referred that fish skin graft showed a high expression of FGF, VEGF and cytokines in the epithelium and underlying connective tissue when used in neovaginoplasty (23).

Conflict of Interest: None

Funding: self

Ethical Clearance: Not required

References


21- Badylak SF. The extracellular matrix as a biologic scaffold material. Biomaterials. 2007 Sep 1;28(25):3587-93.


Review of Fungal Infection in Human Beings and Role of COVID-19 Pandemic

Batool Mutar Mahdi

Consultant Clinical Immunology, Head of HLA Research Unit, Department of Microbiology, Al-Kindy College of Medicine, University of Baghdad, Baghdad – Iraq

Abstract

Background: Fungal infections are common throughout the world. In humans, fungal infections occur when an invading fungus takes over an area of the body and the immune system is unable to handle it and eradicate it. Fungi can live in the air, soil, water, and plants and there are also some fungi that live naturally in the human body.

Aim of the Study: To search and review the current literature surrounding fungal infection in different parts of body and role of fungal infection during COVID-19 pandemic.

Methods: Henari, MEDLINE, EMBASE, PubMed, and Web of Science were searched using search criteria relating to fungal infection and relation with COVID-19 infection. Articles presenting clinical data for patients with fungal infection and coronavirus infection reported in English only were included. Data describing prognosis and outcomes were extracted.

Results: 1100 abstracts were identified. Five full texts reporting fungal infection were included. Analysis showed wide use of empirical broad-spectrum antibacterials drugs in spite of evidence for bacterial coinfection.

Conclusions: Despite frequent prescription of broad-spectrum empirical antibiotics in patients with coronavirus-associated respiratory tract infections, there is a small number of data to support the association with respiratory fungal coinfection. Generation of prospective confirmation to support development of antimicrobial antibiotics strategy and suitable stewardship specific for the COVID-19 pandemic is urgently required.

Keywords: Fungal; COVID-19; antibiotics.

Introduction

The kingdom Fungi (singular: fungus) are a kingdom of usually multicellular eukaryotic organisms that are heterotrophs that cannot make their own food and is a highly various form of eukaryotes found in all environments and fungal infections are common throughout the natural world (1). In humans, fungal infections occur when an invading fungus takes over an area of the body and is too much for the immune system especially the adaptive immunity to handle with this infection (2). Fungi can live in the air, soil, water, plants and some live naturally in the human body (3). Like many microbes, there are many useful fungi at one side and on other side there is a harmful one. When harmful fungi invade the body as opportunistic pathogen, they can be difficult to eradicate due to defect or weak in the immune system (4). Many fungi live with human host without any harm and immune systems cope with constant exposure to them; those known as commensalism (5). Like many microbes, there are helpful fungi and harmful fungi. When harmful fungi invade the body, the patient will complain from redness, itching,
swelling and foul discharge depend on what part of the body is affected like vagina, skin. Some fungi cause reoccurring surface fungal infections while others cause systemic fungal infections like blood and known as fungemia that caused by *Candida albicans* which is less common and constitutes about 70% of the cases, but can have deadly outcomes because of late diagnosis and a rareness of effective treatment present. This nosocomial bloodstream infections (fungemia) occur mainly in patients with haematological malignancies and solid neoplasms in hospitals in the USA (6).

This study tries to review and shed a light on fungal infection in different parts of the body of human beings and different methods used to confirm the diagnosis and effect of COVID-19 on increase fungal infection.

1. Classification of fungi

Fungi classified according to their appearance by microscopy and in culture, and by the method of reproduction, which may be sexual or asexual. They classify them as (7):

1- **Yeast:** form a subtype of fungus characterized by clusters of round or oval cells. These bud out similar cells from their surface to divide and propagate. In some circumstances, they form a chain of cells called a pseudomycelium. (Example: *Candida*, *Cryptococcus*, *Trichosporon*). Yeasts are unicellular fungi. The budding yeasts reproduce asexually by budding off a smaller daughter cell; the resulting cells may sometimes stick together as a short chain or pseudohypha. *Candida albicans* is a common yeast that forms pseudohyphae; it is associated with various infections in humans, including vaginal yeast infections, oral thrush, and candidiasis of the skin. C. albicans isolated from each of vaginal and oral swabs expressed the ALS1 virulence stronger gene of adhesion family (8).

2- **Mould:**

Fungi have well-defined characteristics that set them apart from other organisms. Most multicellular fungal bodies, commonly called moulds, are made up of filaments called hyphae. Hyphae can form a tangled network called a **mycelium** and form the thallus (body) of fleshy fungi. Hyphae that have walls between the cells are called septate hyphae; hyphae that lack walls and cell membranes between the cells are called nonseptate or coenocytic hyphae. Hyphae make up the mycelium (like branches are part of a tree) (9). It is divided into:

- **Septate Hyphae** which is compartmented by cross-walls (called septae). It includes Dimorphic, Dermatophytes and opportunistic Aspergillus. Arthrospores are made up of fragments of the hyphae, breaking off at the septae.

- **Aseptate Hyphae** which include Zygomycetes.

The fungus had asexual spores (conidia) forms on conidiophores and sexual reproductive phase. Reproduction of many fungi is unknown and these known as ‘fungi imperfecta’.

Some fungi are dimorphic. They are capable of changing their appearance in response to environmental changes such as nutrient availability or fluctuations in temperature, growing as a mold, for example, at 25 °C (77 °F), and as yeast cells at 37 °C (98.6 °F). This ability helps dimorphic fungi to survive in diverse environments. For example is *Histoplasma capsulatum*, the pathogen that causes histoplasmosis, a lung infection, is an example of a dimorphic fungus (9).

2. **Cell Structure of fungi**

The fungi consist of cell wall and cell membrane. There are notable unique features in fungal cell walls and membranes. Fungal cell walls contain chitin, as opposed to the cellulose found in the cell walls of plants and many protists. Additionally, whereas animals have cholesterol in their cell membranes, fungal cell membranes have different sterols called ergosterols. Ergosterols are often exploited as targets for antifungal drugs (10). Fungi are eukaryotes, non-motile life form and its basic structural unit consists of either a chain of cylindrical cells (hyphae) or an unicellular form, or both (11).
3. Epidemiology of fungal infection

A better understanding of the epidemiology and clinical presentation of fungal infection is integral to improving outcomes and treatment. Superficial mycoses account for much of the overall global prevalence of fungal infection (12). Invasive fungal infections have emerged in the last decades as an important cause of human disease in aging and immunocompromized patients who use chemotherapy (13). Epidemiology of fungal infection can be divided into Nosocomial fungal infection that occur as opportunistic mycoses and Community fungal infection that occur as Endemic Mycosis that appear in a discrete geographic area (14).

Currently about 180 hospitals participate in the National Nosocomial infections Surveillance (NNIS) system. From January 1980 through April 1990 about 27,200 fungal isolates from these hospitals; Candida species accounted for 19,621 (72.1%) of these isolates in immunocompromised patient who are particularly high risk factor for Candidemia and rapid detection of invasive candidemia in these high-risk patients is particularly important to the improvement of rates of survival and development of new methods for rapid diagnosis and monitoring should help decrease the morbidity and mortality associated with nosocomial fungal infection and invasive fungal infection (15).

4. Types of fungi

Fungi are parasites or saprophytes that live on living or dead organic matter. Mycologists identify five phyla of fungi based on the way the fungus reproduces sexually (16):

1. Chytriomycota: are usually aquatic and microscopic. They are usually asexual, and produce spores that move around using flagella.

2. Zygomycota: cause problems by growing on human food sources. One example of a zygomycete is Rhizopus stolonifer, a bread mold. The hyphae of zygomycetes are not separated by septa.

3. Glomeromycota: make up half of all fungi found in soil.

4. Ascomycota: are pathogens of plants and animals, including humans, in which they are responsible for infections like athlete’s foot, ringworm, and Candida albicans, a yeast which lives in the respiratory, gastrointestinal, and female reproductive tracts. Ascomycetes have reproductive sacs known as ascii, which produce sexual spores, but they also reproduce asexually.

5. Basidiomycota are usually club-shaped, and known as club fungi. Most reproduce sexually. Mushrooms are a common example of basidiomycetes.

5. Immunity to fungi

Most of fungal infections are opportunistic pathogens that do not cause disease unless there are alternations in immune defense mechanism by using immunosuppressive drugs, infection with human immunodeficiency virus (HIV). Fungi also cause nonlethal skin, nail, vagina and mucosal infections that are equally difficult to treat and often recurring (17).

The immune response varies with respect to the fungal species encountered. The relative importance of specific innate and adaptive defence mechanisms differs depending upon the type of fungus and anatomical site of infection. Yeast, pseudohyphae and hyphae of Candida albicans may be an important to stimulate host immune response while yeasts and spores are often effectively phagocytosed while the larger size of hyphae prevent effective ingestion. The location of some fungi intracellular can survive with in phagocytes by using them to evade fungal killing and to disseminate and invade throughout the host (18).

The first line of defense mechanism against fungal infection is innate immunity starting from neutrophils, NK cell, genetically inherited receptors, called pattern recognition receptors (PRRs) (19) on macrophages and monocytes which are important antifungal effector cells that recognize conserved pathogen-associated molecular patterns on the fungus (20). The initiating signals from
these receptors activate cellular responses and killing mechanisms. Theses effector cells are recruited to sites of infection by the action of inflammatory signals like cytokines, chemokines and complement components. Fungi are killed or damaged by production and release of reactive oxygen intermediates and antimicrobial peptides that kill the fungi\(^\text{[21]}\). Once fungal antigen within tissues, macrophages will develop into a distinct functional phenotype, which is determined by the cytokine secretion. Proinflammatory cytokines, particularly interferon gamma (IFN-gamma), drive a classically activated (M1) phenotype, whereas anti-inflammatory cytokines (e.g., TGF-beta) drive alternatively activated (M2) macrophage and macrophage phenotype can have a profound effect on antifungal immunity\(^\text{[22]}\).

Other important cell is dendritic cells that initiate both innate and adaptive immunity \(^\text{[23]}\) (Figure-1-).

These cells phagocytes and process fungal antigens and express these fungal antigen and these cells are capable of taking up fungal antigen and processing antigen for display by major histocompatibility complex (MHC) class I or MHCII molecules to naïve T cells and of mediating fungicidal activity through co-stimulatory molecules leading to migrate and proliferate other lymphocytes and secrete cytokines to initiate immune responses\(^\text{[24]}\). Dendritic cells have an instrumental role in linking innate and adaptive responses to a range of pathogenic fungi including Aspergillus fumigatus, Cryptococcus neoformans and C. albicans \(^\text{[25]}\). Signals transmitted by dendritic cells can vary depending upon the encountered fungus antigen or morphotype of the fungus resultant in differences in the nature of adaptive immune responses obtained \(^\text{[26]}\).

![Figure - 1 - Role of dendritic cell in fungal infection.](image-url)
Then the adaptive immunity will be stimulated through stimulation of naïve T cells and differentiation of CD4+T cells along a T-helper (Th) cell type 1 (Th1) or type 2 (Th2) pathway or Th17 and development of specific Th responses, is an essential determinant of the host susceptibility or resistance to invasive fungal infections. Later on development of Th1 responses is influenced by the action of cytokines, such as interferon (INF-gamma), interleukin(IL-6), tumour necrosis factor (TNF)-alpha, and IL-12, in the relative absence of Th2 cytokines, such as IL-4 and IL-10 (27). In immunocompetent hosts CD4 T-cell-mediated clearance of fungi with limited tissue damage requires a finely cooperated balance among Th1, Th17, and Treg (regulatory cell)(28). Th17 secrets IL-17 which is critical for immunity to fungal infections, particularly mucosal candidiasis (29).

The predominance of Th1 (cellular immunity) over Th2 (humoral Immunity) type cytokines correlates with protection against various mycoses (30). Within this framework of interaction between innate and adaptive immunity relating to quantitative and temporal production of cytokines and the development of particular T-cell responses that modulate immunity to fungal antigen (31).

6. Diseases of fungal infections

There are millions of fungal species but only few of them cause diseases like mold and yeast that affect lung causing pneumonia with symptoms similar to flue or tuberculosis, skin causing skin rashes and nail infections. Fungal diseases can be classified as followings (32):

a- Most common fungal diseases like Dermatophytes that cause nail infection, skin ring worm infection and Candida that affect vagina and mouth.

b- Fungal diseases that affect people who live in certain areas like Blastomycosis, Histoplasmosis, Coccidiomycosis, and Paracoccidiomycosis

c- Fungal diseases that affect people who have weak immune system like Aspergelsonis, Candidiasis, Mucormycosis and Crypyococcus neoformans

d- Health problem diseases caused by fungi like fungal eye infection, Sporotrichosis and Mycetoma.

The most common fungal infection is the followings:

v Dermatophytes: Fungus that inhabit the keratinized tissue of skin, nail and scalp. These include Trichophyton rubrum, Trichphyton mentagrophytes. Microsporum canis and Epidermophyton floccosum that cause cutaneous lesion like nail infection can develop in people at any age, but it’s more common in older adults. As the nail ages, it can become brittle and dry. The resulting cracks in the nails allow fungi to enter (Figure-2-). Other factors like reduced blood circulation to the feet and a weakened immune system may play a role in development of Tinea pedis (Athlete’s foot). Toe nail fungal infection can start from athlete’s foot and it can spread from one nail to another Other fungus is tinea corporis that infect body of human and scalp ringworm that infect scalp (33).

![Figure- 2 - Nail fungal infection caused by Trichophyto.](From Dr. Batool Clinic.)

v Candida species: cause infections in individuals with deficient immune systems. Th1-type cell-mediated immunity (CMI) is required for clearance of a fungal infection. Candida albicans is a kind of diploid yeast that commonly occurs among the human gut microflora. It is an opportunistic pathogen in humans (34).
v Histoplasma capsulatum can cause Histoplasmosis in humans, dogs and cats. The fungus is most prevalent in the Americas, India and southeastern Asia. Infection is usually due to inhaling contaminated air (35).

v Aspergillus: The most common pathogenic species are Aspergillus fumigatus and Aspergillus flavus. Aspergillus flavus produces aflatoxin which is both a toxin and a carcinogen and which can potentially contaminate foods such as nuts (36).

v Cryptococcus neoformans can cause a severe form of meningitis and meningo-encephalitis in patients with HIV infection and AIDS and also isolated from burn patients then infecting C. neoformans cells are usually phagocytosed by killed by the release of oxidative and nitrosative molecules by these macrophages (37).

v Sporotrichosis: Human Sporotrichosis is an infection caused by dimorphic fungus, Sporothrix schenckii by direct inoculation. Sporotrichosis is a rare type of fungal infection that can occur in both humans and animals. Also named “rose handler’s disease,” the fungus can be found in certain plants and their surrounding soil. Sporotrichosis mostly affects people who work with soil like farmers (38).

v Tinea versicolor is a fungal infection of the skin. It’s also called pityriasis versicolor and is caused by a type of yeast that naturally lives on your skin. When the yeast grows out of control, the skin disease will appear as rash (Figure- 3 -) (39).

Figure- 3 - Tinea versicolor of the skin.(From Dr Batool Clinic).

7. Diagnosis of fungal infection

In current years diagnosing fungal infections remains a problem in the management of fungal diseases mainly in the immunocompromised patients with a massive increase in the frequency and severity of fungal infections has been found (40). Signs and symptoms of fungal infection are non-specific in diagnosis and difficult to distinguish from invasive disease particularly when blood cultures are commonly negative. This state has led
to the policy of initiating empirical therapy in the high-risk patient. At the simplest level, the clinician must be familiar with the appearance of various fungi in tissue. Early diagnosis of fungal infection is important to effective and successful treatment. There are many methods to diagnosis like traditional approaches to diagnosis include many methods starting from direct microscopic examination of clinical samples, histopathology, culture, and serology. Nowadays emerging new technologies include molecular diagnostics tests and antigen detection in clinical samples. Rapid and early diagnosis of fungal infections is a key factor for patient outcome and cure. Traditionally, the following are the diagnostic methods to early detect the fungus:

1- Direct microscopic examination of clinical samples under light microscope using KOH preparation and demonstration of broad based budding organisms and hyphi in the sputum or skin. Stains also used like lactophenol cotton blue stain is good for identification mold, Grocott Methanmine Silver (GMS) stain, Periodic acid-Schiff (PAS) stain and Pap stain or Papanicolaou’s stain used in cervical specimen, Hematoxylin and Eosin stain in detection Aspergillosis through finding typical hyphae on staining of respiratory secretions has highly predictive value.

2- Histopathology of biopsy for example coccidioidomycosis of the lung. Histopathologic examination can usually distinguish Aspergillus from other fungi. Tissue biopsy of skin or other organs may be required in order to diagnose extra-pulmonary disease like Blastomycosis is histologically associated with granulomatous nodules.

3- Culture from fungal lesion from lower respiratory tract like bronchoalveolar lavage of leukaemic patients is also very useful which highly predictive of invasive fungal disease like Aspergillosis and Candidiemia. The culture media used are Sabouraud dextrose, malt extract and less commonly brain heart infusion. Culture may take many days to a result with several of the filamentous fungi. In the case of disseminated candidiasis, blood culture used. All above methods depend on the quality of the clinical specimens and the experience of the laboratory personnel. Moreover, these classical methods have previously shown lower sensitivity in fungal detection.

4- Non-culture methods include antibody and antigen based assays for detection fungal infection like cryptococcosis and coccidioidomycosis. Galactomannan antigen testing of blood is used in some European Centres using ELISA or EIA methodology or agglutination method. Tests for mannan antibodies and antigenaemia are also used.

5- Metabolite assays appeared promising but have not been used commercially.

6- Molecular method like PCR based assays that do not require viable cells and have the ability to identify the increasing number of fungi that are clinically relevant pathogens in humans’ and identification of fungal DNA from body fluid samples using specific genome sequences. PCR used genus-specific probes with 100% sensitivity and specificity.

7- Fungal ribosomal genes are multi copy targets enabling early detection using PCR and 18S rRNA gene enables accurate sequence and identification.

8- Detection of glucan in blood has been achieved using crab amoebocyte lysate.

9- CT scan findings enable diagnosis (Figure- 4-).
8. Treatment of fungal infection

Early diagnosis of infection would enable administration of drugs when treatment is most to be effective. Drug like Ketoconazole is an effective treatment for chronic superficial candidiasis and chronic dermatophytosis like for example patients with tinea corporis. Azoles group like Fluconazole is good treatment option for candida albicans and also used for prophylactic antifungal therapy in prolonged neutropenic situations. Other drug like Griseofulvin is also used as antifungal drug. It is possible to maintain some patients with chronic mucocutaneous candidiasis in remission without using prophylactic ketoconazole, although relapses may occur. Empiric treatment of fungal infections like aspergillosis, cryptococcosis, mucormycosis, and invasive candida infections normally doesn’t require species specific identification. Other medication like Creams and ointments are often sufficient to treat many cases of ringworm. Basic hygiene can help treat and prevent ringworm as well. Keeping the skin clean and dry can help avoid infection and wearing sandals into public showers or locker rooms and avoiding shared items and towels (50,51). Some fungal infection like Mucormycosis and Zygomycosis involves a combination of surgical debridement of involve tissues and antifungal therapy like Amphotericin B intravenously with elimination of predisposing factors for infection (52).

Role Of Fungal Infection In COVID-19 Pandemic

Many reports demonstrated that fungal infection had low rate in hospitalized COVID-19 patients but high use of empirical broad-spectrum antimicrobials in hospitals and difficulty in clinically differentiating between COVID-19 and bacterial infection and its progression to fungal infection provides a major challenge to clinicians (53,54). Fungal infection in COVID -19 patients was increased due to immune suppression by viral infection, prolonged length of using ventilators, contaminants in blood and line culture, and excessive empirical use of high dose antimicrobial drugs. Study was reported that fungal infection constitutes 0.7% of infection in Cohort study. Three patients were diagnosed with Aspergillus fumigatus tracheobronchitis and other four patients with Candida albicans bloodstream infection (55). Few data are available about that and any links between them and candidaemia have yet to be explored in details. The WHO recommends against the prescribing of antimicrobials in mild to moderate COVID-19 cases without clear indication of bacterial infection (56). Prospective studies are required to provide greater insight into the risk factors and outcome of fungal infection in COVID-19 and to give evidence-based recommendations. Other report showed no antimicrobial stewardship interventions were described (57).

Conclusions

Fungal infection is a common disease world -wide especially after dissemination of diseases with immune suppression like human immune deficiency diseases and patients on chemotherapy due to transplantation and cancer. It was diagnosed by many methods and treated by many medications.

Declarations

- Conflict of interest: None
- Funding: None
- Ethics approval: Not applicable
- Consent to participate: Not applicable
- Consent for publication: Not applicable
- Availability of data and material: Not applicable
- Code availability : Not applicable
- Authors’ contributions: Dr Batool Collect data , wrote manuscript

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Patient Satisfaction with Hospital Services, Physicians and Nurses Care of Government Hospital in Sulaimani City

Bekhal Abdulwahid Amin¹, Niaz Mustafa Kamal², Bestun Ibrahim Hama Rahim³

¹Assistant Lecturer, Maternity Nursing Department, Sulaimani Polytechnic University, Sulaimani Iraq,
²Lecturer, Pediatric Nursing Department, Sulaimani Polytechnic University, Sulaimani, Iraq
³Lecturer, Community Health Department, Sulaimani Polytechnic University, Sulaimani- Iraq

Abstract

Background: Patients’ satisfaction is a person’s feeling of pleasure or disappointment resulting from a service’s perceived performance or outcome to his or her expectations. As this definition makes it clear, satisfaction is a function of perceived performance and expectations. This study was undertaken to evaluated patients’ satisfaction level with the hospital services and health care providers among four government hospitals in sulaimani city. The data from 311 inpatients admitted to and stay in the hospital for any medical condition were collected by a face-to-face interview from November 2019 to February 2020. Standardized 4-point Likert scales ranging from satisfying to dissatisfying (1 to 4points) were used for all the 24 items in the questionnaire. Data analysis was performed using a statistical package of Stata (14.version). A frequency analysis was used for demographic data analysis. A person Chi-square test was employed to determine the association between the categorical independent variables. The participant’s mean age was 37.9 SD 13.5 years with ages ranged from 14-85 years. The majority (86.5%) of the study participants were satisfied with the hospital services and environment while a small number 42 (13.5%) of them were dissatisfied with the issue. Almost 275 (88.4%) of the patients were revealed excellent nurse care and a very small number 36 (11.6%) was showed dissatisfaction. Also, 245 (79.1%) of the patients were satisfied with doctor care. Only 65 (20.9%) of them have a negative aspect of doctor care. A significant difference was found between the level of education and patients’ satisfaction with doctor care (coefficient 0.88, p<0.05). Besides, a significant association was found between residence and hospital services (coefficient 0.63, p =0.05). In general, we have discovered a satisfaction level among our sample is considered high and indicates good care provided by health facilities in all hospitals. Further study required seeking the least satisfactory factor regarding doctor care.

Keywords: Satisfaction, Shar hospital, nurse, Sulaimani, patients, physician

Introduction

Patient satisfaction has been described as the value and reaction of patients towards the care they received in the hospital and the medical care was performed (¹). Patient satisfaction is a central indicator of health care quality and reflects the ability of the provider to meet the patients’ needs (²). So, it is a process as much as an attitude, so it must be, monitored continuously, and frequently measured. Patient satisfaction is essential and must be taken into account in assessing the quality of health care delivered particularly when decisions are being made about changes and enhancement in services. It also is used as an instrument in determining payment rates, especially in the context of a competitive healthcare atmosphere and consumerism (³, ⁴). Gradually, patients’ satisfaction became an essential component of health care services quality monitoring and improvement processes (⁵). Patient satisfaction with nursing care is of great importance to any health care agency because
nurses comprise most health care providers and they provide care for patients 24 hours a day (6). Assessing to what extent patients are satisfied with health services is clinically relevant, as satisfied patients are more likely to comply with treatment, take an active role in their care, continue using medical care services, and stay within a health provider (where there are some selections to be made) and maintain with a specific system (7). Nurses are the frontline people that patients most likely meet up with, spend the highest amount of time with and rely upon for recovery during their hospitalization. Nursing care plays a prominent role in determining the overall satisfaction of patients’ hospitalization experience (2). Furthermore, maintaining good technical as well as interpersonal skills is essential for doctors to satisfy their patients (8). Besides, the demonstration of professionalism and ethical practice are also required to meet the expectations of patients. The technical expertise of physicians is regarded as consisting of maintaining an appropriate level of experience, ability to diagnose, the performance of clinical procedures, prescribing medicine, and learning about the latest medical developments. Moreover, the success with technical procedures, treatment, and medication depends upon favorable communication with patients (9, 10). Unlike developed countries, doctors are not made to comprehend the importance of ethical practice and communication skills during medical training. Physicians working in public hospitals deal with patients of a lower socio-economic class with negligible health awareness and poor hygiene. Understanding the patients and making them understand is the big challenge with which physicians are confronted in public outpatient clinics in developed countries (10, 11). Patients’ satisfaction of their relation with their doctors is a key element in the efficiency and usage of health services and varies depending on patient characteristics. Each patient has expectations when meeting a doctor and the difference between these expectations and what he obtains represent the perception of the satisfaction (12). Many previous studies have developed and applied patient satisfaction as a quality improvement tool for health care providers. Thus, patient satisfaction is an important issue both for the evaluation and improvement of healthcare services (13). The aims of the study: first, to analyze patient satisfaction with a doctor and nurse-patient interaction and relationship. Second: to determine patient satisfaction with the quality of hospital and services performed to patients during their hospital stay in Sulaimani.

**Methodology**

This cross-sectional study was conducted from November 2019 to February 2020. The study population consisted of all patients admitted to four government hospitals (Shar Hospital, Shahid Hemen Hospital, Teaching Hospital, and Maternity Teaching Hospital) during the period of study. The four hospitals are located in the center of Sulaimani City. Shar, Shahid Hemen, and Teaching hospital are provided a wide range of care and treatment to adult people. Maternity Teaching Hospital is provided a wide range of care and delivers to women. In this study, the participation of the patients was voluntary. The data from 311 inpatients admitted to and stay in the hospital for any medical condition were collected. Convenient sample size was chosen because this is conservative and adequate when the proportion of participants is unknown. Patients selected for the study included those who were at least 2 days of experience of hospitalization and aged 14 years and over. The patient was interviewed when they were in a good situation and conscious. Accompanying patients were excluded from the study. The authors personally visited the hospitals and all of the respondents (patients) after informed consent was taking were told about the aim of the study and they were encouraged to contribute. Furthermore, the authors guaranteed the privacy of the responses of the participants. However, face-to-face interviews were conducted with patients with no education and education. Each interview session took about 10 to 15 minutes and the data collection process was conducted over a period of 16 weeks.

The questionnaire was originally developed in Kurdish for a better understanding of the local people. The questionnaire was shared with the experts to ensure face validity, revised and a pre-tested questionnaire...
was used in this research. A pilot study was done before administering it to a representative sample. The aim was to find out if the questions are clear enough, clearly understood, in the right order and the provided answers are sufficient and detailed enough. The fill-in time was also determined. The questionnaire consists of four sections, the first section include Sociodemographic characteristics (age, gender, residence, occupation, marital state, and education), the second section consists of 11 items on the hospital services and environment, third and four-section each consist of 7 items on physicians and 6 items for nurses care. The study was approved by the ethics committee of the Technical Institute of Sulaimani and permission was also taken from Sulaimani Directory of health and all hospitals.

Data analysis was performed using a statistical package of Stata (14.version) after the data was entered into the Epidata (3.1). A frequency analysis was used for demographic data analysis. A person Chi-square test was employed to determine the association between the categorical independent variables. Standardized 4-point Likert scales ranging from satisfying to dissatisfying (1 to 4 points) were used for all 24 items. Internal consistency was tested using Cronbach’s alpha coefficient. Scales 1, 2, and 3 were interpreted as being satisfied Patients’ and 4 dissatisfied. Binary logistic regression was subsequently conducted to predict the factors which influence the level of satisfaction.

**Results**

A frequency analysis was used for the demographic data analysis and general information about the survey participants. The results of the frequency analysis or demographic assessments are presented in Table1. This includes age, gender, education, residence, marital status, occupation, and education of the participants. One-third of the participants (83.3%) aged 25 and over. The mean age was 37.9 SD 13.5 years with ages ranged from 14-85 years. The majority of the study participants were women (81.1%). A very slight difference was found between inside and outside participants (49.8% and 50.1%) respectively. Of (80.1%) the participants were married while the rest (19.9%) was unmarried. The larger part of respondents had no employment (20.3%). However, most of them were educated (60.1%).

**Table 1: Socio-demographic characteristic of the participants (n=311)**

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Gender</th>
<th>Frequency</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>&gt;25</td>
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<td>16.7%</td>
<td>Male</td>
<td>59</td>
<td>18.9%</td>
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<tr>
<td>&lt;25</td>
<td>259</td>
<td>83.3%</td>
<td>Female</td>
<td>252</td>
<td>81.1%</td>
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<tr>
<td>Residence</td>
<td>Frequency</td>
<td>Percentage</td>
<td>Marital state</td>
<td>Frequency</td>
<td>Percentage</td>
</tr>
<tr>
<td>Inside Sulaimani</td>
<td>155</td>
<td>49.8%</td>
<td>Married</td>
<td>249</td>
<td>80.1%</td>
</tr>
<tr>
<td>Outside Sulaimani</td>
<td>156</td>
<td>50.1%</td>
<td>Unmarried</td>
<td>62</td>
<td>19.9%</td>
</tr>
<tr>
<td>Occupation</td>
<td>Frequency</td>
<td>Percentage</td>
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<td>Frequency</td>
<td>Percentage</td>
</tr>
<tr>
<td>Employee</td>
<td>63</td>
<td>20.3%</td>
<td>Educated</td>
<td>187</td>
<td>60.1%</td>
</tr>
<tr>
<td>Unemployed</td>
<td>248</td>
<td>79.3%</td>
<td>Uneducated</td>
<td>124</td>
<td>39.9%</td>
</tr>
</tbody>
</table>
The distribution of the participants of four hospitals was presented in Figure 1. There was a similar number (32.2%) of the patients who participated from Shar and Teaching hospital. While (25.6%) was from Maternity teaching hospital and fewer patients have participated from Shahid Hemen hospital was (9%).

**Figure 1: Distribution of patient’s participants in four hospitals**

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Participants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternity Teaching</td>
<td>25.6%</td>
</tr>
<tr>
<td>Shar Hospital</td>
<td>32.2%</td>
</tr>
<tr>
<td>Technig Hospital</td>
<td>32.2%</td>
</tr>
<tr>
<td>Shahid Hemen Hospital</td>
<td>9%</td>
</tr>
</tbody>
</table>

**Patient satisfied with hospital services and environment**

Analyses of patients satisfied with hospital services and environment were shown in Table 2. A large number of the patients were satisfied with four hospital services 280(90%) out of 311 patients. We noted most of the respondents were satisfied with the patient’s ward and bedding (61.1%), ward cleanliness (91%), ward comfortable and calmness (71.4%). Of (81.7%) participants were satisfied with providing medical requirements by the staff while only a small number were dissatisfied (18.3%). One-third of the respondents (84.6%) answered that hospital departments (laboratory, radiology, and pharmacy....) can be easily found by the patients. Over half (68.8%) of the patients were satisfied with ward water facilities and sanitary while (31.2%) have a negative aspect. The majority of the patients (89.4%) were satisfied with the patient’s system registration. More than half (59.5%) of the patients were satisfied with the availability of medical requirements (drugs and medical items). A large number of the participants (92.3%) were satisfied with the doctor’s performance for medical procedures at the time needed. A high percentage (90%.vs 91.6%) of the respondents was satisfied with the time patients examine by the physician and the finding of laboratory results respectively.
Table 2: Response to Patient Satisfaction with hospital services and environment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Satisfied N=311 %</th>
<th>Dissatisfied N= 311 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitals services</td>
<td>280 90</td>
<td>31 10</td>
</tr>
<tr>
<td>Number of the patient’s ward and bed</td>
<td>190 61.1</td>
<td>121 38.9</td>
</tr>
<tr>
<td>Patient’s ward cleanliness</td>
<td>283 91</td>
<td>28 9</td>
</tr>
<tr>
<td>Patient’s ward comfortable and calmness</td>
<td>222 71.4</td>
<td>89 28.6</td>
</tr>
<tr>
<td>Providing medical requirements by the staff</td>
<td>254 81.7</td>
<td>57 18.3</td>
</tr>
<tr>
<td>Easy to finding hospital departments (pharmacy, laboratory, radiology…. )</td>
<td>263 84.6</td>
<td>48 15.4</td>
</tr>
<tr>
<td>Water facilities and sanitary</td>
<td>214 68.8</td>
<td>97 31.2</td>
</tr>
<tr>
<td>Patients registration system</td>
<td>278 89.4</td>
<td>33 10.6</td>
</tr>
<tr>
<td>Availability of medical requirements ( drugs, medical items)</td>
<td>185 59.5</td>
<td>126 4.5</td>
</tr>
<tr>
<td>Implementing each procedure at time</td>
<td>287 92.3</td>
<td>24  7.7</td>
</tr>
<tr>
<td>Laboratory results</td>
<td>285 91.6</td>
<td>26 8.4</td>
</tr>
</tbody>
</table>

Patient satisfaction with nurse care

Regarding patients satisfied with nurse care was shown in Table 3. The majority (90.7%) of the respondents were satisfied with nurse behavior and patient respect. Of (89.7% vs 91.6%) were satisfied with nurse responsibility at any time. We noted a participant (92.3%) recorded that nurses have implemented each medical procedure adequately, (89.4%) of them providing a good explanation about the situation to the patients, and (87.5%) of respondents were answered that nurses were medically qualified.

Table 3: Response to Patient Satisfaction with nurse care

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Satisfied N=311 %</th>
<th>Dissatisfied N= 311 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nurse behavior and respect</td>
<td>282 90.7</td>
<td>29 9.3</td>
</tr>
<tr>
<td>Nurse response to the patients</td>
<td>279 89.7</td>
<td>32 10.3</td>
</tr>
<tr>
<td>Nurse response at night</td>
<td>285 91.6</td>
<td>26 8.4</td>
</tr>
<tr>
<td>Implementing each procedure at time</td>
<td>287 92.3</td>
<td>24 7.7</td>
</tr>
<tr>
<td>Nurse providing good explanation to patients</td>
<td>278 89.4</td>
<td>33 10.6</td>
</tr>
<tr>
<td>Nurse are medically qualify</td>
<td>272 87.5</td>
<td>39 12.5</td>
</tr>
</tbody>
</table>
**Patient satisfaction with physician care**

The study findings showed that (91%) of the 282 patients were satisfied with the doctor’s behavior and dealing respectfully with patients during an examination. (87.8%) respondents during visiting hospitals were strongly satisfied with the doctor responded to the patient’s condition and hear them. Also (84.2%), were mostly satisfied with doctors providing a good explanation about the disease condition and the best medical care requirements. Of 90% of the patients were satisfied with the time of examination by the physician. The majority of the patients (91.6%) were satisfied with the easy access to the physician as needed at night shift. Of these (87.5%) were satisfied with physician clarity of drug use and dose. We noticed that (75.6%) of patients were satisfied with the physician’s exam, while (24.4%) of the patients were unsatisfied.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Satisfied N=311 %</th>
<th>Dissatisfied N= 311 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doctors’ behavior and respect</td>
<td>282 91</td>
<td>28 9</td>
</tr>
<tr>
<td>Doctors response and listen to the patient</td>
<td>273 87.8</td>
<td>38 12.2</td>
</tr>
<tr>
<td>Instructions and directives given by doctor</td>
<td>262 84.2</td>
<td>49 15.8</td>
</tr>
<tr>
<td>Does doctor spent adequate time for patient exam</td>
<td>280 90</td>
<td>31 10</td>
</tr>
<tr>
<td>Doctors’ response at night</td>
<td>285 91.6</td>
<td>26 8.4</td>
</tr>
<tr>
<td>Drug use and the dose was explained adequately</td>
<td>272 87.5</td>
<td>39 12.5</td>
</tr>
<tr>
<td>Physician investigation</td>
<td>235 75.6</td>
<td>76 24.4</td>
</tr>
</tbody>
</table>

The score level of patients’ satisfaction with the study subject was presented in Figure 2. The satisfaction score was ranged from 0-10 on a scale of 10. Two hundred ninety-six (86.5%) of the study participants were satisfied with hospital services and environment, while a small number 42 (13.5%) of them were showed dissatisfied with a mean score of 9.5, SD ±1.8. The majority of patients 275 (88.4%) were revealed excellent nurse care while a very small number 36 (11.6%) was dissatisfied, a mean score of 5.4, SD ± 1.3. Patients’ satisfaction with doctor care was 245 (79.1%) and a negative aspect was found among a small number of 65 (20.9%) with a mean score of 4.5, SD ± 1.7.
Chi-square analysis was performed to find the correlation between socio-demographic factors and patient satisfaction level with hospital services and health care (doctors and nurses). Table 5: We found no significant correlation between the participant’s age, gender, marital state, and occupation with the level of satisfaction. Except significant association was found between participant’s residence with hospital services (P-value= 0.04) and between education level with doctor care (P-value= 0.002).

Table 5: Comparison of socio-demographic factors of patients’ satisfaction with hospital services, nurses and doctors care

<table>
<thead>
<tr>
<th>Characters</th>
<th>Hospital services</th>
<th></th>
<th>Nurses care</th>
<th></th>
<th>Doctors care</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Satisfied</td>
<td>Dissatisfied</td>
<td>Chi, P-Value</td>
<td>N=311, %</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside</td>
<td>128 (47.9)</td>
<td>27 (64.3)</td>
<td>4.1, 0.04</td>
<td>133(48.4</td>
<td>14 (38.9%)</td>
</tr>
<tr>
<td>Outside</td>
<td>141 (52.1)</td>
<td>15 (35.7)</td>
<td>142 (51.6)</td>
<td>22 (61.1%)</td>
<td>129 (52.6)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>44 (16.4)</td>
<td>8 (19.1)</td>
<td>0.2, 0.7</td>
<td>45 (16.4)</td>
<td>7 (19.4)</td>
</tr>
<tr>
<td>≥25</td>
<td>225 (83.6)</td>
<td>34 (80.9)</td>
<td>230 (83.6)</td>
<td>29 (80.6)</td>
<td>209 (85.3)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>220 (81.8)</td>
<td>32 (76.2)</td>
<td>0.7, 0.4</td>
<td>221 (80.4)</td>
<td>31 (86.1)</td>
</tr>
<tr>
<td>Female</td>
<td>49 (18.2)</td>
<td>10 (23.8)</td>
<td>54 (19.6)</td>
<td>5 (13.9)</td>
<td>44 (17.9)</td>
</tr>
<tr>
<td>Marital state</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>220 (81.8)</td>
<td>29 (69.1)</td>
<td>3.7, 0.06</td>
<td>223 (81.1)</td>
<td>26 (72.2)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>49 (18.2)</td>
<td>13 (30.9)</td>
<td>52 (18.9)</td>
<td>10 (27.8)</td>
<td>46 (18.8)</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employee</td>
<td>51 (18.9)</td>
<td>12 (28.6)</td>
<td>2.1, 0.1</td>
<td>53(19.3</td>
<td>10 (27.8)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>218 (81.1)</td>
<td>30 (71.4)</td>
<td>222 (80.7)</td>
<td>26 (72.2)</td>
<td>198 (80.8)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Educated</td>
<td>163 (60.6)</td>
<td>24 (57.1)</td>
<td>0.2, 0.7</td>
<td>161(58.6</td>
<td>26 (72.2)</td>
</tr>
<tr>
<td>Uneducated</td>
<td>106 (39.4)</td>
<td>18 (42.9)</td>
<td>114 (41.4)</td>
<td>10 (27.8)</td>
<td>109 (44.5)</td>
</tr>
</tbody>
</table>
Multiple logistic regressions for the level of satisfaction with hospital services and health care providers with socio-demographic factors (residence, age, gender, marital state, occupation, and education) were presented in Table 6. A residence was found significantly associated with hospital services satisfaction the coefficient 0.68, \( P = 0.05 \), and level of education was found significantly associated with doctor care satisfaction the coefficient 0.88, \( P = 0.01 \), while no significant association was found with remained factors.

Table 6: Regression analysis of socio-demographic factors associated with the level of patient satisfaction

<table>
<thead>
<tr>
<th>Socio-demographic factors</th>
<th>Hospital services satisfaction</th>
<th>Nurse care satisfaction</th>
<th>Doctor care satisfaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>( SE )</td>
<td>( P )</td>
</tr>
<tr>
<td>Residence: Inside city</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Residence: Outside city</td>
<td>0.68</td>
<td>0.35, 0.05</td>
<td>0.39</td>
</tr>
<tr>
<td>Age: &lt;25</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Age: &gt;25</td>
<td>0.23</td>
<td>0.50, 0.6</td>
<td>-0.002</td>
</tr>
<tr>
<td>Gender: Male</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Gender: Female</td>
<td>0.09</td>
<td>0.47, 0.8</td>
<td>0.89</td>
</tr>
<tr>
<td>Marital: Married</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Marital: Unmarried</td>
<td>-0.63</td>
<td>0.41, 0.1</td>
<td>-0.54</td>
</tr>
<tr>
<td>Occupation: Employee</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Occupation: Unemployed</td>
<td>0.59</td>
<td>0.46, 0.2</td>
<td>0.61</td>
</tr>
<tr>
<td>Education: Educated</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Education: Uneducated</td>
<td>-0.50</td>
<td>0.39, 0.2</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Discussion

This study examined the level of patient satisfaction with a health care provider (nurses and physicians) and services performed in the non-private hospitals in Sulaimani city. We evaluated 311 Patients among whom were highly satisfied with the nurse’s care (88.4%), physician’s care (79.1%), and hospital services (86.5%). The findings of our study are corresponding with the Saudi experience study and the study of Lagos University Teaching Hospital reported that patients with high levels of satisfaction with nurse care \(^2, 14\) and the study of Southern Saudi Arabia \(^15\). A lower number of patient’s dissatisfaction with hospital services and health care was recorded in this study. Such a low perception of satisfaction with staff care and hospital services by patients may create problems in the health system by decreasing trust and less utilization of services as well as delayed health-seeking with subsequent poor health.
outcomes of the public. We found the study participants were more satisfied with nurse care compared to doctor care. The result of this study appeared consistent with the study done by the Kingdom of Saudi Arabia (16) while is inconsistent with the study reported by Southern Saudi Arabia (15). The study discovered that there were no significant differences between patients’ satisfaction and age and gender. Indeed, older respondents did not affect satisfaction values. However, several studies indicated that older participants are generally more satisfied with the health care provider and hospital services compared to younger respondents (17, 18). More females were participated in this study (81%) but no relationships were found between gender and level of satisfaction. Our results are similar to the study that reported patient gender did not affect satisfaction values (18). The Ha’il City, Saudi Arabia study also reported no relationships were found between gender and patient satisfaction levels (19). The results of this present study indicated that there was a significant difference between the level of education and patients’ satisfaction. Patient with a lower level of education was more satisfied with doctor care services than the higher education the P=0.002. The finding of this study is similar to some studies that reported lower education level was greater satisfaction with health care service (20, 21) and also is consistent with a study that reported a higher level of education was less satisfied with health care (22). This can occur because patients with high educational levels possess more information about treatment alternatives and expect higher care standards and therefore are more critical in this regard. In regression analysis, significant associations between place of residence and patient satisfaction were identified, respondents in the rural area remained significantly associated with respect to satisfaction with hospital services the coefficient 0.63, p=0.05. The result of this study is in line with the study (23). This may be due to that some of the rural places they don’t have large hospitals and if they found, the quality of the services may be at the lowest level compared to urban a place. Our study has much strength including the use of validated measures of patient satisfaction, nurses and physician’s care, hospital services, and environment across four large government hospitals. The study has limitations as well. Data from both health care providers and hospital services are cross-sectional, thus limiting causal inferences about the associations found. The open bedside interview we use to collect data might also influence the participants’ responses to some extent even if the data collectors were not part of the treating team. According to our knowledge, this is the first quantitative study to determine the level of patient satisfaction with health care providers (nurses and doctors) and hospital services in Sulaimaniyah city.

Conclusions

In general, the study revealed that patients were highly satisfied with hospital service and health care providers in Sulaimani city. The proportion of patients satisfied with nurse care was found to be higher than the proportion of patients satisfied with doctor care and those with a lower level of education was more satisfied with doctor care than a higher level of education because the second group have adequate treatment information and predicted adequate care they received. Besides, respondents with rural residents are more satisfied with hospitals’ quality and services because some of the respondents living in rural areas have a lack of large hospitals and inadequate health system services. If we improve the quality of hospital services and health care, it is clear that evaluating patients’ satisfaction should be constant to reformulate the baseline and regulation and to be able to assess interventions and changes in the health system. Further study is suggested on patient satisfaction with doctor care to find factors that influence low patient satisfaction compared to nurse care if we must improve the quality of health.

Acknowledgments

We would like to extend our gratitude to the Sulaimani directorate of health and Sulaimani polytechnic university for their permission. Furthermore, our gratitude also goes to all participants who voluntarily agreed to participate in this study and helping to complete this study.
Conflict of Interest: The authors declare that there was no conflict of interest.

Financial Disclosure: Self

References


Combined Effect of Physical and Psychological Stress Exposure during Pregnancy on the Expression of Caspase-3 Cerebrum and Cerebellum of Newborn Mus Musculus

Binta Dwi Novitasari1, Hermanto Tri Joewono2, Widjiati3

1Postgraduate Student of Reproductive Health Science, Airlangga University, Surabaya, Indonesia 2Lecturer at Department of Obstetrics and Gynecology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia, 3Professor at Department of Embryology, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia

Abstract

Background: Prenatal stress affects fetal development including brain development. When a stressor is felt, the brain as the main target for stress will release a hormone that stimulates the release of pro-apoptotic proteins and activate caspase-3 which acts as an executioner caspase in the cell death process. The aim of the study was to analyze the effect of combined stress during pregnancy on the expression of caspase-3 cerebrum and cerebellum of newborn Mus musculus. Methods: An experimental study using 24 pregnant mice (Mus musculus). Subjects were randomized into four groups, consisting of physical stress exposure group (forced swimming) (G1), psychological stress exposure group (noise) (G2), combination stress exposure group (forced swimming + noise) (G3), and control group (G4). Stress exposure was given on 6th-15th days of pregnancy. From each mother, three newborn of Mus musculus were taken to make preparations from brain tissue. Immunohistochemical examination was performed to assess caspase-3 expression. Results: The study shows that the mean and standard deviation of the expression of caspase-3 cerebrum and cerebellum in the physical stress exposure group is 5.70 ± 0.99 and 5.80 ± 1.35, the psychological stress exposure group is 7.23 ± 1.39 and 7.40 ± 1.24, the combined stress exposure group is 8.67 ± 1.09 and 9.30 ± 1.12, and the control group 4.17 ± 1.18 and 3.90 ± 1.06. ANOVAs statistical test results show significant differences among groups with a value of p = 0.000 in the cerebrum and p = 0.000 in the cerebellum. Conclusion: Exposure to physical and psychological stress during pregnancy increases the expression of caspase-3 in the cerebrum and cerebellum of newborn mice.

Keywords: Mus musculus, pregnancy, stress, cerebrum and cerebellum, caspase-3

Introduction

Stress in pregnancy not only has a negative impact on the survival of the pregnancy, it can also affect fetal development and maternal well-being. Prenatal stress affects the fetus resulting in low birth weight, prematurity, and impaired brain development. Studies report that the prevalence of stress during pregnancy in the world ranges from 5.5 to 78%, while in developing countries it ranges from 6% to 52.9%.

The brain is the main organ that interprets, responds to, and becomes the target of stress marker hormones. When a stressor is felt, the hormone (CRH) which acts on the anterior pituitary to promote the secretion of adrenocortico-tropic hormone (ACTH). This hormone then stimulates the adrenal cortex to release glucocorticoids (GCs) into the bloodstream. High glucocorticoids will reduce BDNF expression and

Corresponding Author:
Widjiati
email: widjiati@fkh.unair.ac.id
stimulate cell apoptosis \(^{(19)}\).

Caspase-3 acts as the executioner caspase in the cell apoptosis process \(^{(12)}\). Caspase-3 when activated has the function of controlling cell death \(^{(21)}\), causing cleavage of protein kinases, cytoskeletal proteins, DNA repair proteins, endonuclease inhibitory subunits and ultimately to deterioration of cellular function \(^{(9)}\). Increased apoptotic activity of brain cells will reduce the number of cells making up the central nervous system in the fetal brain where there are two main types of cells that make up the central nervous system, namely neurons and glial cells \(^{(9)}\). The cerebellum is a part of the brain that contains more neurons than other parts of the brain \(^{(7)}\), the cerebrum and cerebellum are interconnected by means of polysynaptics, forming a system related to cognitive function and neuropsychiatric disorders \(^{(1)}\). There is little literature and researches on the impact of stress and neurobehavioral studies on the impact of stress on the cerebrum or cerebellum.

This study identifies differences in caspase-3 expression in the cerebrum and cerebellum of newborn mice (Mus musculus) whose mothers are exposed to physical and psychological stress during pregnancy.

Materials and Methods

This research is an experimental study on mice (Mus musculus) which was conducted from January to March 2021 at the Laboratory of the Faculty of Veterinary Medicine, UNAIR, Surabaya. This study used 24 adult female mice (Mus musculus) aged 2-2.5 months of pregnancy which were exposed to stress during pregnancy on the 6th until 15th days of pregnancy. The research subjects were divided into 4 groups which were randomly selected (G1, G2, G3, and G4) with 6 mice in each group.

First group: Exposure to physical stress in the form of forced swimming for 5 minutes once a day in a special box measuring 50x30x25 cm filled with water with a height of 18 cm with a water temperature of 24 °-28 °C and put in a dark box cage, exposure time is 09.00 am.

Second group: The group of exposure to psychological stress by giving noise with an intensity of 90 dB once a day for 1 hour successively in a dark and soundproof enclosure measuring 1x1x2 m, TrueRTA software (real time audio analyzer) was used to produce noise. Noise intensity was measured with a real time sound analyzer (TES 1358) each day prior to exposure to experimental animals, by placing the analyzer in animal cages at several locations, and taking the average of the different readings.

The third group: The stress exposure group, a combination of physical and psychological, was given a noise of 90 dB for 1 hour, then given a break of 5 minutes and then be swam for 5 minutes per day, exposure time starts at 09.00 am.

Fourth group: Control group with standard treatment without stress exposure.

Sampling Inspection

Mus musculus mothers were anesthetized then the pups were born by sectio caesarea (SC) on the 16th day of pregnancy. The pups of Mus musculus which were to be sacrificed were anesthetized first, and then the cranium was cut in the sagittal direction from caudal (occipital) to rostral (frontal), right between the two hamisters of their brain. Furthermore, the brain was released. The separated brain was weighed, and then put in a 10% formalin solution for organ preservation; the cerebrum and cerebellum were taken. Furthermore, immunohistochemical preparations and Hematoxylin-Eosin (HE) staining were made.

Data Analysis

To see the normality of the data, the Shapiro-Wilk test was used. If the data obtained are normally distributed, then the ANOVA test is used followed by LSD (Least Significant Difference) to see the differences in all groups. If the data obtained are not normally distributed, the Kruskall Wallis test and the Mann Whitney test are used. This study uses a significance level of P<0.05. To simplify statistical calculations, researchers used the SPPS tool version 21.
Results and Discussion

Results

The results show the highest expression of caspase-3 in the cerebrum and cerebellum in the combination of physical and psychological stress exposure group compared to the physical stress exposure group, psychological stress exposure group, and the control group (Table 1). The results of the normality test using the Shapiro-Wilk test on the treatment group obtained a significance value (p-value) > 0.05, which means that the data distribution is normally distributed, so the Analysis of Variance (ANOVA) test was used to test whether there were differences in the treatment groups on the expression of caspase-3 in the cerebrum and cerebellum.

Based on the ANOVAs test results in table (2), it is known that there are significant differences among the groups in the expression of caspase-3 in cerebrum and cerebellum of newborn *Mus musculus*.

**Table (1) Mean and standard deviation of caspase-3 expression in cerebrum and cerebellum of newborn *Mus musculus*.**

<table>
<thead>
<tr>
<th>Group of Treatment</th>
<th>Mean ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cerebrum</td>
</tr>
<tr>
<td>G.1</td>
<td>5.70 ± 0.99</td>
</tr>
<tr>
<td>G.2</td>
<td>7.23 ± 1.39</td>
</tr>
<tr>
<td>G.3</td>
<td>8.67 ± 1.09</td>
</tr>
<tr>
<td>G.4</td>
<td>4.17 ± 1.18</td>
</tr>
</tbody>
</table>

**Table (2) Anova test results on caspase-3 expression in cerebrum and cerebellum of newborn *Mus musculus*.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression of Caspase-3 in Cerebrum</td>
<td>0.000*</td>
</tr>
<tr>
<td>Expression of Caspase-3 in Cerebellum</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

* Significantly different <0.05
Figure (1) The differences in the description of caspase-3 expression in the cerebrum tissue of newborn mice. The red arrow indicates the presence of Caspase-3 expression which is indicated by the presence of brown chromogen. The combination of physical and psychological stress exposure group (G3) is the strongest among the physical stress group (G1), psychological stress group (G2), and the control group (G4). The expression of caspase-3 in the control group is the weakest. IHC is with a magnification of 400 times.

Figure (2) The differences in the description of caspase-3 expression in the cerebellum tissue of newborn mice. The red arrow indicates the presence of Caspase-3 expression which is indicated by the presence of brown chromogen. The combination of physical and psychological stress exposure group (G3) is the strongest among the physical stress group (G1), psychological stress group (G2), and the control group (G4). The expression of caspase-3 in the control group is the weakest. IHC is with a magnification of 400 times.
Discussion

The developing brain is the most sensitive organ to the effects of stress during the prenatal period, due to substantial changes in structural growth and connectivity during fetal life (2). During the gestational period, there are process of proliferation, differentiation, migration, and aggregation of fetal neurons which are strongly influenced by environmental factors (2). Prenatal stress exposure shows a long-term effect that includes both behavioral and molecular changes (6, 13).

When a stressor is felt, the hypothalamus will release the hormone cortisol. During pregnancy, fetal exposure to maternal cortisol is limited by the placental enzyme 11β-HSD-2 which functions to convert cortisol to cortisone, an inactive glucocorticoid (17). High glucocorticoids under stress reduce placental expression of 11βHSD2 which is associated with intrauterine growth restriction (20).

In acute stress, the binding of glucocorticoids and glucocorticoid receptors increases the tissue-plasminogen activator (tPA) which helps the process of converting proBDNF to mature BDNF (mBDNF) and increases proteolytic processing of proBDNF in mBDNF which can increase BDNF levels during stress, but in chronic stress due to decreased tPA hence the process of proBDNF to mBDNF is inhibited and BDNF expression is reduced. If proBDNF is not processed into mBDNF then proBDNF will bind more highly to the p75NTR receptor which induces a pro-apoptotic signaling pathway (3). The proBDNF binding to the p75NTR receptor will activate the apoptotic pathway through the co-receptor bond, namely sortilin. Sortilin will activate jun-N terminal kinase (JNK) which will then phosphorylate C-Jun which will activate pro apoptotic proteins such as p53, Bad, BIM, BAX so that it will stimulate mitochondria to release cytochrome-C which then activates caspase (12).

It is known that there are two types of caspases that have been identified, namely the initiator caspase and the effector / executioner caspase. Caspase 8 and 9 are the initiator caspases, while caspase-3 is the effector / executioner caspase (23). Caspase executioner mediates cell death during apoptosis, caspase-3 has the ability to partially cleave caspase substrates and its activity is required to induce cell death (10).

The results of our study indicate that there are significant differences in the expression of caspase-3 in cerebrum and cerebellum of newborn mice among treatment groups. Combined stress exposure show the strongest expression of caspase-3 compared to the physical, psychological, and control stress exposure groups. This finding is supported by a study by Qiao Y et al in 2020, where the combination of stress exposure caused significantly more hyper-activity of the HPA system as indicated by increased serum cortisol, CRH and ACTH levels (16).

High caspase-3 expression can cause an increase in apoptotic activity of brain cells and will decrease the number of cells making up the central nervous system in the fetal brain (9). A study conducted by Kinsella et al in 2009 shows that chronic stress during the prenatal period interferes with fetal neurodevelopment (11). Sandman et al, found that prenatal stress impaired cognitive performance during infancy and decreased brain volume (18).

Conclusion

Stress exposure during pregnancy increases the expression of caspase-3 in cerebrum and cerebellum of newborn Mus musculus.

Sources of Funding: This research was self-funded by the author.

Conflict of Interest: There is no conflict of interest in this study.

Ethical Approval : This study has obtained ethical eligibility permit based on the Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University No: 2.KE.001.01.2021.

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Case Study in Fatal Motorcycle Injury Patterns

Budiatri Retno Noormaningrum1, Idha Arfianti Wiraagni1, Dhiwangkoro Aji Kadarmo2

1Researcher, Department of Forensic and Legal Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, 2Researcher, Department of Forensic, Bhayangkara Hospital, Yogyakarta, Indonesia

Abstract

Crash injury is one of the leading causes of premature death globally. In Indonesia, a motorcycle crash is the main cause of road traffic injuries. As a city with a high number of motorcycles, Yogyakarta needs the right mitigation approach based on the local characteristics. The aim of this study was to evaluate the patterns of injury in a fatal motorcycle crash in Yogyakarta. Data were described from five dead motorcyclists that were brought to the mortuary of Bhayangkara Hospital.

Key words: injury; injury patterns; motorcycle accident.

Introduction

Motor crash makes an abundance of needless deaths and disabilities. WHO reported 1.35 million deaths a year or nearly as 3700 deaths every day from road traffic accidents1. In Indonesia, road injuries are the eighth and tenth leading cause of premature mortality and when combined with morbidity, respectively2. As a popular vehicle, the motorcycle demand in Indonesia is very high. Data from 2016 showed that more than 5 million motorcycles were produced annually3.

Yogyakarta is a city in Indonesia that motorcycles were extensively used. Hence, a crash is the greatest cause of road traffic injuries in Yogyakarta4. The right mitigation approaches are needed based on local characteristics. Therefore, we conduct this study to describe the patterns of injury in a fatal motorcycle crash in Yogyakarta.

Material and Methods

All of the deceased that would be discussed was brought to the mortuary of Bhayangkara Hospital in Yogyakarta from the scene of the crash, from April 1st to June 30th, 2020. The deceased were motorcyclists that had perished at the crash scene without received any medical attempts beforehand. The details had been summarized in Table 1. Approval of this study was given by the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health and Nursing University Gadjah Mada/dr Sardjito General Hospital.

Results

Case 1: contusions on the chest

On Monday, April 6th, 2020, the body of 37 years old male was brought by the police after a motorcycle crash that took place in a rural area at 12 PM. The deceased was still wearing an intact helmet on its head, no wounds were found underneath. From the examination, we found bruises, 8x4.2 cm on the right and 1.1x1 cm on the left side of the chest. There was bluish color on the face and nail beds.

Case 2: an app-based motorcycle taxi driver

A dead body of a 25-year-old male was brought to the mortuary on Thursday, April 9th, 2020. The motorcycle crash happened at 5:15 AM in a rural area. The deceased was still wearing an app-based taxi driver jacket and

Corresponding Author:
Idha Arfianti Wiraagni
Email: arfianti.idha@gmail.com
helmet. The examination revealed some abrasions, 10x5 cm on the left side of the chest, 19x15 cm on the left side of the abdomen, and 3x2 cm on the back of the left hand, and also closed fractures on the left upper arm, and left upper leg.

**Case 3: open fracture of the head**

An examination was conducted on Tuesday, May 26th, 2020, on a dead body of 36 years old male. The guy was wearing a helmet while crashed a house wall fence that day, at 2:30 AM under clear weather, as the rider. The pillion passenger was a 19 years old male that survived while suffered from lacerations on the left cheek and the right foot. The crash scene was a dimly lit rural area. The examination exposed an open fracture on the front part of the head that extended to the backside. There was amputation at the first segment of the fifth finger of the left hand, and closed fractures with deformities on the nose, left upper arm, right upper leg, and left knee. We found multiple lacerations on the left side of the head, right and left cheek, and lower lip. There were multiple abrasions on the right shoulder, right hand, left upper leg, right and left knee, and left lower leg.

**Case 4: multiple fractures**

On Sunday, May 31st, 2020, at 10:30 PM, in a rural area, a fatal road crash happened to a 69 years old male. The deceased was brought to undergo a forensic examination. The examination came out with multiple closed fractures on the lower jaw, upper left arm, the right side of the chest, left wrist, left ankle, and right knee. There was a laceration on the back part of the head 3x0.5 cm. We found numerous abrasions on the right side of the head, left knee and left upper arm.

**Case 5: the broken helmet was still attached**

On Monday, June 22nd, 2020, the dead body of 51 years old male that was still wearing a broken helmet on its head was brought by the police. When the helmet had been released, we found an open comminuted depressed fracture 15x13 cm on the frontotemporal region of the skull, with brain tissues scattered around. Other injuries were abrasions and bruises that were spread out on the neck, right elbow, right lower arm, right knee, left lower leg, upper right chest, and back. The crash happened in an urban area that day at 9:55 AM.

**Discussion**

**Demographical characteristics**

All of the cases that were discussed involved male motorcyclists, agreed with other studies that the majority of motorcycle crash casualties were male. This finding happened because there were more male motorcyclists than females, that in accordance with Indonesia’s condition. But for a head injury, there was no considerably different risk for both sexes.

Four from 5 cases (case 1, 3, 4, 5) associated with age $\geq$35, parallel to finding from Malaysia that this age was a significant predictor of death in a motorcycle crash. This similar outcome could be because of the similarity of Indonesia and Malaysia that both countries have large numbers of motorcycles as transportation vehicles for working-class citizens.

**The patterns of injuries**

The main objective of this study was to evaluate the patterns of injury in a fatal motorcycle crash in Yogyakarta. Studies from other countries showed that the most frequent cause of death in a fatal motorcycle crash was head injury. In this study, we described 2 cases (case 3 and case 5) with a devastating fracture on the head that could be easily recognized from external examination.

The actual number of head injuries in this study could be bigger, considered that internal examination had not been done because of the police request. The study in Spain showed that when the internal examination was conducted, the prominent causes of death in a motorcycle crash were subarachnoid hemorrhage, followed by cerebral contusion and skull base fracture. The past study found that the position as the rider had a higher head injury risk, compared to the pillion passenger.
Helmet use had been reported to lower head risk injury, in the amount of 69%, 71%, and 53% reduction in skull fractures, cerebral contusion, and intracranial hemorrhage, respectively\textsuperscript{13}. Contributing factors to the helmet’s failure in preventing injuries in case 1, 2, 3, and 5 could be speeding, or using helmet without proper fixation. As the past studies showed that the helmet protective effect only effective when the speed of the motorcycle was less than 30km/h\textsuperscript{14}, and securely fixed\textsuperscript{15}.

For facial injury, the helmet had a protective effect against maxillary fracture, but for mandible fracture (case 4) was not significantly proven\textsuperscript{16}. This finding might be because there was no consideration to the helmet types. Another study found that the full-face and modular helmet had a higher protective effect against head injury compared to open-face, half-helmet, and novelty helmets\textsuperscript{7}. A study from Thailand reported that a full-face helmet reduced head injury risk by 64% compared with a half-coverage helmet, and 36% compared with a half-coverage helmet\textsuperscript{17}. In Indonesia, wearing a national standardized helmet for motorcyclist was made mandatory\textsuperscript{18}, but the resident’s habit of wearing a helmet while riding a motorcycle was just 33.7% and 41.8% in Indonesia and Yogyakarta in particular based on the report in 2018, respectively\textsuperscript{6}.

Results from the past study showed that the majority of injury types were abrasions or bruises, followed by lacerations and bone fractures. Nearly agreed with our findings that all of the cases involved abrasions or bruises, 3 cases showed lacerations (case 3, 4, and 5), and bone fractures in 4 cases (case 2, 3, 4, 5). Amputation was relatively rare but happened in case 3, which might be because of the crushed effect during the collision\textsuperscript{12}.

After the head injury, injuries in the chest and abdomen caused significant numbers in motorcycle crash mortality\textsuperscript{5}, as we suspected from case 1, 2, and 5. But the actual cause of death could not be defined without internal examination.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Time</th>
<th>Place</th>
<th>Helmet</th>
<th>Crash circumstances</th>
<th>Injury types (sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>37</td>
<td>Monday; 12 PM</td>
<td>Rural</td>
<td>Present</td>
<td>N/A</td>
<td>Bruises (chest)</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>25</td>
<td>Thursday; 5:15 AM</td>
<td>Rural</td>
<td>Present</td>
<td>N/A</td>
<td>Abrasions (chest, abdomen, and left hand) Closed fractures (left upper arm, and left upper leg).</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>36</td>
<td>Tuesday; 2:30 AM</td>
<td>Rural</td>
<td>Present</td>
<td>The guy crashed a fence while riding in a dimly lit area under clear weather.</td>
<td>Open fracture (head) Amputation (the first segment of the fifth finger of the left hand) Closed fractures (nose, left upper arm, right upper leg and left knee) Lacerations (left side of the head, right and left cheek, and lower lip) Abrasions (right shoulder, right hand, left upper leg, right and left knee, and left lower leg).</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>69</td>
<td>Sunday; 10:30 PM</td>
<td>Rural</td>
<td>Absent</td>
<td>N/A</td>
<td>Closed fractures (lower jaw, upper left arm, chest, left wrist, left ankle and right knee) Laceration (head) Abrasions (right temple, left knee and left upper arm).</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>51</td>
<td>Monday; 9:55 AM</td>
<td>Urban</td>
<td>Present</td>
<td>N/A</td>
<td>Open fracture (head) Abrasions (neck, chest, right elbow, right lower arm, right knee, and left lower leg) Bruises (chest and back).</td>
</tr>
</tbody>
</table>
Other factors

The crashes in this study happened at various times and days, with the only case that occurred at the weekend was case 4. A past study in Pakistan found that crash happened on weekdays and during rush hour (6 AM-9 AM in the morning and 3 PM-6 PM in the evening) had more fatal probability than minor injuries that might be because of the high traffic of daily commuters. From this study, there was no crash that happened in rush hour, case 2 befell early in the morning, case 3 before dawn, and case 4 late at night, might have an association with speeding and off-peak hours that increased the probability of fatality.

Application-based taxi drivers (case 2) had a higher risk of mobile phone-related crashed, associated with the high prevalence of mobile phone use while riding. A factor that also contributed to motorcycle crashes was riding under alcohol influence, as it could increase the risk of losing control, and associated with non-helmet use. There were no data related to blood alcohol level obtained from this study.

Conclusions

Knowing injury patterns and associated factors are important in developing mitigation strategy to reduce motorcycle crash fatalities. The demographical characteristics gave us knowledge of the people at risk. Some measures had been made, but the implementation seems still lacking. This study could be developed for a future investigation, with the additions of more samples and variables. Internal examinations and laboratory tests will give objective value that will enrich the study.

Conflict of Interest NIL

Source of Funding The authors received no financial support for the research

Ethical Clearance taken from Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health and Nursing University Gadjah Mada/dr Sardjito General Hospital, Ref no: KE/FK/0369/EC/2021.

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Prevalence of Internet Gaming Disorder (IGD) in University Students and its Relationship with Depression

Bandana Bisht1, Navdeep Kaur2, Sandeep Kumar Goyal3

1Professor & Principal, 2Associate Professor & Vice-Principal, Chitkara University College of Nursing, Chitkara University, Himachal Pradesh, India, 3Senior Consultant, Department of Psychiatry, SPS Hospitals, Ludhiana, Punjab, India

Abstract

Introduction: Internet gaming disorder (IGD) is the emerging disorder in adolescents. The most common motivators behind IGD is coping with stressors, escapism, control over one’s life, excitement and challenges.

Aims: The current study was done with the aims: 1) To find the prevalence of IGD in university students 2) To find the prevalence of depression among university students 3) To find the association of IGD with depression among university students.

Settings and Design: A descriptive research design was used to collect data from a private university. Setting of the study was food court of the selected university.

Methods and Material: A total of 91 college students were recruited from a selected university. Purposive sampling method was used to collect the data. Internet Gaming Disorder addiction was checked with the help of DSM-5 criteria. Depression was checked with the help of PHQ-9 questionnaire.

Statistical Analysis Used: Chi-square was used to check the association between IGD and depression.

Results: Twenty-one (23%) respondents were found to have IGD. Out of 21 respondents who had IGD, 9 were having mild depression, 4 were having moderate and 3 were having severe depression. Out of 70 respondents who did not suffer from IGD, 28 showed no signs of depression, 36 had mild depression and only 6 reported moderate depression. None of them had severe depression. The results demonstrated that internet gaming disorder was significantly associated with depression (p<0.005).

Conclusions: Majority of the respondents having IGD had also reported co-morbid depression on PHQ-9 questionnaire. Thus depression needs to be identified and treated in clients suffering with Internet gaming disorder.

Key words: Internet Gaming Disorder (IGD), Depression

Introduction

The digital world was still in past, is changing dramatically now and will be changing rapidly then. In the past two decades, owing to exponential growth in technology and digital usage, internet gaming has become one of the most popular online activities. Despite its entertainment purpose, excessive engagement in online gaming can cause devastating effect on an individual’s health. In 2013 Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) has labeled it as separate entity ‘Internet Gaming Disorder’ (IGD) with the definition ‘persistent and recurrent use of internet to engage in games, often with other players, which leads to clinically significant distress or impairment.’ Moreover, ‘Gaming Disorder’ has been included as a mental health disorder in International classification of diseases 11th revision (ICD-11) by World Health Organization. Internet gaming disorder is a growing
public health threat that usually affects the adolescents and young adults across the globe and causing a significant decline in an individual’s well-being not only to physical, psychological but also to social and occupational functioning. [4]

Prevalence of Internet Gaming Disorder is increasing worldwide and has been estimated to be between 0.7-27.5 percent. It varies across countries and regions due to difference in various sample characteristics and screening tools being used. As per previous researches, Internet Gaming Disorder is found to be more prevalent in Asian countries than western ones. [5, 6] Further, one of the market surveys quotes India as one of the top five countries for downloading games globally. Also, India being a country with largest youth population and fastest growing smartphone user base, new insights regarding India are being uncovered continuously that it would exceed other countries and become biggest gaming market in future. This would result into more vulnerability to dangers associated with online gaming especially to adolescents in overall population of India. [7, 8, 9, 10] The available literature suggests that Internet Gaming Disorder is more prevalent in males and in adolescents brought up by divorced parents and reared up in broken homes. It is reported that increasing age, low self-esteem and poor daily life satisfaction usually cause more severe online gaming addiction particularly among males. [11, 12, 13, 14]

The predictive factors associated with problematic online gaming are not well established. However, researchers state that Individual factors (gender and age), social interactive factors (family atmosphere, social anxiety and self-esteem), cognitive factors and psychopathological conditions deeply influence the development of Internet Gaming Disorder. Depression, ADHD, anxiety and impulsivity are considered as the strongest risk factors. [15, 16] Also, an individual with higher psychopathology is more prone to online gaming addictions in an attempt to escape through emotional difficulties. [17, 18, 19] Furthermore, Addicted gamers often exhibit more irritability, aggression, low mood, and confusion between real and virtual world. Problematic gaming is also associated with low academic performance and substance abuse. [20]

In India, there is a scarcity of studies regarding prevalence and associated factors with digital gaming despite of the fact that internet usage is rising day by day in the country. Therefore, exploring the field of research regarding online gaming disorders will provide necessary hub of knowledge in an order to formulate health policies to prevent and treat Internet Gaming Disorder. With this background, present study was conducted to know Internet Gaming Disorder prevalence among university students and its association with depression.

**Aim of the Study**

1) To find the prevalence of Internet Gaming Disorder in college students

2) To find the prevalence of depression

3) To find the association of Internet Gaming Disorder with depression, if any

**Materials and Methods**

**Setting & Participants**

A total of 91 college students were recruited from a selected university. The setting of the study was Food court of the selected university. Purposive sampling method was used to collect the data. For the present study, those students were enrolled who gave the consent for the study and who met the inclusion criteria which was: a) The volunteer college students studying in undergraduate program of any stream b) Students using mobile phones or/laptops and also having access to internet since last one year.

The design of the study was descriptive research design.

**Tools**

1. Socio-demographic Performa: A socio-demographic performa was used.

2. PHQ-9:PHQ-9 [21] is a subset of Patient health
questionnaire. PHQ is a self-report version of Primary Care Evaluation of Mental Disorders (PRIME-MD). The PHQ-9, a tool specific to depression, simply scores each of the 9 DSM-IV criteria based on the mood module from the original PRIME-MD. PHQ-9 total score for the nine items ranges from 0 to 27. Scores of 5, 10, 15 and 20 represent cut points for mild, moderate, moderately severe and severe depression, respectively.

3. IGD Assessment tool: Internet gaming disorder was assessed by the researchers according to DSM-5 criteria by clinical interview.\[24\] There were nine items related to gaming behavior. Participants were supposed to respond in Yes or No. For every “Yes”, score of 1 was given. Any participant having score 5 or more was categorized as having IGD. The data so generated was subjected to statistical analysis.

The assessment was made on the basis of above instruments and scales, supplemented with a clinical interview.

**Results**

**Table 1: Socio-Demographic Characteristics of Participants**

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>N=91</th>
<th>%age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>- Female</td>
<td>61</td>
<td>67</td>
</tr>
<tr>
<td>Type of Family</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Nuclear</td>
<td>69</td>
<td>76</td>
</tr>
<tr>
<td>- Joint</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Type of residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Urban</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>- Semi-urban</td>
<td>68</td>
<td>75</td>
</tr>
<tr>
<td>- Rural</td>
<td>19</td>
<td>21</td>
</tr>
</tbody>
</table>

The socio-demographic characteristics are shown in Table 1. Majority of the participants were females (67%), having a nuclear family (76%) and residing in semi-urban areas (75%).

![Fig. 1 Depression in College Students](image-url)
Fig. 1 shows the results of PHQ-9 assessment in college students. Out of 91 participants, 45 students were found to have mild depression, 12 moderate and 2 were having moderately severe depression. None of the student had severe depression.

Fig. 2 Prevalence of Internet Gaming Disorder among College Students

Fig. 2 shows the prevalence of Internet gaming Disorder among college students. 21 (23%) students were having Internet gaming disorder out of 91 participants.

Table 2. Association between Internet gaming disorder and Depression among college students

<table>
<thead>
<tr>
<th>Internet Gaming Disorder (n)</th>
<th>No Depression (n)</th>
<th>Mild Depression (n)</th>
<th>Moderate Depression (n)</th>
<th>Moderately severe Depression (n)</th>
<th>Total (N)</th>
<th>Chi Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGD Present</td>
<td>5</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>21</td>
<td>13.021* (p 0.005)</td>
</tr>
<tr>
<td>IGD Absent</td>
<td>28</td>
<td>36</td>
<td>6</td>
<td>0</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>45</td>
<td>10</td>
<td>3</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 depicts that out of 33 students who did not have depression, 28 students were also not having IGD. Similarly, students with mild depression also have less cases of IGD. Out of 10 students who had moderate depression, 4 were having co-morbid IGD. All the students who were having moderately severe depression also had co-morbid IGD. Chi-square analysis found that there is a significant association between Depression and IGD.

Discussion

The current study, to the best of our knowledge,
is the first study to report prevalence of IGD and its association with depression among university students in India. This study found that overall prevalence of IGD was 23% in students of university. Similarly, a review literature conducted by Mihara and Higuchi found a prevalence of IGD ranging from 0.7% to 27.5%. In contrast to developed countries where the prevalence of IGD is reported only between 0.3%-1% by Przybylski AK et al.[25] It is hypothesized that unlike the western countries, students of Asian countries face more academic and societal competition. Thus, digital gaming provides an escape into a virtual world where they can relieve stress from such competitions.[10, 26]

Individuals with internet addiction exhibit more psychopathology, as revealed by Yen J et al.[27] For instance, Whang et al,[28] found significant association between degree of depression, anxiety and problematic internet use. A systemic review on the association between disordered online gaming and mental health issues found the correlation of 92% between anxiety and IGD, 89% between depression and IGD, and 85% between ADHD and IGD.[29] Hence, Depression is considered as a co-morbid psychiatric symptom of disordered digital gaming. Similarly, in current study, relationship between IGD and depression was also examined and it was found to be significantly associated (p value ≤ 0.005) as majority of the respondents had reported co-morbid depression on PHQ-9 questionnaire. These results are in line with the existing researches on relationship between pathologic internet gaming and psychiatric symptoms.[30, 31, 32,33,34] The literature has consistently demonstrated that online game addicts report more depression, loneliness, anxiety and lower psychological well-being.[28, 35]

Furthermore, in past, it was proposed by investigators that an individual with depression use Internet excessively as the means of self-medicating and Internet addiction itself can lead to depression symptoms because of social isolation resulting from spending much time in online gaming, which, in turn, causes undermined psychological health.[36, 37] Achab et al[38] compared the characteristics of addict vs non-addict online gamers and found significantly higher rates of irritability, emotional changes, low mood since online gaming onset. In addition to this, online gaming disorders and depression may share common risk factors, like, genes, environmental, educational, deficit social support, stress coping skills, and each might serve to aggravate of severity of other.[36,39] However, more researchers are needed to explicate the common mechanism underlying IGD and depression.[40]

Conclusions

In conclusion, this research demonstrated the most recent prevalence of Internet gaming disorder and its association with depression in University students of India. It shows continuous increase in prevalence of digital gaming and has strong association between depression and IGD. Thus depression needs to be identified and treated in clients. Furthermore, the direction of relation between IGD and depression needs to be explored.

Limitation

- Sample size was small
- Randomization was not done

Financial support and sponsorship

Nil

Conflicts of Interest

There are no conflicts of interest.

Ethical Clearance: taken from institutional review board

References


Pattern of Drowning Cases at a Tertiary Care Hospital, Khammam

Pondurthi Srinivas Rao¹, Bharath Kumar Guntheti²
¹Asst Professor, Government Medical College, Siddipet; Telangana, ²Professor, Dept. of Forensic Medicine & Toxicology, Mamata Medical College, Khammam; Telangana

Abstract

The diagnosis of death by drowning is one of the hardest challenges in forensic pathology. Circumstantial factors and physical evidence such as autopsy findings are both important in drowning. There were 68 cases of drowning deaths during one-year study period from Jan 2019 to Dec 2019, at dept of Forensic Medicine MMC&MGH, Khammam, consisting of 60 male and 8 female victims. The largest number of victims, 26 [38.23%] were found in the age group 31 to 40 years. Males [n=60, 88.23%] were predominantly affected. 37 [54.41%] cases were married, in which 32 were males (47.05%) and females were 5 (7.35%) and 31 cases are unmarried out of which 28 [41.17%] are male and 3 females. Most of the victims were follows Hindu belief, habitat of rural. Approximately 30 [44.11%] of victims come from the student unemployed group. Incidence of fresh water drowning is more than sea water drowning. The wet drowning accounted more than dry drowning. Highest number [n=32, 47.05%] occurred in afternoonthan other timings. The incidence of drowning was more in summer. Major fatalities occurred within one to three days of hospitalization. Accidental falls 56 [82.35%] were the commonly occurring incidents that leads to drowning death and most of drowning deaths 58 [70.58%] occurred in the lakes. Majority of victims died at the place of drowning. Most of the victims witnessed respiratory complications [55.2%]. 10.00% of the deceased were the tested positive for alcohol, while 4 [6.66%] were confirmed as drug abusers. Non swimmers were victims more than swimmers. Decomposition changes on the body were found in 10 % cases. Externally, frothy or blood-stained discharge at mouth and nostrils and “washer-women” appearance on the hands and feet were the common postmortem findings; while internally, congestion, edema of the lungs and presence of foreign bodies in an airway, were the common findings. Out of 68 cases 62 [91.17%] cases were showing positive results for same diatoms in bone marrow and sample water, in 45.88% cases, only sample water showed positive results, 22.94% cases were showing no diatoms in bone marrow and sample water. The aim of the study is to analyzed different main objective of the study is epidemiological factors and pattern of drowning, contributing factors, and arriving diagnosis of ante mortem drowning by diatom test.

Keywords: Pattern of Drowning, River, Canals, Wells, Diatoms, Fresh water

Introduction

Drowning is a type of asphyxia due to aspiration of fluid in to air passages, caused by submersion in water or any other fluid.

There are four types of drowning:
1. Wet [primary] drowning
2. Dry drowning
3. Secondary drowning [Post immersion syndrome or near drowning]
4. Immersion syndrome [Hydrocution, submersion inhibition]¹.

Corresponding Author:
Dr Bharath Kumar Guntheti
Professor, Dept. of Forensic Medicine & Toxicology, Mamata Medical College, Khammam; Telangana.
India Pin code: 5070002. MobileNo: 09908339507. Gmailid: bk62743@gmail.com
The main question in case of a body recovered from water is whether the individual was alive at the time he/she entered water. Circumstantial evidences, external appearances like cutis anserine, washer woman feet, and internal findings like emphysema aqueosum and froth in airways up to the terminal bronchioles give substantial amount of evidence for the diagnosis of ante mortem drowning. The discovery of diatoms and its application in diagnosis of drowning has increased the corroborative evidences for drowning².

During autopsy, one must focus attention to distinguish between changes that are due to drowning and those that are otherwise, i.e., those that occur in bodies immersed/submersed/disposed in water after death from causes other than drowning. The autopsy diagnosis of drowning is one of the major problems in forensic medicine, especially when there is delay in recovering the corpse. In advanced decomposed and skeletonized bodies, the only method of identifying the classical ante mortem drowning is estimation of the diatoms from the bone marrow and of comparing them with the diatoms available in the water².

Drowning is the third leading cause of unintentional injury death worldwide, accounting for 7% of all injury related deaths worldwide³.

According to National Crime Records Bureau, about 12 people end life every hour, and 83 people drown every day in India. The total number of accidental drowning deaths in India was 32,671[7.9%] during 2019.⁴

Accidental deaths due to drowning are common in India and suicidal drowning is one of the most common means to commit suicide. Homicidal drowning occurs less commonly. Some murderers dispose of the dead bodies of their victims in the rivers, seas and wells to simulate death due to drowning. Sometimes unwanted bodies are thrown into water.

The main objective of this retrospective study is profile, pattern of drowning, circumstances, location, month wise, contributing factors for drowning, and arriving diagnosis of ante mortem drowning by diatom test and suggestive preventive measures.

**Aims & Objectives**

Study of sociodemographic profile, pattern & present trends in drowning.

Types of submersion media and seasonal variation

Study the water diatoms and tally them with sternal bone marrow diatoms.

To know the validity of diatom test.

Cause and manner of death.

**Materials & Methods**

The present study was conducted in the Dept of Forensic Medicine, Mamata Medical College, Khammam, between January 2019 to December 2019. A total of 68 cases of drowning were statistically analyzed with regard to general incidence, profile, pattern, and drug/alcohol abuse, contributing factors after complete and detailed postmortem examination. Viscera were preserved for chemical analysis. Intact sternum and two-liter control sample of water from site of drowning was also preserved for comparative study with diatoms test. The diatoms from the sternal bone marrow of the cases were analyzed by acid digestion and were compared with the water diatoms from the same source.

**Result and Discussion**

Total 768 autopsies were conducted during the period of one year in the Dept of Forensic Medicine, MGH, Khammam. Out of these cases, drowning was concluded as cause of death in 68[8.85%] cases. These are similar to studies by authors.⁴⁻⁶

When identity is taken in to consideration, nearly all the cases were of known type. The above distribution may be because most of the accidental drowned deaths occurred in a close place from their residence, where they were brought to the mortuary as known bodies. 4[5.88%] cases are brought to the mortuary as unknown identity by investigating officers and in stage of decomposition, in those cases the early signs of drowning, postmortem
examination was masked and diatom test becomes the only means of knowing whether death was due to antemortem drowning or postmortem disposal. These are similar to authors.5, 6

In this study, peak incidence is observed during second [n=27, 39.70%] and third [n=26, 38.23%] decades of life, after which a gradual decline was evident. 2nd and 3rd decades together accounted for 77.94% of the total cases. These are consistent with authors1-6. Young people are at greatest risk of drowning owing to their energy and curiosity that can easily lead them to jump into water source from which they cannot escape. In teenagers and adults, drowning has been associated with drugs and intoxication.

In our study, deaths were more among males by drowning [n=60, 88.23%] as compared to females [n=8, 11.76%], male-female ratio being 7.5:1. This may be because of extensive outdoor activity of men. These are similar to another authors1-8. Married victims [n=37, 54.41%] outnumbered unmarried [n=31, 45.58%]. A total of 37 married people out of which male were 32 [47.05%] and 5 [7.35%] were female. Whereas unmarried males 28 [41.17%] were more than the unmarried females 3 [4.41%]. Amongst female victims the percentage of married are more. In the males no much difference between married and unmarried. As depicted in table no.1. Same findings were noted by authors.6-8

44 [64.70%] constituted from rural population. This distribution is because of the area under our jurisdiction consists of mostly rural, sub rural area surrounding the Khammam. Which are supported by authors.5, 7

Occupation wise, students were top among the victims. Whereas laborer was 19 [27.94%], employees were 10 and house wives were 5. This might be because of over enthusiasm of students in swimming and lack of proper supervision as felt by authors4-6. Chart no.1

Majority of people drowned in daytime as compared with night. Highest incidences reported in afternoon [n=40, 58.82%] followed by evening [n=23, 33.82%] and least [n=4, 5.88%] in night time. Same findings were made by authors7-9.

In this study, the incidence of drowning was more in summer [n=46, 67.64%] when compared to other seasons. These are consistent with authors6-9.

The month wise occurrence of drowning was more [n=46, 67.64%] in April-June months followed by July-August months [n=10, 14.70%] and Sept-Nov months [n=9, 13.23%] and least in Dec-Feb months [n=34.41%]. The high incidence of drowning in summer season might be because, victims being students in vacation, high temperatures in this season forced them into swimming to beat the heat and lack of proper supervision. These are consistent with authors6-9.

The commonest place of submersion was well/lakes [n=40, 70.58%] followed by river [n=10, 14.7%] and canals [n=7, 10.29%]. The reason might be due to jurisdiction of Khammam being surrounded by rural and semi-rural habituation. Place of incident and media of submersion was known in 68 cases. Chart no.3

Fresh water drowning was more commonly encountered with drowning in wells/lakes predominating 67 [98.52%] when compared with sea water drowning 1 [1.47%]. The reason might be due to presence of no sea in this jurisdiction. Similar observations were made by other studies.5-9

Pattern of drowning, 56 [82.35%] of deaths occurred due to accidental drowning, while swimming in wells/lakes, canals and river water. The victims are usually children, fisherman, and waterfront workers, intoxicated or epileptic subjects. Unintentional drowning was reported in maximum studies2-10. Chart no.4

Suicidal drowning was seen in about 8 [11.76%] of the cases. Drowning remains a relatively popular method of suicide in this region. This trend is because of increasing social, family, financial responsibilities and adjustment problems in the society, which might lead to increased suicidal tendencies. Suicidal episodes are fairly common amongst women or disabled persons.
These are consistent with studies by authors 7-10.

In our study only four homicidal cases [5.88%] of drowning was reported. The reason is intentionally persons being drowned by others with various grounds such as business, financial problems, extra marital affairs, family quarrels, and property disputes. Same results are obtained by authors 7-10.

In this study, wet drowning 54[79.44%] is commonest type of drowning, followed by dry drowning [n=10, 14.70%] and post immersion syndrome [n=4, 5.88%] in present study. These are consistent with authors 9-11.

In this study non-swimmers [n=58, 85.29%] dominated swimmers [n=10, 14.17%]. These are supported by authors 8-12.

In our study, most [n=64, 94.11%] of the persons were alive at the time drowning when compared with post mortem drowning 4[5.88%]. We could prove scientifically by positive diatom test and similarity of diatoms in test sample of bone marrow and control sample of water as ante mortem drowning, especially in putrefied corpses where no other signs of ante mortem drowning are present. We have also made an attempt to find out that in four cases the cause of death was not drowning, in the bodies recovered from water, as the victims were thrown into the water after killing them by some other means. These are consistent with authors 9-18.

In this study, out of 68 cases, 62(91.17%) cases are showing positive results for same diatoms in bone marrow and sample water, in 4[2.94%] cases, only sample water showed positive results, 2 (3.3%) cases showed no diatoms in bone marrow and sample water. The analysis of diatoms found in body may draw a fairly sound conclusion related to drowning as a mode of death. These are consistent with studies by authors 8-18. We analyzed the relationships between the numbers of the diatoms in the lung tissues and the drowning medium. Also, we made a comparative analysis between the diatoms in the lung tissues and the drowning medium using the ratio of diatom numbers in both samples. Quantitative diatom analysis in the lung tissues, especially combined with the diatom analysis of the drowning medium, provides supportive evidence in determining if a body recovered in water was due to drowning or not.

We observed associated injuries in 12 cases. This is supporting that the person was alive at the time of drowning. Middle ear hemorrhage was present in 72% of cases. Similar findings were made by authors 14-17.

Duration of hospital stay, we observed that the maximum number [n= 6, 8.82%] of victims stay in hospital for 1-2 days, followed by 2-3 days [n= 4, 5.88%] and up to seven days [n=22.94%] while spot dead were 56[82.35%] cases and brought dead were 2 [2.94%] cases. These are consistent with authors 9,16-20. Period of survival in fatal cases varied from 2 -7 days. The chances of survival depend on the duration of submersion, the water temperature, the person’s age, and how soon resuscitation begins.

In this study, 8[11.76%] people were positive for alcohol in their body. People who have consumed alcoholic beverages before submersion are more prone to develop brain or lung damage. Similar findings are made by authors 9,11, and 21.

Respiratory complications were encountered in maximum number of cases [n=50, 73.52%] followed by 10[14.70%] victims who did not aspirate water but died of asphyxia due to laryngospasm, neurological complications were seen in 3[4.41%] cases and post immersion syndrome in 4[[5.88%] cases. While seven victims [10.29%] died in the hospital. These are consistent with authors 18-22. The original concept of drowning deaths was that they were asphyxial in nature with water occluding the airways, death was due to electrolyte disturbance and or cardiac arrhythmias, produced by large volume of water entering the circulation through lungs. Chart no.8
Conclusion

Drowning deaths were more common in males, among 21 to 30 years of age group. A majority of them were married rural people and unemployed. Majority of drowning deaths were accidental in nature by falling into the wells/lakes and fresh water drowning deaths. Accidental drowning deaths were more common in males and children, suicidal drowning deaths were more in females. Almost all the deceased died at the scene and day time drowning deaths. Maximum number of drowning deaths were reported in summer and non-swimmer victims.

Froth or blood-stained discharge at mouth and nostrils, congested, edematous and voluminous lungs were common autopsy findings. Alcohol intake, disease status was found in 7.2% and 10.29% of cases respectively. Middle ear hemorrhage was present in 72% of cases. Postmortem aquatic bite marks and ante
mortem ingestion of water was found. The diatom test was still considered as golden standard. As drowning is a major cause of mortality among teenagers after road traffic accidents, all over the world, more in developed countries, this matter should not be overlooked.

**Recommendations:** Preventive measures need to be taken, based on the risk factors that had been identified. Therefore, a more comprehensive and strict approaches should be amended to prevent drowning death.

Supervision and surveillance of any child in proximity to any aquatic environment, including swimming pools, baths, beaches, buckets containing water, etc., is an essential strategy for the prevention of drownings ...

The presence of a lifeguard is the best way to avoid water accidents that end in drowning.

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**Ethical Clearance:** Not Required

**Conflict of Interest:** Nil

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The Pattern of Physical Injuries among Victims of Fatal Accidents in Bhopal, Madhya Pradesh

Brinda Patel¹, SK Asawa²

¹Senior Resident, Dept of Forensic Medicine & Toxicology, AIIMS, Bhopal, M.P., ²Professor & Head, Dept of Forensic Medicine & Toxicology, Government Medical College, Dungarpur, Rajasthan

Abstract

Background: The pattern of injuries is unique to the mode of the accident as well as to the causative agent. The objective of the present study was to describe the pattern of injury among victims of a fatal accident and draw a medicolegal conclusion from the pattern of injury. Methods: A total of 145 dead bodies brought for autopsy at the two selected post-mortem centres were included in the present study. The socio-demographic data like age, sex, religion, occupation as well as the circumstances leading to the death of the individual were gathered from documents and detailed interview of the friends/relatives/eyewitnesses etc. Among study participants, burns were the single largest category of accidents closely followed by road traffic accidents. The brain was the most common internal organ injured and the skull was the most common major bone fractured among the victims. One-fourth of all study participants were dead before being brought to hospital and one-third of all study participants survived for more than 48 hours after the incident. Conclusion: Septicaemic shock and craniocerebral injury were the most common cause of death among study participants.

Keywords: Accidents, Injury, Burns, Fall, Fatal, India.

Introduction

Trauma is an injury inflicted on a living tissue caused by the application of external force.¹ As per section 44 of the Indian Penal Code, an injury is defined as any harm illegally caused to any person in body, mind, reputation, or property.² Injuries can be classified as physical (or mechanical), chemical, thermal & miscellaneous depending on the nature of the causative agent.³ For medico-legal purposes, injuries can also be classified as suicidal, homicidal, accidental, fabricated, self-inflicted & defence. Traumatic instances can be unintentional (accidental) or intentional (suicidal or homicidal).⁴ It is estimated that worldwide about five million people die each year due to some type of injury.⁵ Moreover, different types of injuries collectively account for about 9% of global mortality & 12% of DALY (disability-adjusted life years).⁵

An accident is the occurrence of a series of events resulting in unintended injury, death, or property damage. It is an unplanned event, occurring suddenly, unexpectedly & inadvertently in an unforeseen circumstance.⁶ An accident is a result of defects laying either in the host (victim), agent (vehicle) & environment (road condition, traffic, weather conditions etc.) or a combination of defects at each of these three levels. Accidents represent an endemic of the modern and fast-changing world. There are different types of accident viz. road traffic accidents (RTA), railway accidents, falls from height, accidental fires, occupational accidents. In India, RTAs has the maximum share of unnatural deaths followed by burns.⁷ Globally, RTAs are considered as the 3rd leading cause of death after heart disease & cancer⁸ & the 3rd major preventable cause of unnatural deaths.⁹ ‘Fall from height’ is defined by ICD-9 as an event where a person falls to a ground-level from an upper level, whereas FICSIT (Frailty & Injuries Cooperative Studies) defines them as unintentionally coming to rest on the ground or lower level.¹⁰ The frequency, type & extensiveness of an injury depend on body weight, velocity, height, nature of surface impacted, body’s orientation at impact.¹¹ The most important among all these factors is the ‘height’ of
the fall.[11] During the autopsy, the most important thing to do is to determine the primary site of impact to permit the forensic reconstruction of the event to form a medico-legal opinion whether the fall was suicidal, homicidal, or accidental.

The objective of the present study is to identify and describe the pattern of injuries sustained by victims of fatal accidents.

Material and Methods

Study Setting: The present study was carried out at the mortuary of People’s College of Medical Sciences, Bhopal and Medico-legal Institute of Madhya Pradesh, Bhopal. Study Duration: The total duration of the study was one & a half year; from 1st January 2014 to 30th June 2015. Sample Size: For this study, we sampled and collected data from a total of 145 dead bodies on which the autopsies were conducted during the period of data collection.

Inclusion Criteria: Deaths due to accidents (e.g., road traffic accidents, falls from height & thermal injuries), who either died before reaching the hospital or were admitted to hospital before their death. Exclusion Criteria: (I) deaths due to non-accidental trauma (interpersonal or self-harm)(ii) Decomposed bodies preventing a valid autopsy, (iii) deaths due to causes other than trauma.

Data collection: The detailed medico-legal postmortem examination was carried out in the two selected centres after receiving requisition from the concerned police official, the inquest report & the dead body challan. The socio-demographic data like age, sex, religion, occupation as well as the circumstances leading to the death of the individual were gathered from documents like inquest report, dead body challan and through detailed interview of the friends, relatives, neighbours, eyewitnesses, and police officials accompanying the dead bodies. In cases where the death of the victim occurred in the hospital, the treatment records available were also studied. All medico-legally important findings were documented by taking scaled photographs, whenever & wherever possible.

Data Analysis: All the data were collected in a paper-based data collection form. Thereafter, the data will be coded and entered in Microsoft Excel. The coded data were imported into Stata 15.1 version for analysis. For the continuous data, the author calculated the mean, median, and standard deviation. For discrete data, the author calculated and reported frequency, proportion, and percentage.[12] Any statistical difference between the two proportions will be estimated using the Chi-square test.[13] Any statistical difference between the two means will be estimated using the T-test.[13]

Results

During the period of data collection, a total of 145 dead bodies fulfilling the selection criteria were brought to the mortuaries for autopsy. Table 1 gives details about the type of accident. As can be inferred from table 1, burns were the single largest category of accidents followed by road traffic accidents resulting in death among study participants. There was only a single case of death due to lightning.

<table>
<thead>
<tr>
<th>Type of accident</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Road traffic accident</td>
<td>53</td>
<td>36.6</td>
</tr>
<tr>
<td>Railway accident</td>
<td>7</td>
<td>4.8</td>
</tr>
<tr>
<td>Fall from height</td>
<td>18</td>
<td>12.4</td>
</tr>
<tr>
<td>Burns</td>
<td>65</td>
<td>44.8</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>Total</td>
<td>145</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 2 give details about the socio-demographic details of the study participants. The mean and the median age of the study participants was 38.6 and 28 years, respectively. Most participants were between 10-30 years of age followed by 30-60 years of age. Occupationally, most of the study participants were field workers and met with an accident while on their job. This was closely followed by females working as a homemaker.

| Table 2: Distribution of study participants by their socio-demographic data (n=145) |
| Age |
| 0-10 | 5 | 3.4 |
| 10-30 | 77 | 53.1 |
| 30-60 | 44 | 30.3 |
| >60 | 19 | 13.1 |
| Gender |
| Male | 100 | 69.0 |
| Female | 45 | 31.0 |
| Religion |
| Hindu | 123 | 84.8 |
| Muslim | 19 | 13.1 |
| Others | 3 | 2.1 |
| Marital Status |
| Unmarried | 56 | 38.6 |
| Married | 79 | 54.5 |
| Other | 10 | 6.9 |
| Occupation |
| Field workers | 51 | 35.2 |
| Factory worker | 7 | 4.8 |
| Official/clerical | 18 | 12.4 |
| Housewife | 34 | 23.4 |
| Other | 35 | 24.1 |
Table 3: Distribution of study participants based on injury to internal organ and fracture of major bones (n=80***)

<table>
<thead>
<tr>
<th>Fracture of Major Bones</th>
<th>n^</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skull &amp; Face</td>
<td>48</td>
<td>26.2</td>
</tr>
<tr>
<td>Neck and Thorax</td>
<td>18</td>
<td>9.8</td>
</tr>
<tr>
<td>Pelvis</td>
<td>31</td>
<td>16.9</td>
</tr>
<tr>
<td>Upper Limb</td>
<td>39</td>
<td>21.3</td>
</tr>
<tr>
<td>Lower Limb</td>
<td>47</td>
<td>25.7</td>
</tr>
</tbody>
</table>

INTERNAL ORGAN INJURY

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>n^</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>41</td>
<td>30.4</td>
</tr>
<tr>
<td>Lung</td>
<td>23</td>
<td>17.0</td>
</tr>
<tr>
<td>Heart</td>
<td>23</td>
<td>17.0</td>
</tr>
<tr>
<td>Liver</td>
<td>17</td>
<td>12.6</td>
</tr>
<tr>
<td>Other</td>
<td>31</td>
<td>23.0</td>
</tr>
</tbody>
</table>

***- Includes study participants without burn injury.
^- sample exceeds 145 because of multiple injuries.

Table 3 illustrates the injury sustained by bones and internal organs among victims of the non-burn accident. Among study participants, skull & facial bones were the most commonly (26.2%) injured major bones closely followed by bones of lower limb (25.7%), neck & thorax bones were least commonly injured bones (9.8%). Similarly, the brain was the most commonly injured organ (30.4%). Collectively, the hollow viscera of the abdomen were the second most common organ injured among the victims.

Table 4: Distribution of study participants based on the cause of death and survival after the accident (n=145)

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cranio-Cerebral Injury</td>
<td>44</td>
<td>30.3</td>
</tr>
<tr>
<td>Septicemic Shock</td>
<td>53</td>
<td>36.6</td>
</tr>
<tr>
<td>Polytrauma</td>
<td>21</td>
<td>14.5</td>
</tr>
<tr>
<td>Hemorrhagic Shock</td>
<td>25</td>
<td>17.2</td>
</tr>
<tr>
<td>Cardiac Arrest</td>
<td>32</td>
<td>22.1</td>
</tr>
</tbody>
</table>
Table 4 highlights the cause of death and the time for which the victim survived after the accident. Among study participants, septic shock was the most common cause of death. This is hardly surprising as most study participants suffered a burn injury. This was closely followed by cranial injury. In our study, most victims survived for more than 48 hours and about one-fifth of victims were declared dead on arrival at the hospital. Most participants who survived for more than 24 hours after the incident suffered from burn and most participants who were dead before reaching hospital suffered from poly-trauma.

**Discussion**

The present study included data from a total of 145 victims of fatal accidents whose dead bodies were brought to the mortuary of People’s College of Medical Sciences & Research Centre (PCMS & RC), Bhopal and Medicolegal Institute of Madhya Pradesh (MLI), Bhopal, for autopsy, during the period of data collection. The participants included victims of road traffic accidents, falls from height, railway accidents, accidental burns & other accidents. Most deaths were attributed to burns (44.8%), followed by road traffic accidents (36.6%), falls from height (12.4%), and railway accidents (4.8%).

In our study, out of a total of 145 victims, the majority were males (69.0%). In Indian society, men are generally the more active group (both economically and physically), thereby exposing themselves to various injury-causing agents. This may explain the observed male preponderance among study subjects. In our study, the single most common age group was 20-60 years, which also corresponds to the age group of maximum economic and physical activity. Children <10 years of age constituted the least common age group among study participants. All these participants (children) exclusively suffered from either burn or fall from height. The majority of the victims belonged to the Hindu community followed by Muslims. Similar results of Hindu predominance followed by Muslim were also reported by Gowri S et al. and Mazumdar A et al.

In the present study, most of the victims were married (54.5%), followed by unmarried (38.6%). This is easily understood as the most common age group in the present study was 21-40 years and this represents the bulk of the married population. Similar findings were also reported by other researchers e.g., Gowri S et al., Harish D et al. & Mangal HM et al.

In cases of death due to burn injury, women (61.53%) outnumbered men (38.46%). This female preponderance can be explained by the fact that in
our study most accidental burn were kitchen related. Furthermore, in our study about 23% of all participants were homemaker. Such female predominance among victims of accidental burns injuries is also reported by other researchers viz. Dhillon S et al (2005) [18], Gowri S et al, [14]& Bharadwaj SD et al [19]. In contrast,Rani A et al [20]and Chaudhary BL et al [21]reported a slight male preponderance among burn victims. In our study male predominance was seeing among victims of accidents that happened outdoors such as RTA. Moreover, in our study all the victims of fall from height were men. Similar findings were also reported by Kumar M et al [22], Naik BV et al [23], and Satish NT [24].

In the present study, we observed that the most commonly affected region,bearing external injuries was head, neck & face/HNF, involving 74 cases(26%), followed by lower limb (25%), and upper limb (21%). Involvement of HNF & extremities was most common because of the obvious facts that all these are exposed & projecting body parts & most active parts at the time of infliction of trauma.Khan MK et al. observed a similar pattern of distribution, having a larger proportion of injuries in HNF & extremities as compared to thorax & abdomen.[25] Merchant SP et al, Patil AM et al,also describes quite a similar pattern of injuries in conformity as observed1 in the present study. [26, 27](Out of all the internal organs, the brain was the most injured organ (30%). Both lungs and heart were injured in 17% of cases each, whereas the liver was injured in about 12% of cases. The brain was found to be the most injured organ in many studiesMandal BK et al (2012) of RTAs, falls from height & assault.[28]

Most cases of fracture were noted in the skull (26%), followed by lower limb (25%) followed by upper limb fractures (21%). As the head is the most prominent of the exposed parts of the body under its situation, it bears the brunt of violence in traumatic cases. Bairagi KK et al. (2010) also reported that most fractures were seen in the skull and face.[29] Similarly, Ravikumar R (2013) found skull fractures in 67.75% of cases of RTA, especially among two-wheeler accidents.[30] Mandal BK et al, also found the skull to be mostly fractured, followed by thoracic bones & long bones respectively in RTA cases. [28] Kumar JVK et al (2013) observed that there were skull fractures in almost all cases of fall from height wherever primary head impact was present.[31]

In the present study, one-third of all participants remained alive for >48 hours after the incident and about one-fourth of participants were declared dead on arrival at the hospital. The longest period of survival was predominantly seen among the victims of burn injury whereas in cases of head injury most victims survived <48 hours. Patil AM et al had more or less similar results. [27] Khan MK et al. in their study on fatal head injury victims found that the greatest number of victims survived for 1-6 hrs (27.17%).[25] Manish K. et al (2012) found that the maximum number succumbed to death within 6 hours.[32] The variation noted may be due to the difference in the type of trauma, type of organs involved & time of medical attention etc. Among burn-related deaths, the majority survived for 3-7 days (48.64%); followed by spot deaths (22.97%). Such a long survival period was because the burnt tissue acts as a nidus for infection & sepsis sets in as a delayed complication leading to death in approximately 3-7 days. This was consistent with the results of Gadge SJ et al (2014).[33] Chaudhary BL et al [21] state that the maximum victims died on the spot, followed by a survival period of 3-7 days. Harish D (2013) on the other hand found that maximum victims survived for more than 1 week (24%).[16]

**Conclusion**

Accidents constitute a major chunk of preventable deaths. Most of the participants in our study were either physically or economically active or both. The burn injuries were most common among housewife as most burn accidents were domestic incidents. Among victims of non-burn trauma, the most common injured internal organ was the brain and the most common major bone fractured was the skull.

**Ethical Clearance:** The protocol for the present study was approved by the Ethical Committee on Human Research of the People College of Medical Sciences,
Bhopal.

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**Conflict of Interest**: Authors declare they have no conflict of interest.

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Demographic Characteristics as Predictors of Medicine and Health Services Access Difficulties and Economic Problems during Covid 19 in Java, Indonesia

Cati Martiyanat, Leny Latifaht, Yusi Dwi Nurcahyant, Diah Yunitawatit, Marizka Khairunnisat

1Magelang Health Research and Development Center, Ministry of Health, Indonesia

Abstract

Background. The very rapid spread of COVID-19 infection, causing large-scale social restriction in many parts of the world. A lot of businesses and working places closed. Health services and medicine were in high demand. Objective. This study aims to determine the demographic characteristics that predict medicine and health services access difficulties and economic problems. Method. This was cross-sectional research conducted in Java, Indonesia. The research sample was 1,385 individuals aged 15 years and over who lived in Java and had filled out a survey via a google form. Data analysis using multivariate logistic regression. Results. The risk of difficulty accessing medicine and health services and economic difficulties was higher for males, had income < 3 million per month, and do not have health insurance. Conclusion. Male, had low income, and do not have health insurance are at higher risk for having difficulty accessing medicines/health services and experiencing economic difficulties. Meanwhile, respondents under 25 years of age have a higher risk of experiencing difficulties in accessing medicines and health services. The government needs to implement a strategy to reduce health and economic problems due to COVID-19 and pay attention to individuals under 25 years of age to access health services/medicines.

Keywords: Covid-19, medicine and health services, economic difficulties

Introduction

COVID-19 has spread widely in the world, at least in 216 countries and other territories with confirmed numbers of COVID-19 were 6,057,853 people and the number of deaths as many as 371,166 people as of June 1, 2020. After almost 3 months of mass social distancing, on 1st June 2020, Indonesia entered the new phase of transition into new normal or called new habit adaptation. A study showed timely control strategies using epidemiological measures in reducing social mixing, closely related to the outcomes of the COVID-19 epidemic. Despite the epidemiological concern, Indonesia has to juggle with economical calculation when declaring transition into the new normal phase. It is explained why the relaxing distancing control measures took place in a time of the still-raising confirmatory person contracting COVID-19.

During the COVID-19 pandemic, a lot of businesses and working places closed, mainly in the informal work sector. This made people lose their job and impacted on financial income. The increase in unemployment, poor households, the problem of people’s purchasing power, and the empowerment of micro, small and medium enterprises has become the focus of the Indonesian government. The vaccination program for certain age groups has begun to be carried out by the Indonesian government starting in early 2021, however, the economic sector in Indonesia has not shown significant progress. COVID-19 and its countermeasures have caused economic shocks that have an impact on unemployment, poverty, economic crises, and health inequalities. The government is faced with controlling the rate of cases and the economic situation to address health disparities and economic impacts due.
to the COVID-19 pandemic.\textsuperscript{11}

Research in several countries has identified mixed results for groups that are vulnerable to a more severe economic impact than for other groups. Economic health problems that arise due to COVID-19 are various, such as work and income problems\textsuperscript{12}, job losses and increased poverty \textsuperscript{13}, access to health services\textsuperscript{14}, inequality of health services between rural\textsuperscript{13}, and fulfillment of basic or daily necessities\textsuperscript{14}. Identification of vulnerable groups is important so that interventions in dealing with the economic and health impacts of COVID-19 are right on target and according to needs. So far, no research has been found on demographic characteristics that are predictors of access to medicines and health services and economic difficulties due to COVID-19 in Java, Indonesia. Given the diversity of vulnerable groups affected and indicators of economic problems that arise in various countries, studies are still needed on predictors of access to medicines and health services and economic difficulties due to COVID-19. This study aims to determine the demographic characteristics that predict economic impacts and community groups that require special attention regarding the economic impact of COVID-19 in Java, Indonesia. The demographic variables including age, gender, education level, employment status, monthly income, and marital status. Economical difficulties including questions about decreased income, difficulty in meeting daily needs, health insurance ownership, and difficulty accessing medicine and health services. The age variable is categorized into 3 categories: \( \leq 25 \) years old, 26-45 years old, and > 45 years old. Gender consists of males and females. The education level consists of 2 categories: high school and below and bachelor and above. Work status is divided into categories of health workers and non-health workers. Monthly income consists of 3 categories: < IDR 3 million, IDR 3-10 million, and > IDR 10 million. Marital status consists of the categories of non married and married. Statistical analysis used descriptive analysis and multivariate logistic regression using SPSS v.16.

\section*{Method}

This research is a quantitative study with a cross-sectional design. The research sample is individuals aged 15 years and over who live in Java, Indonesia and have filled out a survey via google form which is distributed by the research team through various cellular networks and social media. The number of samples obtained was 1,385 people. The study was conducted from June - July 2020. The inclusion criteria included individuals aged 15-64 years, willing to be respondents, able to read and understand questions. This study aims to determine demographic characteristics that predict economic impacts and community groups that require special attention regarding the economic impact of COVID-19 in Java, Indonesia. The demographic variables including age, gender, education level, employment status, monthly income, and marital status. Economical difficulties including questions about decreased income, difficulty in meeting daily needs, health insurance ownership, and difficulty accessing medicine and health services. The age variable is categorized into 3 categories: \( \leq 25 \) years old, 26-45 years old, and > 45 years old. Gender consists of males and females. The education level consists of 2 categories: high school and below and bachelor and above. Work status is divided into categories of health workers and non-health workers. Monthly income consists of 3 categories: < IDR 3 million, IDR 3-10 million, and > IDR 10 million. Marital status consists of the categories of non married and married. Statistical analysis used descriptive analysis and multivariate logistic regression using SPSS v.16.

\section*{Result}

\section*{Respondent Characteristics}

\begin{table}[h!]
\centering
\caption{Distribution of respondent characteristics}
\begin{tabular}{|l|c|}
\hline
Characteristics & n (%) \\
\hline
Age groups & \\
\hline
\( \leq 25 \) years old & 234 (16.9) \\
\hline
26 – 45 years old & 876 (63.2) \\
\hline
>45 years old & 275 (19.9) \\
\hline
Sex & \\
\hline
\end{tabular}
\end{table}
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>360 (26.0)</td>
</tr>
<tr>
<td>Female</td>
<td>1.025 (74.0)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
</tr>
<tr>
<td>High school and below</td>
<td>191 (13.8)</td>
</tr>
<tr>
<td>Bachelor and above</td>
<td>1194 (86.2)</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
</tr>
<tr>
<td>Non married</td>
<td>408 (29.5)</td>
</tr>
<tr>
<td>Married</td>
<td>977 (70.5)</td>
</tr>
<tr>
<td>Type of occupation</td>
<td></td>
</tr>
<tr>
<td>General public</td>
<td>911 (65.8)</td>
</tr>
<tr>
<td>Health worker</td>
<td>474 (34.2)</td>
</tr>
<tr>
<td>Type of occupation</td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>125 (9.0)</td>
</tr>
<tr>
<td>Student/college student</td>
<td>141 (10.2)</td>
</tr>
<tr>
<td>Employee</td>
<td>318 (23.0)</td>
</tr>
<tr>
<td>Government employee</td>
<td>498 (36.0)</td>
</tr>
<tr>
<td>Farmer/laborer/fisherman</td>
<td>10 (0.7)</td>
</tr>
<tr>
<td>Entrepreneur</td>
<td>78 (5.6)</td>
</tr>
<tr>
<td>Others</td>
<td>194 (14.0)</td>
</tr>
<tr>
<td>Income</td>
<td></td>
</tr>
<tr>
<td>&lt; IDR 3 million</td>
<td>593 (42.8)</td>
</tr>
<tr>
<td>IDR 3-10 million</td>
<td>630 (45.5)</td>
</tr>
<tr>
<td>&gt;IDR 10 million</td>
<td>162 (11.7)</td>
</tr>
<tr>
<td>Health insurance</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>218 (15.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>1167 (84.3)</td>
</tr>
</tbody>
</table>
The total number of respondents who filled out the questionnaire was 1415 people. A total of 30 people filled out the double questionnaire so that the remaining 1385 respondents could be processed. The majority of the sample were women (74.0%), aged 26-45 years (63.2%), married (70.5%), and highly educated (86.2%). Most of the respondent’s occupations as a government employee (36.0%) with the type of occupation as non-health workers (65.8%), earn <10 million per month (45.5%), and do not have health insurance (84.3%). The next table presents a multivariate logistic regression test to describe demographic characteristics as predictors of access to medicines and health services and economic difficulties due to COVID-19 in Java, Indonesia.

Table 2. Multivariate logistic regression estimates for factors associated with the economic stressor

<table>
<thead>
<tr>
<th>Variables (N=1,385)</th>
<th>Difficulty accessing medicines and health services</th>
<th>Difficulty in meeting daily needs</th>
<th>Decreased income</th>
<th>Losing job</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25 years old (ref)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 – 45 years old</td>
<td>0.540 (0.330-0.884)*</td>
<td>1.088 (0.849-1.849)</td>
<td>1.185 (0.766-1.832)</td>
<td>1.198 (0.771-1.861)</td>
</tr>
<tr>
<td>≥ 45 years old</td>
<td>0.335 (0.181-0.621)**</td>
<td>0.645 (0.372-1.119)</td>
<td>0.990 (0.608-1.612)</td>
<td>1.497 (0.906-2.473)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.789 (1.294-2.474)**</td>
<td>1.754 (1.281-2.402)***</td>
<td>1.380 (1.056-1.805)*</td>
<td>1.216 (0.914-1.618)</td>
</tr>
<tr>
<td>Female (ref)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General public</td>
<td>1.191 (0.855-1.659)</td>
<td>0.901 (0.666-1.219)</td>
<td>0.808 (0.630-1.035)</td>
<td>1.185 (0.898-1.564)</td>
</tr>
<tr>
<td>Health worker (ref)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school and below</td>
<td>1.083 (0.705-1.665)</td>
<td>2.233 (1.525-3.268)***</td>
<td>1.459 (0.971-2.192)</td>
<td>1.945 (1.343-2.816)***</td>
</tr>
<tr>
<td>Bachelor and above (ref)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non married</td>
<td>0.933 (0.611-1.425)</td>
<td>1.068 (0.735-1.551)</td>
<td>0.893 (0.644-1.239)</td>
<td>1.008 (0.712-1.427)</td>
</tr>
</tbody>
</table>
**Cont..** Table 2. Multivariate logistic regression estimates for factors associated with the economic stressor

<table>
<thead>
<tr>
<th></th>
<th>AOR (95% CI)</th>
<th>AOR (95% CI)</th>
<th>AOR (95% CI)</th>
<th>AOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married (ref)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Income</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; IDR 3 million</td>
<td>2.035 (1.128-3.672)*</td>
<td>5.069 (2.753-9.334)***</td>
<td>3.643 (2.429-5.463)***</td>
<td>2.444 (1.503-3.975)***</td>
</tr>
<tr>
<td>IDR 3-10 million</td>
<td>1.353 (0.772-2.374)</td>
<td>1.657 (0.908-3.025)</td>
<td>1.323 (0.928-1.886)</td>
<td>1.498 (0.946-2.372)</td>
</tr>
<tr>
<td>IDR &gt; 10 million</td>
<td>(ref)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health insurance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.446 (1.003-2.086)*</td>
<td>1.683 (1.201-2.360)*</td>
<td>1.639 (1.162-2.311)*</td>
<td>1.261 (0.909-1.750)</td>
</tr>
<tr>
<td>Yes (ref)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.001, ***p<0.000

Logistic regression results showed that male (AOR 1.754; 95% CI: 1.281-2.402; p <0.000), had low education (AOR 2.233; 95% CI; 1.525-3.268; p <0.000), had income <3 million per month (AOR 5.069; 95% CI: 2.753-9.334; p <0.000), and do not have health insurance (AOR 1.683; 95% CI: 1.201-2.360; p <0.05) are more at risk of experiencing difficulties in meeting daily needs. Being male (AOR 1.380; 95% CI: 1.056-1.805; p<0.05), had income < 3 million per month (AOR 3.643; 95% CI: 2.429-5.463; p<0.000), and do not have health insurance (AOR 1.639; 95% CI: 1.162-2.311;p<0.05) are more at risk of experiencing decrease income. The risk of losing a job is higher for people with low education (AOR 1.945; 95% CI: 1.343-2.816; p<0.000) and had income < 3 million per month (AOR 2.444; 95% CI: 1.503-3.975; p<0.000).

The risk of difficulty accessing medicines and health services is higher for male (AOR 1.789; 95% CI: 1.294-2.474; p<0.000), had income < 3 million per month (AOR 2.035; 95% CI: 1.128-3.672; p<0.05), and do not have health insurance (AOR 1.446; 95% CI: 1.003-2.086; p<0.05). But, older age (25-45 years old), AOR 0.540 95% CI: 0.330-0.884, p<0.05; > 45 years old, AOR 0.335 95% CI: 0.181-0.621, p<0.001) become protective variable in facing difficulty accessing medicines and health services.

**Discussion**

This study found that the risk of accessing drugs and health services was higher for men, had income < IDR 3 million per month, and not having health insurance. The absence of health insurance generally occurs in low-income. Populations that are vulnerable to COVID-19, including those in low socioeconomic groups are at risk of difficulty accessing health care services. Low-income families have 3 main barriers to accessing health care including lack of insurance coverage, poor access to health care facilities, and unaffordable costs. The results of Shadmi’s research in Zambia found that primary health services are free, but other costs must be borne by patients when accessing health services such as transportation costs, medicines, and diagnoses that cannot be carried out in health facilities that have the potential to continue to increase as the spread of
COVID-19 and poor households in urban and rural slum areas are the vulnerable groups to this financial risk. In this study, older age (25-45 years old) and 45 years old become protective variables in facing difficulty accessing drugs and health services. Previous research has found that access of young adults to health services is lower. The reasons that young people do not access health care include feeling unneeded, awareness of seeking treatment, structural weaknesses and existing care systems, or economic factors. Different from the results of this study, Bambra’s study found that lockdown policies had an impact on women’s access to health care, such as preventive care against breast cancer or cervical cancer screening, which was restricted in European countries.

This study found that male, had low education, had income < IDR 3 million per month, and do not have health insurance are more at risk of experiencing difficulties in meeting daily needs. lower-income earners are twice as likely to experience economic difficulties as those in the top income quintile. Wolfson’s research also found that households in the low-income, low-educated (not university) group, and who do not have health insurance are struggling to meet their basic needs because of COVID-19. Karpman’s study suggests that low-income people spend less on food, delay buying expensive goods, use up savings, or increase credit card debt at the start of the COVID-19 pandemic. Power’s research also found that people with low incomes employ complex food management and shopping strategies to maintain food security by buying goods at low prices, choosing local produce, shopping at multiple supermarkets for the cheapest items, and budgeting for the long term. Sarma’s survey in the United States found 6.3% of respondents were concerned about financial stability, 42.5% about jobs, 69.4% about food availability, 31.0% about housing stability, and 35.9% about access to health care due to COVID-19.

This study found that male, had income < IDR 3 million per month, and do not have health insurance are at higher risk for having difficulty accessing medicines and health services and experiencing economic difficulties. Meanwhile, respondents under 25 years of age have a higher risk of experiencing difficulties in accessing medicines and health services. This research identifies groups that need special attention because they are more economically affected and have difficulty accessing health due to the COVID-19 pandemic. The government needs to implement a strategy to reduce health and economic disparities because COVID-19 in male groups with low-income who do not have health insurance and pay attention to individuals under 25 years of age to access health services and medicines.

**Conclusion**

The male gender with low income (<3 million per month), without health insurance are at higher risk for having difficulty accessing medicines and health services and experiencing economic difficulties. Meanwhile, respondents under 25 years of age have a higher risk of experiencing difficulties in accessing medicines and health services. This research identifies groups that need special attention because they are more economically affected and have difficulty accessing health due to the COVID-19 pandemic. The government needs to implement a strategy to reduce health and economic disparities because COVID-19 in male groups with low-income who do not have health insurance and pay attention to individuals under 25 years of age to access health services and medicines.

**Conflict of Interest:** The authors have no conflict of interest with the material presented in this paper

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**References**


The Association of Adiponectin Serum Level and Body Mass Index among Javanese Obese Adolescents

Christine Florens¹, Nur Aisiyah Widjaja², Roedi Irawan³, Meta Herdiana Hanindita²

¹Resident, ²Senior Staff, ³Head of Department, Division of Nutrition and Metabolic Diseases, Child Health Department, Faculty of Medicine, Airlangga University, Dr. Soetomo General Hospital, Surabaya, Indonesia

Abstract

Introduction: The adiponectin level should decrease in obese people, however, many previous studies’ results about its role in obesity still remain controversies. The present study aimed to evaluate the profile of adiponectin serum level among obese adolescents in Javanese population and its correlation with body mass index (BMI).

Methods: A cross-sectional study involving obese adolescents aged 13-18 years old from some schools was conducted from May to September 2020. The subject were determined by using a total population sampling method that met inclusion and exclusion criteria. Anthropometries were measured to count BMI and determine obesity according to CDC 2000. The adiponectin serum level was examined by ELISA from blood samples. Data were analyzed using Mann-Whitney Test and Spearman correlation, with a significance value at \( p < 0.05 \).

Results: There were 240 obese adolescents involved (52.1% boys) in the present study. There were significant differences in the mean of body-weight and body-height according to gender (\( p<0.05 \)), however, there was no significant difference in BMI based on genders. The median of adiponectin serum level was 13.9 (1.5-46.6) µg/ml, which had no correlation with BMI (\( p=0.98; r=0.002 \)).

Conclusion: The adiponectin serum level had no significant correlation to body mass index. More studies are suggested to find out several factors that might influence the adiponectin serum level in Javanese population.

Keywords: Adiponectin, Body Mass Index, Obese Adolescents, Javanese

Introduction

Adiponectin is an endocrine factor that is synthesized and released from adipose tissue, especially in subcutaneous and visceral fat.¹ Several studies have shown that adiponectin serum level has negative correlation with body fat mass.²,³ However, others still show controversial results.⁴,⁵

In obesity, there are adipocytes hypertrophy. This condition, accompanied with high fat diet, might cause DNA methylation. DNA methylation is the addition of methyl group to the atom carbon number 5 from cytosine ring or atom nitrogen number 6 of adenine. Furthermore, it will decrease the adiponectin expression. The same methylation might be happened in adiponectin receptor (AdipoR1 and AdipoR2), thus, it will prevent...
the adiponectin to bind its receptor.\textsuperscript{6}

The adiponectin increases insulin sensitivity and glucose uptake, decreases gluconeogenesis, decreases triglyceride production and lipogenesis on the liver and skeletal muscle.\textsuperscript{7}

Adiponectin and insulin resistance in obese adolescents will increase glycolysis, gluconeogenesis, hyperlipidemia, and endothelial dysfunction. The process, at last, results in metabolic syndrome.\textsuperscript{8} The obesity management and decreasing body weight hopefully will increase adiponectin serum level.\textsuperscript{9}

Therefore, it is essential to see the profile of adiponectin serum level in obese adolescent. In conjunction with its association to metabolic syndrome from previous studies, the obese children with lower adiponectin serum level will be more susceptible to develop metabolic syndrome.\textsuperscript{10}

The correlation of adiponectin serum level and BMI might differ according to race, age, and gender. To the best of the researchers’ knowledge, the study of adiponectin serum level among obese adolescents in Javanese population, Indonesia, is still limited.

**Materials and Methods**

*Cross-sectional research* was conducted on obese adolescents from 12 junior and senior high schools in Surabaya and Sidoarjo city, East Java, Indonesia, from May to September 2020. The subjects were determined using a total population sampling method that met the inclusion and exclusion criteria. The inclusion criteria were adolescents aged 13-18 years old with obesity problems. Moreover, both students and their parents voluntarily participated in the study. Adolescents with a history of corticosteroid consumption for more than two to six months before the study was carried out or the subjects got sick were excluded.

Obesity was established based on the CDC 2000 criteria, which was body mass index (BMI) for age and gender above the 95\textsuperscript{th} percentile. Body weight was measured using a digital weight scale (Seca, Germany No ref. 224 1714009) with a precision of 0.1 kg. The subjects were standing with barefoot and using thin clothes during bodyweight measurement. Height measurement was performed using stadiometers (Seca, Germany No ref. 224 1714009), with an accuracy of 0.1 cm. During height measurement, the subjects were standing with barefoot without using hat. The stadiometer was used to measure the height from heel to vertex. The results were presented as ‘meter’. BMI was calculated by the following formula:

\[
BMI = \frac{\text{Body weight (kg)}}{\text{Body height (m)}^2}
\]

The subjects had been fasting for 12 hours before the blood samples were taken. The adiponectin serum level was examined using 5 ml venous blood. The blood samples were centrifuged until the serum were achieved and saved in -70\textdegree C for further analysis. The analysis of adiponectin serum level used ELISA (Enzyme Linked Immuno-Sorbent Assay) of DBS (Diagnostic Biochem Canada CAN-APN-5000) kit and presented as µg/ml.

Bodyweight, body height, and adiponectin were described as ± mean (\(M\)) and standard deviation (\(SD\)). The correlational analysis between adiponectin serum level with gender and age group was carried out using Mann-Whitney test. The correlation between BMI and adiponectin serum level was analyzed using spearman rho, \(p<0.05\) considered as significant. All statistical analysis were conducted using SPSS 21.0.

This study had got permission from the ethics committee of The Faculty of Medicine, Airlangga University No. 115/EC/KEPK/ FKUA/2020. Before the subject recruitment, the researchers had explained to the subjects and their parents about the general research information and the consent.

**Results**

There were 240 obese adolescents involved in this study, consisting of 125 (52.1%) boys and 115 (47.9%) girls. Based on age group, there were two groups namely those less than 15 years old and those 15 years old or older. 44.6% subjects were less than 15 years old.
Table 1. Characteristic of the subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Gender</th>
<th>Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>Boys</td>
<td>87.4 ± 14.6</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>81.4 ± 11.7</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>Boys</td>
<td>164.1 ± 7.9</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>156.9 ± 8.1</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>Boys</td>
<td>32.4 ± 4.2</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>32.9 ± 4.7</td>
<td></td>
</tr>
</tbody>
</table>

The median of adiponectin serum level from all subjects was 13.9 (1.5-46.6) µg/ml and the mean amounted of 15.7 µg/ml±7.6. Table 2 shows that the mean of adiponectin serum level among boy adolescents was lower than girls and it was not statistically significant. Furthermore, from the classified age, the mean of adiponectin serum level in less than 15 years old group was slightly lower than the others. Figure 1 confirms that there was no correlation between adiponectin serum level with body mass index ($p=0.98; \ r=0.002$) from this study.

Table 2. The adiponectin serum level according to gender and age groups.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Mean of Adiponectin Serum Levels±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boy</td>
<td>14.8 ± 6.8</td>
<td>0.09</td>
</tr>
<tr>
<td>Girl</td>
<td>16.7 ± 8.4</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Mean of Adiponectin Serum Levels±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 15 years old</td>
<td>15.3 ± 7.3</td>
<td>0.59</td>
</tr>
<tr>
<td>≥ 15 years old</td>
<td>15.9 ± 7.9</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The decrease of adiponectin serum level in obesity is caused by the failure of transcription regulation with unclear mechanism. Generally, the adiponectin expression is ruled by transcription and translation process. In obesity, adipocyte hypertrophy and inflammation disturb the process. The DNA-methyltransferase-1 (DNMT1) expression is increasing and causes hyper methylation on adiponectin promoter. As a result, it can inhibit adiponectin production.11

This study gave the result of median adiponectin serum level of 13.9 (1.5-46.6) µg/ml and the mean of 15.7 µg/ml±7.6 from all subjects. This value was higher than previous studies conducted by Asayama et al, who stated 6.4 µg/ml±0.6, Lanas et al with 9.9 µg/ml±3.2, and Widjaja et al with 7.84 µg/ml±3.8.

Several factors might influence adiponectin serum level such as gender, obesity, physical activity, dietary pattern, and genetic.13,14 Ethnicity is one of the independent factor that might influence adiponectin level. The adiponectin serum level was significantly different between Iranian, Indian, and European although they were living in Australia. The Europeans had the highest mean of adiponectin serum level of 16.8 (14-20.2) µg/ml.15

Similar to the result of the present study in adiponectin serum level according to gender, several previous studies also showed that it was lower in boys than girls.13,16,17 The insignificant difference of our result was the same as Woo et al, and might be happened due to our samples only included obese adolescents.18

Meshkini et al found the adiponectin serum level was initially lower in boys. However, after it was corrected with age, BMI, and fat mass, and waist circumference, there were no differences between both genders. Based on that result, the adiponectin serum level might be influenced by percentage of fat mass and pubertal state. The percentage of fat body mass in girls is increasing during puberty. On the contrary, it is constant in boys.19

Estrogen might increase subcutaneous fat, on the other hand, testosterone is more influencing the visceral fat. Cnop et al observed that visceral fat had association with lower adiponectin level.20,21

The adiponectin level in this study was not significantly different according to age classification.
In contrast, other studies could describe the decrease of adiponectin level during puberty. Thus, the older adolescent would have lower adiponectin serum level. Otherwise, on adults, Obata et al found the positive correlation between adiponectin and age, either in healthy or patient with type 2 diabetes mellitus.

In this study, there was no significant correlation between serum adiponectin and body mass index. Awede et al found the same result in west African without diabetes. In male subjects, the lower adiponectin level was in subjects with normal BMI and obesity. On the other hand, on female group, subjects with overweight had the highest adiponectin level but subjects with obesity had the lowest adiponectin serum level. Study in Indian population also portrayed the same result. Our result was in contrast with other studies that stated the BMI had negative correlation with adiponectin serum level.

The racial distinction might play an important role. Mente et al found negative correlation between BMI and adiponectin serum level on subjects from Chinese, European, and Aborigin population, whereas the same correlation did not find in South Asian population. Khoo et al, also found different rate of negative correlation between BMI and adiponectin serum level in the Chinese, Malay, and Indian population that lived in Singapore. The most significant decrease of adiponectin serum level based on BMI happened in Chinese population.

The insignificant correlation from this study might be caused by the difference of fat mass distribution, dietary patterns, and genetic factor among our subjects with other studies. The limitation of this study was only involved obese adolescents. Moreover, the physical activity and dietary patterns have not assessed in this study yet. Further studies should include normal and overweight adolescents, evaluation of physical activity and dietary patterns.

Conclusion

The concern to adiponectin serum level in obese adolescent is increasing due to its impact on insulin resistance and developing metabolic syndrome. Results of the decrease of adiponectin level related to obesity is still controversy. The adiponectin serum level in this study had a wide range, with no significant correlation to body mass index.

Our average result of adiponectin serum level among obese adolescents was higher than other previous studies. It also did not have significant correlation with age and gender. More studies are needed to find out several factors that might influence the adiponectin serum level in Javanese population such as physical activity, dietary patterns, and genetic.

Acknowledgements: Authors are grateful to the 12 high schools in Surabaya and Sidoarjo who volunteered to become the research respondents. Moreover, they would thank all headmasters and counselling teachers who gathered our respondents and their parents with us before and during the data collection.

Ethical Clearance - This study had got permission from the ethics committee of The Faculty of Medicine, Airlangga University No. 115/EC/KEPK/ FKUA/2020. Before the subject recruitment, the explanation was done to the subjects and their parents about the general research information for getting their consent.

Source of Funding – Self

Conflict of Interest – Nil

References


The Gloves as Effective Personal Protective Equipment (PPE) of Indonesian Batik Workers

Cita Rosita Sigit Prakoeswa, Damayanti, Sylvia Anggraeni, Menul Ayu Umborowati, Sri Awalia Febriana

1Professor, 2Researcher, Department of Dermatology and Venereology, Faculty of Medicine Universitas Airlangga/ Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

Abstract

Background: Batik substances may increase the risk of biological function disruption to batik workers.

Objective: To Determine the effectiveness of using test gloves in transepidermal water loss (TEWL), skin hydration level, and skin acidity (pH).

Methods: This study was one group pretest-posttest design of 16 batik workers. Subjects use test gloves made from neoprene on right hands and personal gloves made from thermoplastic polymer of vinyl chloride on left hands in first and second week. Washed out for 2 weeks, last 2 weeks use test gloves on left hand and personal gloves on right hand. TEWL, skin hydration level, and pH was examined by Cutometer dual MP-580

Result: First and second week using test gloves on the right hand, significantly differences of TEWL level on extensor and dorsum manus; skin hydration level on flexor, extensor, palmar, and dorsum manus; pH on extensor, palmar and dorsum manus (p<0.05; CI 95%). Fifth and sixth week using test gloves in the left hand, significantly difference of TEWL level on dorsum manus, all area of skin hydration level and pH results (p<0.05; CI 95%). No significant differences of comparison right and left hand in the first, second, fifth, and sixth week, that showed any types of gloves can protect batik workers from skin barrier disruption.

Conclusion: Personal gloves and test gloves usage for protection doesn’t have much significant difference. Wearing gloves may also give risk to trigger contact dermatitis if not used properly or using incompatible material.

Keyword: gloves, Indonesian batik workers, skin barrier function
During the production of batik, synthetic dyes usage increases exposure to harmful pollutants such as heavy metals, suspended solids, or organic substances. These exposures might enter human’s body through the skin and respiratory mucosa. That exposure can be contact dermatitis. Occupational contact dermatitis is the most common in occupational skin disease in developed countries for between 70% and 90%. The incidence in Europe reported that 0.5 to 1 per 1000 workers. In Indonesia, study that had been conducted by researchers in batik workers population in Yogyakarta shows that the contact dermatitis prevalence due to work is quite high (10.36%). This shows the importance of personal protective equipment (PPE) in batik workers.3–6

PPE is important to prevent contact dermatitis. One of the most important PPE item in batik industry is gloves, that is recommended when wet work is performed. The wearing of gloves may improve hydration and pH of the skin.6,7 The purpose of this study was to determine the effectiveness of using test gloves which can provide protection for skin, especially for batik workers, from irritation due to long-term working.

Methods

Sixteen female batik workers who were involved in dyeing process from batik center in Tanjung Bumi District, Paseseh Village were included as samples in this clinical trial. Inclusion criteria in this study were age 15-50 years, involved in dyeing process, and were willing to participate in this study. There were 2 types of gloves evaluated in this study. The first type was personal gloves (their own gloves that batik workers had worn, made from thermoplastic polymer of vinyl chloride). The second type was test gloves that made from neoprene. In the first 2 weeks, the subjects were asked to use test gloves on their right hands and personal gloves on their left hands. Daily usage of the gloves were 8 hours a day and they were changed every 3 days. Washed out period was conducted for 2 weeks, it was a period when subjects were given with no gloves. Finally, in the last 2 weeks the subjects were asked to use test gloves on their left hand and their personal gloves on their right hand. At the beginning and end of each treatment clinical examination as well as skin biological function tests (TEWL level, skin hydration level and skin acidity) were conducted on both flexors, extensor, palmar, and dorsum of the hands. Biological function of the skin (TEWL level, skin hydration level, and skin acidity) was examined using Cutometer dual MP-580, the examination takes three times per examination and the final result is an average. Analysis will be carried out to compare skin function between batik worker who wore test gloves and personal gloves. The correlation between pre and post exposure of the right/left hand was analyzed by t dependent test, while the correlation between right and left hand was analyzed by independent t-test.

Results

This study involved 16 batik workers who were involved at dyeing process as subjects. Subjects were both male and female, aged 15-50 years. The results for mean TEWL level on flexor, extensor, palmar, and dorsum of right hands that used test gloves on the first week of study were 27.99 g/m2/h, 26.07 g/m2/h, 48.57 g/m2/h, and 40.78 g/m2/h, respectively; meanwhile mean TEWL level on flexor, extensor, palmar and dorsum of left hands that used personal gloves from the workers on the first week of study were 29.64 g/m2/h, 27.09 g/m2/h, 47.05 g/m2/h, 33.16 g/m2/h, respectively.

Skin hydration level measurement results on flexor, extensor, palmar and dorsum of right hands on the first week were 58.77 AU, 63.80 AU, 46.53 AU, and 63.93 AU, respectively, meanwhile on the left hands the results were 61.61 AU, 59.74 AU, 45.40 AU, and 61.88 AU, respectively. Furthermore, the skin pH measurement results on flexor, extensor, palmar and dorsum of right hands were 5.18, 5.22, 5.25, and 5.04, respectively; meanwhile on the left hands the results were 5.19, 5.25, 5.36, and 5.16, respectively (Table 1, Table 2).

On the second week after the use of test gloves on the right hands for two weeks, TEWL level results on flexor, extensor, palmar, and dorsum manus of right hands were 24.66 g/m2/h, 19.77 g/m2/h, 54.49 g/m2/h and 23.61 g/m2/h, respectively; meanwhile on the right
hands the results were 27.24 g/m²/h, 18.72 g/m²/h, 50.98 g/m²/h and 20.54 g/m²/h, respectively. Skin hydration level results on right and left hands flexors were 44.13 AU and 38.74 AU; extensor 35.38 AU and 37.06 AU; palmar 29.47 AU and 27.98 AU; and dorsum 36.67 AU and 39.11 AU. The skin pH measurement on right and left hands’ flexor were 5.37 and 5.40; extensor 5.49 and 5.43; palmar 5.81 and 5.83; and dorsum 5.51 and 5.51 (Table 1, Table 2).

Table 1. Comparison of the test gloves on the right hand at first week, second week, fifth week and sixth week

<table>
<thead>
<tr>
<th></th>
<th>First week</th>
<th>Second week</th>
<th>p</th>
<th>Fifth week</th>
<th>Sixth week</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td></td>
</tr>
<tr>
<td>TEWL level (g/m²/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Flexor</td>
<td>27.99 9.10</td>
<td>27.24 14.94</td>
<td>0.881</td>
<td>35.99 29.38</td>
<td>27.92 8.88</td>
<td>0.607</td>
</tr>
<tr>
<td>Right Extensor</td>
<td>26.07 9.83</td>
<td>18.72 8.43</td>
<td>0.008</td>
<td>28.00 13.61</td>
<td>23.12 8.26</td>
<td>0.142</td>
</tr>
<tr>
<td>Right Palmar</td>
<td>48.57 11.33</td>
<td>50.98 9.67</td>
<td>0.367</td>
<td>52.29 11.16</td>
<td>56.48 9.12</td>
<td>0.109</td>
</tr>
<tr>
<td>Right Dorsum manus</td>
<td>40.78 10.25</td>
<td>20.54 8.54</td>
<td>0.000</td>
<td>35.91 11.08</td>
<td>30.84 10.17</td>
<td>0.073</td>
</tr>
<tr>
<td>Skin hydration level (AU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Flexor</td>
<td>58.77 14.46</td>
<td>44.13 15.60</td>
<td>0.002</td>
<td>77.00 23.67</td>
<td>57.95 12.10</td>
<td>0.002</td>
</tr>
<tr>
<td>Right Extensor</td>
<td>63.80 16.28</td>
<td>35.38 13.86</td>
<td>0.000</td>
<td>73.30 23.54</td>
<td>55.64 12.30</td>
<td>0.004</td>
</tr>
<tr>
<td>Right Palmar</td>
<td>46.53 15.34</td>
<td>29.47 11.58</td>
<td>0.001</td>
<td>55.62 22.88</td>
<td>54.76 15.92</td>
<td>0.795</td>
</tr>
<tr>
<td>Right Dorsum manus</td>
<td>63.93 15.10</td>
<td>36.67 12.84</td>
<td>0.000</td>
<td>72.43 31.51</td>
<td>50.50 16.24</td>
<td>0.001</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Flexor</td>
<td>5.19 0.16</td>
<td>5.37 0.42</td>
<td>0.055</td>
<td>5.43 0.17</td>
<td>5.57 0.25</td>
<td>0.072</td>
</tr>
<tr>
<td>Right Extensor</td>
<td>5.22 0.14</td>
<td>5.49 0.30</td>
<td>0.014</td>
<td>5.32 0.22</td>
<td>5.46 0.27</td>
<td>0.066</td>
</tr>
<tr>
<td>Right Palmar</td>
<td>5.26 0.19</td>
<td>5.81 0.24</td>
<td>0.000</td>
<td>5.54 0.27</td>
<td>5.56 0.26</td>
<td>0.972</td>
</tr>
<tr>
<td>Right Dorsum manus</td>
<td>5.03 0.29</td>
<td>5.51 0.30</td>
<td>0.002</td>
<td>5.32 0.27</td>
<td>5.63 0.28</td>
<td>0.000</td>
</tr>
</tbody>
</table>
On the fifth week after washed out, the test gloves were used on the left hand and the personal gloves were used on the right hands. TEWL level results on right and left hands flexor were 35.99 g/m²/h and 26.80 g/m²/h; extensor 28.00 g/m²/h and 27.31 g/m²/h; palmar 52.29 g/m²/h and 53.18 g/m²/h; and dorsum 35.91 g/m²/h and 38.16 g/m²/h. Skin hydration level results on right and left hands flexor were 77.00 AU and 70.66 AU; extensor 73.30 AU and 72.51 AU; palmar 55.62 AU and 64.02 AU; and dorsum 72.43 AU and 69.35 AU. Furthermore, pH results on right and left hands flexor were 5.43 and 5.32; extensor 5.32 and 5.31; palmar 5.54 and 5.52; and dorsum 5.32 and 5.38 (Table 1, Table 2).

On the last week of measurements, TEWL level results on right and left hand’s flexor were 27.92 g/m²/h and 27.53 g/m²/h; extensor 23.12 g/m²/h and 22.06 g/m²/h; palmar 56.48 g/m²/h and 56.67 g/m²/h; and dorsum 30.84 g/m²/h and 33.30 g/m²/h. Skin hydration level results on right and left hand’s flexor were 57.95 AU and 57.93 AU; extensor 54.38 AU and 54.31 AU; palmar 59.62 AU and 59.60 AU; and dorsum 53.84 AU and 53.73 AU.
AU and 56.66 AU; extensor 55.64 AU and 56.59 AU; palmar 54.76 AU and 55.18 AU; and dorsum 50.50 AU and 50.88 AU. The skin pH results on right and left hand flexor 5.57 and 5.48; extensor 5.46 and 5.52; palmar 5.56 and 5.74; dorsum manus 5.63 and 5.60 (Table 1, Table 2).

In this study, the results at fifth and sixth on the TEWL level measurement were not significantly different except for the left dorsum (p=0.040; CI 95%). Meanwhile there were significant differences of skin hydration level results in all area (p<0.05) except the right palmar (p=0.795; CI 95%). There were significant differences of skin pH on the right dorsum manus (p=0.000; CI 95%), left flexor (p=0.029; CI 95%), left extensor (p=0.022; CI 95%), left palmar (p=0.006), and left dorsum manus (p=0.009; CI 95%).

| TEWL level (g/m²/h) | First week | | | Second week | | | | | | | |
| | Right hand | Left hand | | Right hand | Left hand | | | | | | | |
| | Mean ±SD | Mean ±SD | P | Mean ±SD | Mean ±SD | P |
| **Flexor** | 27.98 ± 9.10 | 29.64 ± 14.36 | 0.925 | 27.24 ± 14.94 | 24.66 ± 13.65 | 0.474 |
| **Extensor** | 26.07 ± 9.82 | 27.09 ± 11.92 | 0.794 | 18.72 ± 8.43 | 19.77 ± 12.04 | 0.777 |
| **Palmar** | 48.56 ± 11.33 | 47.05 ± 12.11 | 0.718 | 50.98 ± 9.67 | 54.49 ± 9.20 | 0.301 |
| **Dorsum manus** | 40.77 ± 10.25 | 33.16 ± 12.52 | 0.070 | 20.54 ± 8.54 | 23.61 ± 9.23 | 0.336 |

| Skin hydration level (AU) | First week | | | Second week | | | | | | | |
| | Right hand | Left hand | | Right hand | Left hand | | | | | | | |
| | Mean ±SD | Mean ±SD | P | Mean ±SD | Mean ±SD | P |
| **Flexor** | 58.77 ± 14.46 | 61.61 ± 19.25 | 0.640 | 44.13 ± 15.60 | 38.74 ± 11.03 | 0.418 |
| **Extensor** | 63.80 ± 16.28 | 59.74 ± 12.16 | 0.436 | 35.38 ± 13.86 | 37.06 ± 10.60 | 0.703 |
| **Palmar** | 46.53 ± 15.34 | 45.40 ± 13.04 | 0.812 | 29.47 ± 11.58 | 27.98 ± 23.12 | 0.214 |
| **Dorsum manus** | 63.93 ± 15.09 | 61.88 ± 9.96 | 0.653 | 36.67 ± 12.84 | 39.11 ± 17.13 | 0.626 |

| pH | First week | | | Second week | | | | | | | |
| | Right hand | Left hand | | Right hand | Left hand | | | | | | | |
| | Mean ±SD | Mean ±SD | P | Mean ±SD | Mean ±SD | P |
| **Flexor** | 5.18 ± 0.15 | 5.19 ± 0.16 | 0.974 | 5.37 ± 0.42 | 5.40 ± 0.33 | 0.774 |
| **Extensor** | 5.22 ± 0.13 | 5.25 ± 0.18 | 0.911 | 5.49 ± 0.30 | 5.43 ± 0.30 | 0.696 |
| **Palmar** | 5.25 ± 0.18 | 5.36 ± 0.27 | 0.215 | 5.81 ± 0.24 | 5.83 ± 0.29 | 0.831 |
| **Dorsum manus** | 5.03 ± 0.28 | 5.15 ± 0.20 | 0.059 | 5.51 ± 0.30 | 5.51 ± 0.28 | 0.928 |
Table 4. Comparison of using gloves in the right hand and left hand at fifth week and sixth week.

<table>
<thead>
<tr>
<th></th>
<th>Fifth week</th>
<th></th>
<th></th>
<th>Sixth week</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right hand</td>
<td>Left hand</td>
<td>p</td>
<td>Right hand</td>
<td>Left hand</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td></td>
</tr>
<tr>
<td>TEWL level (g/m²/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flexor</td>
<td>35.99</td>
<td>29.38</td>
<td>0.228</td>
<td>27.92</td>
<td>8.88</td>
<td>0.919</td>
</tr>
<tr>
<td>Extensor</td>
<td>28.00</td>
<td>13.61</td>
<td>0.821</td>
<td>23.12</td>
<td>8.26</td>
<td>0.689</td>
</tr>
<tr>
<td>Palmar</td>
<td>52.29</td>
<td>11.16</td>
<td>0.819</td>
<td>56.48</td>
<td>9.12</td>
<td>0.959</td>
</tr>
<tr>
<td>Dorsum manus</td>
<td>35.91</td>
<td>11.08</td>
<td>0.601</td>
<td>30.84</td>
<td>10.17</td>
<td>0.508</td>
</tr>
<tr>
<td>Skin hydration level (AU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flexor</td>
<td>77.00</td>
<td>23.67</td>
<td>0.430</td>
<td>57.95</td>
<td>12.10</td>
<td>0.760</td>
</tr>
<tr>
<td>Extensor</td>
<td>73.30</td>
<td>23.54</td>
<td>0.880</td>
<td>55.64</td>
<td>12.30</td>
<td>0.844</td>
</tr>
<tr>
<td>Palmar</td>
<td>55.62</td>
<td>22.88</td>
<td>0.319</td>
<td>54.76</td>
<td>15.92</td>
<td>0.880</td>
</tr>
<tr>
<td>Dorsum manus</td>
<td>72.43</td>
<td>31.51</td>
<td>0.734</td>
<td>50.50</td>
<td>16.24</td>
<td>0.950</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flexor</td>
<td>5.41</td>
<td>0.17</td>
<td>0.117</td>
<td>5.57</td>
<td>0.25</td>
<td>0.303</td>
</tr>
<tr>
<td>Extensor</td>
<td>5.32</td>
<td>0.22</td>
<td>0.914</td>
<td>5.46</td>
<td>0.27</td>
<td>0.503</td>
</tr>
<tr>
<td>Palmar</td>
<td>5.54</td>
<td>0.27</td>
<td>0.664</td>
<td>5.56</td>
<td>0.26</td>
<td>0.082</td>
</tr>
<tr>
<td>Dorsum manus</td>
<td>5.32</td>
<td>0.27</td>
<td>0.500</td>
<td>5.63</td>
<td>0.28</td>
<td>0.763</td>
</tr>
</tbody>
</table>

Discussion

Batik workers are directly and indirectly exposed to irritant materials of dyes. Batik candles consist of coconut oil, bee hive, animal fat, paraffin. This ingredient may increase the risk of skin dryness and irritant contact dermatitis. Clinical manifestations of contact dermatitis are pruritus, roughness, tightness, burning sensation and pain, followed by lack of elasticity, texture coarsening, and skin wrinkle.\(^8\)\(^9\) Occupational contact dermatitis is the most common occupational diseases. That can be prevented by elimination, substitution, engineering controls, and personal protective equipment (PPE).

Using gloves is one of prevention modality, that may reduce the risk of chemical exposure.\(^8\)\(^,10\)

In second week after using test gloves in right hand there were significant difference in TEWL level, skin hydration level, and pH results except TEWL level on flexor, TEWL level on palmar and pH on flexor. Not only in the right hand in first week, but also left hand using personal gloves showed significant difference on TEWL level, skin hydration level, and pH results, except TEWL level on flexor and palmar. This suggests that the use of gloves on batik workers can improve the skin barrier function. Some literature showed clinical
improvement of contact dermatitis after using PPE. PPE can vary in form such as gloves, aprons, overalls, hats, safety boots and masks. There are many types of gloves such as type I (disposable gloves), type II (household gloves), type III (industrial gloves), and type IV (special gloves). There are also various kinds of gloves materials, made from rubber, plastic, textile, or combination. Some literatures said that protective gloves may give protection for working, involving direct contact with solvent for several hours.

Protective gloves must be periodically checked and inspected if any holes are found, that condition can absorb the allergens or irritants. Effectively prevention of contact dermatitis may use disposable gloves and frequent changes; but as long as the gloves are intact, frequent changes are not necessary to prevent contact dermatitis. Any substances in the gloves that should be used for dry work are fabric gloves (e.g., cotton or leather), and for wet work should are cotton gloves which absorb sweat. Furthermore, gloves that can protect from solvents and inks are nitrile and neoprene gloves.

However, the use of waterproof gloves over period time must be considered hazardous to the skin, due to occlusive effect in moist chamber. Some literatures reported although the gloves can protect from irritant, allergen and microbial agents, gloves can be a etiologic factor in occupational contact dermatitis if not used properly. Kwon et al, reported a case of irritant contact dermatitis caused by chronic cutaneous exposure to solvents on the inner surface of gloves.

Some mechanisms by which gloves can be exposed to chemicals are contamination, permeation, and penetration. When workers open the gloves incorrectly without first cleansing of the hand and the contamination of gloves interiors are very common problems. The physical defect of gloves can be a process of solvent penetration, like a hole in the gloves. One study shows evidence that the use of cotton gloves liners can prevent form contact dermatitis rather than wearing occlusive gloves. In other studies reported that appropriate PPE use is highly recommended due to batik workers might be exposed any substances and materials. There are many factors can be a protective gloves include gloves material, the additive used in manufacturing, the manufacturing process, and the thickness and homogeneity of the material. Choosing suitable gloves for chemicals or solvent is very important, that there are some chemicals for which no gloves can provide protection for long periods time.

This study showed that any types of gloves may give protection for batik workers in batik production, since there were no significant difference between the skin barrier function after wearing test gloves and personal gloves. However, the length of the gloves may also play role in the protection itself. Short gloves may increase the risk of allergens or irritant contamination inside the gloves.

**Conclusion**

Personal gloves and test gloves usage for protection doesn’t have much significant difference. Glove usage can reduce TEWL that can give a protective effect, however, there is a risk of contact dermatitis because it can increase pH and reduce skin hydration. Wearing gloves may also give risk to trigger contact dermatitis if not used properly or using incompatible material.

**Acknowledgement:** We gratefully thank to Riset Kolaborasi Indonesia-World Class University (RKI-WCU), Universitas Airlangga Surabaya, Dr. Soetomo General Academic Hospital Surabaya, Batik Zulpah Tanjungbumi, and other parties that support this research.

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**Conflict of Interest:** There is no conflict of interest

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Qualitative Phytochemical Screening and In Vitro Antibacterial Activities of Crude Akaziraruguma Leaf Extracts

Cyuzuzo Callixte¹, Heny Arwati², Dusabimana Jean Damascene³

¹Postgraduate Student, Department of Immunology, Postgraduate School, Universitas Airlangga, Indonesia, ²Doctor, Department of Parasitology, Faculty of Medicine, Universitas Airlangga, Indonesia, ³Research Scholar, Department of Food and Drugs Assessment and Registration, Rwanda Food and Drugs Authority, Rwanda

Abstract

The resistance of pathogenic bacteria is a global health dynamic and the time is now to find alternative solutions from plant secondary metabolites. Ageratum conyzoides L. is a plant known as akaziraruguma in Rwanda and used to treat wounds and ulcers. This study aimed to evaluate the phytochemical profile and germicidal efficacy of ethanolic and aqueous leaf extracts of Ageratum conyzoides L. grown in Rwasave wetland. The leaves were collected and dried under the shed for 10 days, blended into powder by electric blender and macerated with water and 96% ethanol. Phytochemical screening was performed by following the standard procedures and antibacterial activity of the extracts was examined by agar well diffusion method and the inhibition zones were recorded. Phytochemical screening revealed the presence of different secondary metabolites including alkaloids, tannins, flavonoids, steroids, terpenoids and saponins. In this assessment, aqueous and ethanolic extracts exhibited significant inhibitory activity against tested pathogens with inhibition zones that ranging from 6 ± 0.9 mm to 20 ± 0.5 mm of diameter. The minimum inhibition concentrations range between 0.47 mg/mL and 15.00 mg/mL. The results confirm that the leaves of akaziraruguma could be the credible source of antibacterial agents that should be used for therapeutic purposes and in production of pharmaceuticals.

Keywords: Ageratum conyzoides L., Antibacterial, Phytochemical screening, Zone of Inhibition

Introduction

Akaziraruguma (Ageratum conyzoides L.) is an ethnomedical plant by which one or more parts can be used for therapeutic intentions. It was named akaziraruguma by Rwandan citizens due to the fact that it is mostly employed to treat wounds. It is believed to have originated from America yet, nowadays it is able to be cultivated all over demographic areas including tropical and subtropical regions.

The leaves of Ageratum conyzoides L. contain flavonoids (eupalestin, kaempferol, sinensetin, quercetin), alkaloids (echinaline and lycopsamine), steroids (β-sitosterol, stigmastanol, brassicasterol) and sesquiterpenes (linalool, limonene, eugenol) which possess various medicinal importance including antioxidant, analgesic, anti-inflammatory, anti-malarial, anti-cancer due to its potent hepatotoxic and carcinogenic nature associated with pyrrolizidine and antihyperglycemic, anti-ulcerogenic, antimicrobial and insecticidal properties.

This plant has an ability to heal wounds and the haemostatic effects by decreasing the bleeding time through the production of vasoconstriction, precipitate proteins at the bleeding sites and promote the natural
process of blood coagulation. The hemostatic activity of *Ageratum conyzoides* L. could be observed through the lowering of prothrombin and clotting times and also by increasing plasma fibrinogen concentration in rats of the experimental group. Indeed, this treatment is not only used in modern medical treatment but also people are familiar with it in traditional ways to cure different types of diseases.

It has been used to treat pneumonia in Cameroon, to cure tetanus, itchiness and leprosy in India and it is as well considered as an anti-gynecological disease in Vietnam. The leaves of this plant are used to treat headache and skin diseases, ringworm infections and have anti-nematocidal activities against *Taenia solium*. The leaves of this plant have active pharmacological substances such as chromenes and chromans. Chromans consist of 6-amino and 6-acetamido derivatives, which can help to relieve the symptoms of depression and also demonstrated the ability to reduce fever by causing the hypothalamus to override a prostaglandin-induced increase in temperature. They also used to treat the infections caused by worms that are classified in the order of trematodes. The other secondary metabolites that are available in *Ageratum conyzoides* L. are the derivatives from 2,2-dimethyl chromene like 6-(1-hydroxyethyl)-7,8-dimethoxy-2,2-dimethylchromene and 6-hydroxy-7,8-dimethoxy-2,2-dimethyl chromene which demonstrate the capability to impede the life processes of various microorganisms.

The polyhydroxyflavones include scutellarein-5,6,7,4’-tetrahydroxyflavone, quercetin, quercetin-3-rhamnopyranoside, kaempferol-3-rhamnopyranoside and kaempferol3,7-diglucopyranoside which have virustatic ability to inhibit HIV syncytium and viral p24 antigen formations. They also contain afzelin and quercetin 3-O-α-arabinopyranoside which have repulsive potential against herpes simplex virus type 1, Aujeszky’s disease virus and adenovirus type-3 by inhibiting acyclovir-resistant HSV-1. The leaf extracts of this plant have demethoxyageratochromene with antifungal activity against *Penicillium chrysogenum* and *Paphiopedilum javanicum* and antibacterial potential against *Vibrio cholerae*, *Streptococcus pyogenes*, *Corynebacterium diphtheriae* and *Salmonella typhi*. By considering all scientific researches done in different parts of the world, there are no published findings on phytochemical evaluation and germicidal efficacy of ethanolic and aqueous leaf extracts of *Ageratum conyzoides* L. grown in Rwase wetland, Rwanda. The novelty of this study is to provide the primary data on medicinal profile of this plant by taking into account that the abundance of bioactive substances in plants depend on seasonality, climate, geographical location and other environmental conditions. From that standpoint, the current study is specifically aimed to evaluate phytochemical constituents and antibacterial activity of *Ageratum Conyzoides* L. leaves grown in Rwase wetland, Rwanda.

### Materials and methods

#### Collection of plant materials

The mature healthy fresh leaves of *Ageratum conyzoides* L. were collected from Rwase wetland which is located in Huye district, Southern province, Rwanda. The plant was independently authenticated by Chief Herbarium Officer at National Herbarium of Rwanda which is located in Huye district, Rwanda. The leaves were washed thoroughly with running tap water and rinsed properly with distilled water. The leaves were air-dried at room temperature for 10 days and blended into powder using electric blender.

#### Plant material extraction

During extraction, twenty grams of *Ageratum conyzoides* L. leaf powder was macerated with 100 mL of 96% ethanol (1:5) and water for 3 days using rotary shaker for better extraction. After extraction, the extracts were decanted and then filtered through Whatman filter paper N°1. Ethanolic crude extract was obtained by evaporating the solvent using rotary evaporator. Aqueous crude extract was obtained by lyophilization process and the yielded thick
extracts were dissolved in 10% DMSO and kept in labelled containers at 4°C for future use.

**Sterility proofing of the extracts**

To be sure about the sterility of the extracts, 2mL of the extracts were introduced into 10 mL of Mueller Hinton broth and incubated at 37°C for 24 hours. The absence of microorganism growth on the broth after the period of incubation signifies the presence of a sterile extract. After such observations, sterilization of the extracts under UV light was not carried out.

**Phytochemical screening**

Qualitative phytochemical screening was carried out to evaluate the presence of bioactive components in crude leaf extracts according to standard method. Qualitative analysis tests were performed for various phytoconstituents such as flavonoids (Shinoda’s test), steroids (Salkowski test), tannins (Ferric chloride test), alkaloids (Wagner’s test), saponins (Froth’s test), proteins (Xanthoproteic’s test), and terpenoids. The formation of brown ring confirmed the availability of terpenoids in the examined extracts.

**Source of test bacteria**

*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* are the bacteria that were used in this work and they were obtained from University Teaching Hospital of Butare. Each bacterium was tested for viability by reviving it in peptone broth and finally sub-cultured into nutrient agar followed by incubation at 37°C for 24 hours. A single colony of each microorganism was diluted in 9 mL of peptone water and eventually acclimatized to give the equal concentration of bacterial cells to density of 10⁴ CFU/mL.

**Antibacterial and antifungal assay of extracts**

The antibacterial assay of extracts was examined by agar well diffusion method according to National Committee for Clinical Laboratory Standards. About 20 µL of diluted bacterial cultures were swabbed on respective nutrient agar plates. After spreading, Pasteur pipette was used to create 3 wells in the inoculated agar and filled up with 20mg/mL, 40mg/mL and 60 mg/mL, respectively. During this experiment, 30 µL of 30mg/mL vancomycin was used as a standard antibiotic. The plates were incubated in the upright position at 37°C. *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* plates were incubated for 24 hours. Following the incubation, the antifungal and antibacterial assays of the extracts were evaluated by observing and measuring the inhibition zones. These assays were repeated three times each and the data were noted as mean±standard deviation. The ration between inhibition zone of the sample and the inhibition zone showed by the standard antibiotic was reported as the activity index of the extracts.

**Determination of minimum inhibition concentration (MIC)**

The MIC is explained as the lowest concentration that inhibits the observable growth of microorganism after nightlong incubation. In this study, the MIC was examined by preparing the inoculum of microorganisms from nutrient broth cultures. With broth dilution technique, the extracts were serially diluted from 60mg/mL to 0.0585mg/mL with 2mL of distilled water.1 milliliter suspension of the test microorganisms was inoculated with Mueller Hinton broth as a positive control and Vancomycin as a standard reference antibiotic. It was incubated for 18-20 hours at 37°C and determined the MIC by observing the presence or absence of turbidity in the test tubes. The least concentration where no turbidity observed was noted as the MIC value.

**Results**

**Phytochemical analysis**

The yields of the ethanol and water extraction were 12.5 and 7.5mg/g, respectively. Ethanol disclosed the highest capability to extract bioactive substances from plant leaves. In reference to the results presented in table 1, phytochemical screening clearly confirmed the
presence of alkaloids, proteins, terpenoids, tannins, flavonoids and steroids in ethanolic extracts and absence of terpenoids and tannins in aqueous extracts.

**Table 1: The results of the chemical tests of the crude epicarp extracts of Ageratum conyzoides L.**

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Flavonoids</th>
<th>Steroids</th>
<th>Terpenoids</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Proteins</th>
<th>Alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Aqueous</td>
<td>+</td>
<td>+</td>
<td>_</td>
<td>+</td>
<td>_</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Key:** (+): Presence, (-): Absence

The findings of antimicrobial assay revealed that vancomycin had more potential compared to ethanolic and chloroform extracts. Both extracts were able to inhibit Gram positive bacteria (*Staphylococcus aureus* ATCC 25923) stronger than Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC25922) in Table 2. This potential was also confirmed by their activity indexes presented in Table 3 and their respective MICs as shown in Table 4.

**Table 2: Antimicrobial activity of leaves extracts of Ageratum conyzoides L.**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Inhibition zones (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol extract (mg/mL)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>16±3.3</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>20±0.5</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC25922</td>
<td>14±0.2</td>
</tr>
</tbody>
</table>

**Table 3: The activity indexes of each extract in accordance to the standard antibiotic**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Activity index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inhibition zone of extracts/ inhibition zone of standard antibiotic</td>
</tr>
<tr>
<td><strong>Activity index</strong></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>0.59</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>0.83</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC25922</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Table 4. The Minimum Inhibitory Concentrations of the extracts against the tested pathogens

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Ethanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa ATCC 27853</td>
<td>3.75</td>
<td>7.50</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25923</td>
<td>0.47</td>
<td>15.00</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>1.88</td>
<td>3.75</td>
</tr>
</tbody>
</table>

**Discussion**

The current investigation was carried out to evaluate the phytoconstituents and antibacterial activity of ethanolic and aqueous leaf extracts of *Ageratum conyzoides* L. grown in Rwasave wetland, Rwanda. The core findings of this study revealed that leaf extracts of *Ageratum conyzoides* L. contain secondary metabolites with antiseptic properties.

The results disclosed the availability of tannins, steroids, saponins, alkaloids and flavonoids in ethanolic extract which have ability to inhibit the visible growth of various pathogens. These findings are in conformity with the results published by other researchers which highlighted that the secondary metabolites from plants have ability to hinder the cell wall synthesis of different microorganisms by creating substances that are rich in prolene growth factor. 24

Once the plant extracts associate with the compounds that do not have slightly negative and positive charges in the water-fearing interior of the membrane, they impede the microbial growth. This growth should also be hindered through the formation of hydrogen bonds between the polar lipids and the hydrophilic flavonoids at the interface of the membrane. The antimicrobial activity of flavonoids is explained by the fact that they decrease elasticity in hydrophilic and hydrophobic regions of cell membrane and induce biofilm disturbance. 25

The observed outstanding antibacterial capacity of flavonoids is attributed to the presence of 3-O-octanoyl-epicatechin which enhance membrane affinity of their long acyl chains. The absence of hydroxyl groups on the B rings of the flavonoids play major role to inhibit microbial membranes than the flavonoids which have hydroxyl(OH) groups on their B rings. 26

The plant derived antimicrobials control bacterial growth by altering their membrane permeability or decreasing their pH. 8 These membrane disruptions along with the activity of β-lactams on the transpeptidation of the cellular membrane increase the inhibitory activity of the extracts. 28 The extracts evidently demonstrated extensive capability to cause leakage of different growth factors and enzymes from the cell. These plant secondary metabolites perform antibacterial activity by agitating cellular binary fission, interacting with extracellular proteins and by damaging the integrity of bacterial cell walls. 29,9

The differences in antimicrobial potential of the extracts is also attributed to the amount of secondary metabolites available in tested plant parts. This finding could be explained by the fact that the distribution of bioactive substances in roots, leaves, fruits, stems and seeds are different. 31 The abundance of the bioactive compounds in plants depends on the stage of maturity, rainfall, seasonality, soil salinity and other agroecological
conditions which repress or induce water absorption, physiological and chemical processes during plant metabolism.

Both ethanolic and aqueous extracts demonstrated great antimicrobial activity against tested microorganisms but organic solvents demonstrated high ability to dissolve plant secondary metabolites due to their polarity. This finding is as well in agreement with the findings of Idris who published that the ability of extractants to extract the compounds from the leaves plays a pivotal role during extraction of plant metabolites. This finding is in contrast with the research published by Cowan which highlighted that water may not be able to extract aromatic and saturated antibacterial compounds that can inhibit the growth of microorganisms.

The results obtained from this study showed that all leaf extracts exhibited high antibacterial activities against Gram-positive than Gram-negative bacteria. This statement could be explained by the fact that cell wall make-ups of Gram-positive and Gram-negative bacteria are slightly different. Gram-negative outer membrane consists of phospholipids and lipopolysaccharides that act as a barrier which block the entrance and reaction of antimicrobial agents through cell envelope. The diversity in antimicrobial activity of extracts to Gram-positive and Gram-negative bacteria can be as well explained by the fact that catechins induce an oxidative stress due to reactive oxygen species (ROS) that cause changes in the membrane porosity and its destruction.

Gram-negative were not highly sensitive due to the liposomes which contain high amounts of negatively charged lipids which make catechins weak to inhibit Gram-negative bacteria due to negatively charged lipopolysaccharides of the outer bacterial membrane. Staphylococcus aureus ATCC 25923 as a Gram-positive bacterium was more sensitive to the ethanolic extracts with the activity index of 0.83. This finding is absolutely correlated with the results of Zaika who reported the significant sensitivity of Staphylococcus species due to their cell walls and outer membranes. This result is also in line with the research of Bravo and Anacona which demonstrated that Mn\(^{2+}\), Hg\(^{2+}\), Co\(^{2+}\), and Cd\(^{2+}\) complexes of quercetin exhibit bactericidal upshot against Staphylococcus aureus, Bacillus cereus and Klebsiella pneumoniae.

In this research, antibiotic disc of vancomycin manifested high inhibitory activity than the prepared plant extracts. This outstanding effectiveness of antibiotic than the plant extracts is obviously correlated with the fact that antibiotics are refined and naturally purified while plant extracts are crude states.

**Conclusion**

The conclusion that can be drawn from the findings of the current study is that *Ageratum conyzoides* L. has high medicinal importance and also give a great promise that the leaves of this plant should be considered in production of antibacterial agents to fight off the pathogens that are resistant to typical antibiotics in current use.

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**Ethical Clearance:** Not applicable

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A Review of the Use of Strategic Management Tools in the Health Sector

Dani Sahirul Alim¹, Nur Wening²

¹Doctoral Student, ²Associate Professor, Post Graduate Program of Management, University of Technology Yogyakarta

Abstract

This paper is a review and study of various research related to tools of strategic, and factors that influence both of internal and external strategic in medical facilities research. The purpose of the review is to explore and connecting theories from various research. This paper identified 51 articles with related topics published the last decade. The results of this study that research related to implementation tools of strategic in health facilities still rarely. The use of SWOT (strengths weaknesses opportunities threats) analysis is the most popular tool used (42.86%), followed by Break-even analysis (28.56%), Balanced scorecard (BSC) and PEST (political, economic, socio-cultural, technology) each (14.29%). Based on the analysis of environmental factors related to internal factors in the health facility amount of 33.33%. External environmental factors in health facilities that have been studied amounted to 66.66%. Based on the summary of research related to internal and external, it is known that industry environment factors and rivalry environment factors are still rarely studied. The general environmental factors of demography and epidemiology are still rare studies and have the opportunity to be researched and developed.

Keywords: strategic management, medical, strategic, tools, environment factors

Introduction

Strategic management is very important to decisions making and to determine the future of the hospital. Due to the carefully conducted strategic analysis, a hospital can define:

- Tasks that must be addressed as well as opportunities and threats that emerge in the surrounding environment
- Possibilities arising in the surrounding environment,
- The present and future position in the managed market of health certificates
- The projected strategy that must be implemented, answering the challenges and flowing the chances arising from its immediate and more distant environment ¹

Because of the introduction of (regulated) market competition and self-regulation, strategy in becoming and importance management field for health care sector ². Healthcare organizations today are facing a series of problems such as patient dissatisfaction, demanding user, lack of medical personnel and others, demand the need for a strategic management in health service ³. Changes in the external environment (i.e., demographic and epidemiological transitions, economic fluctuations, public and political expectations) and health care systems (i.e., health market, demands, costs, new technologies, regulations) have put pressure on hospital managers to implement strategic management programs responding to environmental challenges⁴,⁵. According to studies, most health care facilities develop strategic plans (71.4%). For 21.4% of the studied facilities, the strategic plan is known mainly to management. In contrast, 28.6% of entities do not have a strategic plan. Health facilities that have implemented of strategic management using methods SWOT analysis 64.2%, break-even point analysis 42.9%, PEST analysis 14.3%, with factors that influence strategies such as political and
legal, economic, sociocultural, technology, demographic and epidemiology. Previous research conducted in Indonesia comparing Muhammadiyah Hospital in East Java with Muhammadiyah Hospital in Central Java showed that hospitals in one organization may not necessarily have the same management standards in hospital management, so it cause the different speeds development.

**Literature Review**

**Strategic Management Models**

The business model is a template that depicts the way the firm conducts its business. It describes the system of interdependent activities that are performed by the firm and by its partners and the mechanisms that link these activities to each other. Strategic management in health facility research is very important for its future development and for maintaining competitiveness. According to Fred David’s strategic model, strategic management process is comprised of three stages that include strategy formulation, strategy implementation and strategy evaluation. It drives all the activities of an organization. Strategy formulation also familiar as strategic planning involves the steps: development of vision and mission statements, audit of internal and external environment, long term objectives, and generation, evaluation and selection of strategies respectively. The second stage of strategic management process is commonly known as strategy implementation. In this stage, business strategy is actually implemented. It involves the activities like establishing annual objectives, devising policies for each business function, and allocating resources etc. for achievement of organizational objectives. The third stage is the strategy evaluation (strategy evaluation) this stage is carried out by evaluating work and taking corrective action in each stage of the strategy. Organization should continuously update and its internal and external environments by knowledge management and competitive intelligence respectively. Any change in important internal and external factors or identification of new ones can lead to change in business strategy. Currently the process of making a management model is developing, observations of important information to make a business strategy model can using computer including to see environmental factor are most influential in determining strategy management. The process of developing information systems in making strategic management of the synergy Decision Support Systems (DSS) with Knowledge Management (KM) in a Management Information Systems (MIS). The important factors in Knowledge Management (KM) are environmental factors that influence management strategies to make organization competitive advantage.

**Tools of Strategic Management Formulation Research**

According to previous research in Lubin region, most health care facilities develop strategic plans (71.4%). For 21.4% of the studied facilities, the strategic plan is known mainly to management. In contrast, 28.6% of entities do not have a strategic plan. Health facilities that have implemented of strategic management using methods SWOT analysis 64.2%, break-even point analysis 42.9%, PEST analysis 14.3% and none of the surveyed health care facilities applies the balanced corecard. This may indicate a relatively low knowledge of this tool among health care managers. The most common tool used in strategic management is the analysis of strengths and weaknesses. External factors are related to opportunities and threats, while internal factors are related to strengths and weaknesses. Research related to SWOT analysis in health facilities that have been carried out include strategies in medical tourism, improved dengue mapping, information technology development in the health industry, translation evidence and guidelines into medical strategies for individual patients, development of targeted health promotion strategies, facility strategic management (buildings, infrastructure, organization and environment) public hospitals.

Hospitals are complex organizations that have many professionals and resources in a variety of medical therapy services, so we need tools to measure.
performance indicators systematically. For this reason, the Balanced Scorecard (BSC) tool is used to measure performance management or performance effectively and efficiently in hospitals. Balanced scorecard makes the strategic target of an enterprise into some balanced indexes of performance evaluation, and examines the state of the indexes in different time. In this way, a reliable performance evaluation system is built to achieve the strategic target. The performance evaluation of an enterprise is divided into four indexes: finance, customer’s satisfaction degree, internal process, innovation and improvement. The main advantage of the method consists in guiding managers, departments, human resources, technological and financial toward the strategy organization. Unfortunately BSC is mainly used in private companies, because high costs and lack of specialists pose a real obstacle in implementing this instrument. Balanced Scorecard (BSC) is used to achieve an operational strategic vision at all levels of the organization regarding issues related to performance, strategy, communication, resource allocation, decision-making and competitiveness. The research related to BSC analysis in health facilities that have been conducted include to measure performance against the national standards and guidelines in the Essential Package of Hospital Services, used to assess the quality of health services to strengthen health services in the development of a country.

Break-even analysis is the use of a simple mathematical formula to determine the sales level in which the business. Break-even analysis, sometimes called cost volume profit analysis, is an important analytical technique used to study relations among costs, revenues and profits. The research related to Break-even analysis in health facilities that have been conducted include immunization development programs for children to find out the relationship between fixed and variable costs to income received from immunizations. This analysis is also used to assess procurement of equipment such as MRI scans in health facilities, cost analysis to determine the most effective and efficient method for treating dementia patients, Analyze the risk of companies that can be reduced by health promotion programs.

Most of the studies use PEST analysis to analyze the general environment which represents political, economic, sociocultural, and technological factors. Describes a framework of macro-environmental factors used in the environmental scanning component of strategic management but the word PEST is no more than a convenient mnemonic. The underlying thinking of the PEST analysis is that the enterprise has to react to changes in its external environment. This reflects the idea that strategy must involve an analysis of the external environment in determining strategy. The research related to PEST analysis in health facilities includes analysis of environmental factors that affect mental health, use of PEST analysis to find out important factors that influence the pharmaceutical sector, PEST analysis to create a competitive advantage in the health industry. The used tools of strategic management is based on the purpose of the strategy because they have their respective functions and strengths.

Factors That Affect Strategic Management

Internal and external environment is one of the factors that influence the evaluation and performance of an organization. Internal and external environmental factors are factors that influence the strategy. Internal environmental factors include:

1. Resources (Finance, physic, technology, human resource, innovation dan reputation)

2. Value chain analysis (infrastructure, MSDM, technology development, procurement, Inbound logistics, operations, Outbound logistics, marketing and selling, dan service)

While external environmental factors allow:

1. General environment (politic, economy, socio
cultural, technology, demography, environment)

2. Industry environment (threat new entrants, bargaining power suppliers, bargaining power of buyers, threats of substitutes products)

3. Rivalry environment

Analysis of environmental factors, can be analyzed by PESTEL analysis, i.e. Politic (P), Economic (E), Social (S), Technological (T), Environmental (E), and Legal (L). PESTEL analysis can be combined with SWOT analysis to make better and prudent decisions and strategic solutions. External environmental factors have a significant effect on the company's value. Therefore external environmental factors (i.e. politic and legal, economy, socio cultural, technology, demography dan epidemiology) it is important to be analyzed and controlled for the achievement of successful strategy formulations for the company. Analysis of external environmental factors allows the organization to extract timely information about the environment, the business environment at the regional level and to develop the development programs of the organization in the short and long term. The choice of strategy is influenced by the business environment. Companies must be able to identify these factors, especially external factors because these factors are outside the company and difficult to be managed by the company. Then an analysis of environmental factors is used in determining strategies that will have a positive impact on the company. Analysis of environmental factors must also be integrated to determine the strategy will be developed by the company.

In previous studies, internal factors affecting health facilities include research with value chain management to compare the speed of development of Muhammadiyah Hospital in East Java and Central Java Indonesia. Resource analysis of hospital performance. External factors that have been studied in health facilities include political and legal, economic, socio-cultural, technological, demographic and epidemiological factors.

**Method**

This paper is a review and study of various research related to models of strategic, tools of strategic, and factors that influence both internal and external strategic, especially in the scope of health research. The articles collecting by the google database, google scholar and mendeley. A review was conducted on articles with topic tools of strategic and factor that affect strategic especially in the scope of health research. We identified approximately 51 articles that were published in the last decade. The review in this article is divided into several sections that are strategic management models, tools of strategic, environmental factors that affect strategic management as well as conclusions and possible research that can be developed.

**Results and Discussion**

In hospitals, as highly pluralistic contexts, organizational knowledge, legitimacy and social capital play an important strategic role. However, their accumulation involves a variety of internal and external stakeholders concerned with both internal conditions and the external environment. The strategy management process revealed many similarities in how this process occurs at both hospitals. The main similarities are: (a) only the partial adoption of the SP; (b) the relevance of political interactions (more than plans) in strategic decisions; and (c) the influence of the institutional environment in strategic decisions and actions. Only the partial hospital adoption of the Strategic planning (SP) this is an appropriate other research in Lubin region, most health care facilities develop strategic plans (71.4%). For 21.4% of the studied facilities, the strategic plan is known mainly to management. In contrast, 28.6% of entities do not have a strategic plan. Health facilities that have implemented of strategic management using methods SWOT analysis 64.2%, break-even point analysis 42.9%, PEST analysis 14.3%, with factors that influence strategies such as political and legal, economic, sociocultural, technology, demographic and epidemiology. Based on a summary of studies, using tools to determine the strategies in health facilities, as in the table 1:
Table 1. Summary of research using tools of strategic in health facilities.

<table>
<thead>
<tr>
<th>Strategic tools</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWOT (strengths weaknesses opportunities threats)</td>
<td>42.86</td>
</tr>
<tr>
<td>Balanced scorecard (BSC)</td>
<td>14.29</td>
</tr>
<tr>
<td>Break-even analysis</td>
<td>28.56</td>
</tr>
<tr>
<td>PEST (politic, economic, socio-cultural, technology)</td>
<td>14.29</td>
</tr>
</tbody>
</table>

Source^{4,14,29–33,37,39,51,16–21,26,27}

Based on table 1. It is known that SWOT analysis is the most popular strategy tool used in the health sector, while other formulation tools of strategic are still relatively unpopular. Based on previous research conducted on health facilitation research in the Lubin area, it is known that there are six environmental factors that influence the strategy including political and legal factors, economic factors, social culture, technology, demography and epidemiology. What is the reason why SWOT analysis is used the most while PEST analysis is the least used should be able to be addressed in further research. The results in table 1 also show that research related to PEST and BSC in the health sector is still very minimal and is a research opportunity that is still very likely to be developed.

Conclusion

For a company to have a competitive advantage and be able to compete for a long term, the company must have the tools of strategic. Various strategies that can be used such as SWOT analysis, Break-even analysis, PEST analysis and balanced scorecard (BSC) analysis. The used tools of strategic management is based on the purpose of the strategy because they have their respective functions and strengths. On formulating the strategy there are environmental factors that influence such as internal factors and external factors. Internal factors include resources (Finance, physic, technology, human resource, innovation dan reputation) and value chain analysis (infrastructure, MSDM, technology development, procurement, inbound logistics, operations, outbound logistics, marketing and selling, dan service). While external environmental factors include the general environment (politic, economy, socio cultural, technology, demography, environment), industry environment (threat new entrans, bargaining power suppliers, bargaining power of buyers, threats of substitutes producs), rivalry environment.

The research related to implementation tools of strategic in health facilities still rarely. Previous research conducted at health facilities in the Lubin area can be developed to see variations of tools of strategic applied in different regions or countries. Is this related to internal and external environmental factors in the region. What are the internal and external factors that most influence can be studied. Previous studies that no one has researched what sub factors most influence the socio-cultural, demographic and epidemiological factors. The use of BSC and PEST analysis tools are still rarely used in health facilities and have the opportunity to be researched and developed. Based on a summary of research related to internal and external factors that have been carried out in health facilities, it is known that industry environmental factors and rivalry environment are still rarely to be studied. The general environmental factors of demography and epidemiology are still rarely research that explores it in health facilities so it can be a future research opportunity.
Ethical Clearance: No Need Ethical Clearance on this Research

Source of Funding: This research was funded privately and did not receive funding from any party.

Conflict of Interest: The author confirm that there are no conflicts of interest to disclose.

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Peutz Jeghers Syndrome in 47 Years Old Woman (Histopathological Review) : Case Report

Danu Yuliarto¹, Prasetyadi Mawardi², Ambar Mudigdo³

¹Recidency Program, ²Associate Professor in Department of Dermatology and Venereology, Faculty of Medicine, Sebelas Maret University/ Dr. Moewardi General Hospital, Surakarta, Indonesia, ³Professor in Department of Anatomical Pathology, Faculty of Medicine, Sebelas Maret University/ Dr. Moewardi General Hospital, Surakarta, Indonesia

Abstract

Background: Peutz-Jeghers syndrome (PJS) is an autosomal dominant inherited disorder, characterized by intestinal hamartoma polyps in association with distinct patterns of skin and mucosal macular melanin deposition. Patients with PJS have a 15-fold increased risk of developing bowel cancer compared to the general population.

Case: A 47-year-old woman complained of black patches appearing on the lower and upper lips. Several months ago, the patient said that black patches appeared on the gums and inner cheeks. Gastrointestinal complaints such as nausea or vomiting were previously denied. Dermatological examination in the oris et gingivae et buccalis region showed multiple well-defined hyperpigmented macules. Histopathological examination found an increase of melanocytes in the stratum basale with rows of melanocytes. Patient had an endoscopy in the gastrointestinal tract and histopathological examination of the colonic mucosa, no abnormalities were found and no signs of malignancy were found on histopathological examination.

Conclusion: Result of histopathological examination showed an increase in the number of melanocytes in the stratum basale with lined melanocytes. Based on histopathological examination in this patient more leads to the diagnosis of PJS

Key words: Peutz-jeghers Syndrome, Histopathology, Melanocytes

Introduction

Peutz-Jeghers Syndrome (PJS) is an autosomal dominant inherited disorder characterized by intestinal hamartoma polyps in association with a distinct pattern of association of skin and mucosal macular melanin deposition.¹⁻⁹ This syndrome was described in 1921 by Jan Peutz (1886-1957), a Dutch physician who noted the association between intestinal polyps and mucocutaneous macules in Dutch families. Harold Jeghers (1904-1990), an American physician, contributed with a definitive descriptive report of this syndrome, when he published “generalized intestinal polyposis and melanin spots of the oral mucosa, lips and fingers”, in 1949, with McKusick and Katz. The eponymous Peutz-Jeghers syndrome was introduced by the radiologist Andre J. Bruwer in 1954.¹⁻³⁻⁵

Forty-eight percent of patients with PJS develop cancer and die from cancer at 57 years old. Cancers that develop in the gastrointestinal tract of patients with PJS have a higher frequency than that occurring in the
general population. During the first 3 decades of life, anemia, anal bleeding, abdominal pain, obstruction or intussusception are common complications in patients with PJS. Nearly 50% of patients experience intussusception during their lifetime, most often in the small intestine. The median age at first diagnosis of cancer is 42.9 years, ± 10.2 years.

Peutz-Jeghers syndrome should be diagnosed in patients as early as possible. Genetic counseling should also be provided. Many of the gastrointestinal lesions begin to develop early in life, even if the syndrome does not become clinically apparent until the second and third decades of life. Appropriate screening for colon cancer and extra intestinal cancer must be carried out. This paper will report a case report with the results of anamnesis, physical examination, dermatological examination and histopathological picture of melanocytes lined up in the stratum basale. The purpose of this case report is to increase knowledge about diagnosis through histopathological examination of PJS.

Case

A 47-year-old woman from Sukoharjo came to the skin and genital polyclinic of the dr. Moewardi Regional General Hospital Surakarta on March 29th, 2016 with the main complaint of black spots appearing on her lips and gums.

The current history of the disease began about a year ago where the patient complained of black patches appearing on the lips. Initially, the complaint of spotting appeared only on the lower and upper lips, but several months ago the patient said black spots appeared on the gums and inner cheeks. There are no complaints of pain in the spot and never bleed.

This is the first time for the patient has experienced this type of complaint. No family member is sick like this. A history of taking drugs in the long term, a history of wounds, canker sores or previous skin diseases in the area was denied. There was no history of asthma, allergies to certain foods or drugs in the patient or family. Gastrointestinal complaints such as nausea or vomiting were not previously found.

Physical examination showed good general condition with vital signs still within normal limits. Dermatological examination of the oris et ginggiva et buccalis regions showed multiple well-defined hyperpigmented macules (Figure 1). This case was differentially diagnosed with PJS and Cronkhite-Canadian syndrome (CCS).

Subsequently, this patient underwent a skin biopsy in the oris region. After histopathological examination, the epidermis showed an increase in the number of melanocytes in the stratum basale with rows of melanocytes (Figure 2).

The patient is planned for consulted to the internal medicine department, then endoscopy is done by the internal medicine department. This patient received therapy in the form of cryotherapy for lesions on the lip mucosa from the skin.
Figure 1. A-D The oris *et* gingival region shows multiple hyperpigmented macules with well-defined borders. E-H The *region buccalis dextra et sinistra* showed multiple hyperpigmented macules with well-defined borders.

Figure 2. A-D Melanin cells at the base are lined up. Melanin in the papillary dermis.
Discussion

Peutz-Jeghers syndrome consists of two main components, hamartomatous polyposis of the gastrointestinal tract and mucocutaneous pigmentation. The incidence of PJS is reported to be 1 in 150,000 to 200,000 people.\textsuperscript{18} Initial manifestations of PJS are melanin spots, i.e. 1-5 mm hyperpigmented macules on the lips, buccal mucosa, periorbital area, nose, genitals and fingers, usually present in the first year of symptoms and represent mucocutaneous marker of this syndrome. This sign is most commonly seen on the mucous membranes of the lips and cheeks as well as the anal and intestinal mucosa, nose, periorbital area and hands and feet. In some observations there is pigmentation on the nails, however this finding is rare.\textsuperscript{16} This case was differentially diagnosed with CCS. Cronkhite-Canada syndrome was first described by Cronkhite and Canada in 1995; so far, about 400 cases mainly of European or Asian descent with a mean age of 59 years have been reported. Cronkhite-Canadian syndrome is also characterized by intestinal polyposis and lentigo-like macules, usually on the face, extremities and palms. Pigmentation tends to be more diffuse than in PJS. Symptoms of CCS may vary, but classic symptoms are characterized by the presence of diffuse gastrointestinal tract polyposis, dystrophic changes in the nails, alopecia, skin hyperpigmentation, diarrhea and weight loss. Other symptoms such as hypogeusia and xerostomia have also been described in the literature.\textsuperscript{21} On physical examination in this patient found black patches on the lips, patches on the upper and lower lips, black spots also appeared on the gums and inner cheeks, so in this case it is more suitable with a picture of PJS.

The results of microscopic examination of the skin of patients with PJS who were biopsied gave an abnormal picture of increased melanin deposition, there was basal hyperpigmentation in the pigmented macula. There is conflicting opinion as to whether an increased number of melanocytes, basal hyperpigmentation with or without increased melanocyte proliferation, that seen in CCS.\textsuperscript{16} In this patient’s biopsy, histopathological examination results in the epidermis showed an increase in the number of melanocytes in the stratum basale with rows of melanocytes. This is more likely with the histopathological picture in PJS, whereas in CCS when histopathological examination is carried out, hyperpigmentation results in the stratum basale without an increase in the number of melanocytes.

Autosomal dominant PJS is characterized by the association of gastrointestinal polyposis with the presence of pigmented macules, this syndrome has an increased risk of developing cancer at a relatively young age. There is genetic heterogeneity, although the majority of cases involve the serine/threonine kinase (STK11/LKB1) gene on chromosome 19p13.3. This gene encodes the protein LKB1 that regulates the p53 mutation pathway. Apoptosis in gene regions involved in substrate recognition is more often associated with malignancy than mutations in gene regions involved in ATP binding and catalysis. A case has been reported in association with primary melanoma of the rectum.\textsuperscript{16,19} In the majority of cases, multiple polyps develop in the small intestine which may become malignant in 2-3% of patients.\textsuperscript{20} According to Giardiello et al, endoscopic examination is a surveillance for cancer detection. The age range of finding colorectal cancer in PJS varies from 21-71 years, with an overall risk of 39%, the majority being in males. Hearle et al found that colorectal cancer was the most common cancer of the gastrointestinal tract. The risk of colorectal cancer is 3%, 5%, 15% and 39% at the age of 40, 50, 60 and 70 years. In this large series only one case of sigmoid cancer was detected during surveillance. Less common upper gastrointestinal cancer. Gastric cancer is much more common than oesophageal and the median age at diagnosis of cancer of the gastrointestinal tract is 30 years. Although very rare, upper gastrointestinal cancers have been reported during the first and second decades of life.\textsuperscript{12,22} Upper GI colonoscopy and endoscopy are sometimes performed as early as 8 years of age. It is possible that polyps are detected, this should be repeated every 3 years. If no significant polyps are found on initial endoscopy, routine surveillance is repeated at age 18 or earlier depending on symptoms, then repeated every three years. We
recommend that after the age of 50 years the frequency of examinations increases to every 1–2 years because of the increased risk of malignancy at this age.\textsuperscript{22} While the endoscopic appearance of CCS may vary, colonic polyps have been characterized by a “strawberry-like” appearance in one study.\textsuperscript{21} The etiology of CCS is not well understood but several studies have shown an association with elevated antinuclear antibody (ANA) and IgG4 levels, there is also an association between CCS and hypothyroidism and various autoimmune diseases such as rheumatoid systemic lupus, rheumatoid arthritis, and scleroderma, all of which lead to autoimmune etiology.\textsuperscript{16,19} In this patient, an endoscopy of the gastrointestinal tract and histopathological examination of the colonic mucosa was performed, from the two investigations, no abnormalities were found on gastrointestinal endoscopy and no signs of malignancy were found on histopathological examination of the colonic mucosa.

Although the mucocutaneous pigmentation seen in PJS may fade with age, it can be psychologically disturbing for the patient. The use of intense pulse light (IPL) with a 590nm cut-off filter was reported in one case where it led to cosmetic improvement of the lesions. Similar improvements also have been described with Q-switched ruby lasers and CO2-based lasers. In addition, in the study reported, cryotherapy was performed on 15 patients and gave satisfactory results. Cryotherapy has a mechanism of tissue destruction by rapid clotting. The lesion froze and produced necrotic tissue which then sloughed off spontaneously.\textsuperscript{22} In this patient’s case, cryotherapy was administered to the skin and genitals.

**Conclusion**

It has been reported a case of PJS in a 47-year-old woman with complaints of black spots appearing on the lips, the spots appearing only on the lower and upper lips, the complaints have been felt since one year ago, the spots do not increase. On dermatological examination in the oris et gingiva et buccalis region, multiple hyperpigmented macules were clearly demarcated. After histopathological examination, the epidermis showed an increase in the number of melanocytes in the stratum basale with rows of melanocytes, this picture supports the diagnosis of PJS. No abnormalities were found on gastrointestinal endoscopy examination from the internal medicine department. Furthermore, this patient received therapy in the form of cryotherapy on the skin and genitai.

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**Ethical Clearance:** This study did not use ethical clearance

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**References**


Readiness to Change, Medication Adherence and Quality of Life among Alcoholic Patients at Selected Hospitals, Bangalore

Dayananda Bittenahalli Omkarappa¹, Rajratan Gupta², Reema Jacqueline Andrade³

¹Professor, HOD, Department of Psychiatric Nursing, Kempegowda College of Nursing, K.R. Road, V.V. Puram, Bangalore, ²Assistant Professor, Dept. of Psychiatric Nursing, Nursing College, All India Institute of Medical Sciences, Bhopal, ³Registered Nurse, Northern Devon District Hospital, Barnstaple, United Kingdom

Abstract

Background of the Study: Alcohol affects virtually every organ system, and alcoholics are at increased risk medical problems. Many factors contribute for poor response to treatment and outcome. The factors like poor adherence and motivational level by patients with alcohol use disorders (has often been raised in the literature. However, there has been a limited effort to measure the problem. Hence the investigator felt that there is need to assess the level of readiness to change, Medication adherence and Quality of life among alcoholics.

Methods: A descriptive Survey research design was adopted for the present study. 100 alcohol dependent individual were recruited by simple random sampling method. The Stage of Change Readiness and Treatment Eagerness Scale, Morisky Medication Adherence Scale and WHO quality of life-BREF were used to assess readiness to change, medication adherence and quality of life. The study was conducted at selected Hospitals, Bangalore.

Results: The result shows that majority (73%) of respondents are having low level of readiness to change, medication adherence (67%) and quality of life (56%). A moderate positive correlation was found between readiness to change and medication adherence ($r=0.67$), a negligible positive correlation found between readiness to change with quality of life ($r=0.032$) and a high positive correlation was found between quality of life with medication adherence ($r=0.71$).

Conclusion: The study concludes that alcohol dependent individual had low level of readiness to change, medication adherence and quality of life.

Key words: Readiness to change, medication adherence, quality of life and Alcoholics

Introduction

Alcoholism is a broad term for any drinking of alcohol that result in problems. Alcohol affects virtually every organ system, and alcoholics are at increased risk for cirrhosis, gastrointestinal bleeding, pancreatitis, cardiomyopathy, trauma, mental health disorders, and a wide variety of cancers. Patients with viral hepatitis who abuse alcohol significantly increase their risk for cirrhosis and ensuing hepatocellular carcinoma.

W.H.O ranked alcohol dependence as nine among ten medical disorders causing morbidity in the world. As many as 80% of men and 60% of women in

Corresponding author: Rajratan Gupta
Assistant Professor, Dept. Of Psychiatric Nursing Nursing College, All India Institute Of Medical Sciences, Bhopal-462020
M: +91 9845869630
E-Mail: Rajratan.nursing@Aiimsbhopal.edu.in
developed countries drink at some time during their lives. In the United States, 138.3 million people age 12 and older, surveyed, report that they actively use alcohol, in addition, 40% of patients have experienced complications of alcohol misuse in the world.

In India 85% of men who are violent towards their wives were frequent or daily users of alcohol. 3 to 45% of household expenditure is spent on alcohols, use of alcohol increases and reduces the ability to pay for food and education. The National Household Drug Use Survey done in India found that alcohol (21.4%) was the primary substance used (apart from tobacco) followed by cannabis (3.0%) and opioids (0.7%). 17 to 26% of alcohol users qualified for ICD 10 diagnosis of dependence and low of 7% in the western state of Gujarat (officially under Prohibition) to 75% in the North-eastern state of Arunachal Pradesh.

The stigma associated with substance use, the guilt and shame resulting from inappropriate use and the lack of awareness about the part drugs play in the problems they face –all these lead to a denial of the problem of addiction. In an attempt to protect the dignity of the family, in most cases family members also deny the existence of any serious problem. Hence, Readiness to change (RTC) becomes one of the key issues in the treatment of alcohol dependent persons.

Motivation to change had a significant association with complications of alcohol use, medical comorbiditly, onset and severity of alcohol dependence, socioeconomic status, religion, and mode of referral. The higher RTC scores were associated with improved alcohol use outcomes and the strongest effects were for confidence. Even RTC score translates into short-term reductions in alcohol use and in turn alcohol consequences, and highlight important of motivation. Medication adherence is also one of factor which determines the treatment effectiveness along with motivational factor.

Nonadherence diminishes the effectiveness of treatment for all chronic diseases, including alcohol dependence. Rates of adherence to pharmacotherapies for alcohol dependence range widely, both within and among agents. The factors like use of external reinforcers, such as positive and negative contingencies, and involvement of family members or significant others; and specific prescribing and dosing practices that may improve adherence and there by improve the quality of life among alcohol dependent patients.

There is sound evidence that both higher levels of alcohol consumption and the severity of drug dependence correlate with poorer Quality of life (QoL). Assessment of QoL, medication adherence and motivational level in alcohol dependence is a valuable measure of clinical status and also helps to identify predictors of relapse. Early identification of factors influencing relapse helps in prevention of complications; hence the investigator felt there is need to “assess to readiness to change, medication adherence and quality of life among alcoholic patients”.

Materials and Methods

A cross sectional descriptive study was conducted on alcohol dependent patient who admitted at KIMS hospitals Bangalore, from December 2017 to July 2018. The simple random sampling technique was used to collect the data from 100 samples. The inclusion criteria include patients who are diagnosis with alcohol dependence syndrome aged between 25 to 50 years and Free from alcohol withdrawal symptoms. The exclusion Criteria includes Patient suffering from mental and behavioural disorders due to use of alcohol and individual with other sever life threatening illness. The study protocol was approved fromInstitutional human ethics committee and permission was taken from hospital authority. Informed consent was obtained from the patients and their care givers.

Assessment

Subjects were administered with socio-demographic data sheet, The Stage of Change Readiness and Treatment Eagerness Scale, Morisky Medication Adherence Scaleand WHO quality of life-BREF.

1. Socio-demographic data sheet: It includes questions on age, sex, religion, area of residence,
educational status, occupation, marital status and family income. Clinical parameters included duration of alcohol intake, no of previous hospitalization, duration of treatment and family history of alcoholism.

2. The Stage of Change Readiness and Treatment Eagerness Scale: There were 19 items for SOCRATES questionnaire. Each item has five options with relevant answers. The most relevant answer for each item was five and least relevant answer for each item was one. Thus for 19 items maximum score is 95 and minimum score is 19. The readiness to change was classified based on scores as low level for score up to 48, a score of 49-71 as medium level and a score higher than 71 as classified as high level of readiness to change.

3. Morisky Medication Adherence Scale (MMAS): is used to assess the drug non adherence and consists of eight items, that assess the medication-taking behaviour with a scoring scheme of “Yes”=1 or “No”=0 for the first seven items and a 5-point Likert response for the last item. The items are summed to give a range of scores from low adherence to high adherence though they are dichotomized in this study by considering scoring greater than 3 were low adherence, 1-2 considered as medium adherence and score of zero considered as high adherence.

4. WHOQOL-BREF: It contains 26 items; each item has five options with relevant answers. The most relevant answer for each item was five and least relevant answer for each item was one. Thus for 26 items maximum score is 130 and minimum score is 26. The quality of life was classified as low level for score up to 52, a score of 53-78 as medium level and a score higher than 78 as classified as high level of quality of life.

**Statistical Analysis**

Data were analysed using Statistical package for the Social Sciences Software Package (Version 23), and results were presented in table form. Descriptive statistics were used for study variable. Correlation coefficient was calculated to find out relationship between readiness to change, medication adherence and quality of life among alcoholic patients.

**Results**

In the present study, majority (42%) of the subjects were from 35-45 years age group, most (92%) of the respondents were male and majority (70%) of the respondents belongs to Hindu religion. Majority (60%) of the subjects from rural area, most (52%) of the samples were studied up to PUC and 40% of samples were having private job as occupation. Majority (96%) of the respondents were married and most (65%) of the respondent’s monthly family income ranges less than Rs 20,000. The mean duration of alcohol intake is 5±0.96 years, mean duration of treatment is 20±3.96 days and most (53%) of respondents admitted twice previously. Majority (56%) of the respondents had family history of alcohol consumption.

With regards to classification of respondents scores, majority (73%) of respondents are having low level of readiness to change, medication adherence (67%) and quality of life (56%). (Table 1)

A moderate positive correlation was found between readiness to change and medication adherence(r=0.67), indicating that as the readiness to change scores increased medication adherence scores also increased. A negligible positive correlation found between readiness to change with quality of life (r=0.032) and a high positive correlation was found between quality of life with medication adherence(r=0.71). (Table 2)

**Discussion**

Motivation and medication adherence are the key element in treatment and recovery that influences a patient’s progression through the stages of change—from considering change, to making the decision to change, to following the planned action into sustained recovery. The present study shows that majority of respondents were in low level of readiness to change, medication adherence and quality of life.

Our findings echo previous research evidence which shows that alcohol dependent patient shows
poor readiness for change their behaviour towards drinking.17-18 The studies have shown that effectiveness of treatment modality mainly dependent upon individual readiness to change.19 The study conducted by Bertholet N et al-2012 shows that among the 377 subjects reporting unhealthy alcohol use, 216 (58%) reported low level of readiness to change.20

Concern about poor adherence with medication by patients with alcohol use disorders (AUD) has often been raised in the literature. Nevertheless, there has been a limited effort to measure the problem, despite the recognition that it adversely affects treatment outcomes. The study conducted by KranzlerHR et al -2008 shows that less than 15% of patients treated with oral naltrexone for AUDs over a 6-month period were able to persist with refills for at least 80% of the time, while more than 85% were identified as non-persistent.21

The substance abuse treatment seeks to promote abstinence or at least significant reductions in substance use, its ultimate aim is to improve the patient’s quality of life. The present study shows that poor quality of life among alcoholics. This finding is supported by Pasareanu AR et al study shows that majority of patients had Low QoL at inpatient and need active intervention to improve their condition.22 The studies have shown that QoL measurements were useful for providing evidence of therapeutic benefit in the ADS field.23-24 The study also found that there is significant correlation between the readiness to change, medication adherence and quality of life. The study suggests that intervention should be aimed at improving the factors which helps in improving well-being of alcohol dependent patients.

Limitations: The study is limited to small sample size (100) and small number of subjects’ limits generalization of the study.

Conclusion

The study concludes that alcohol dependent individual had low level of readiness to change, medication adherence and quality of life. Health care members should focus on the factors which improves the quality of life of patients along with prevention of relapse.

Ethical Consideration: Ethical approval was obtained from the institutional ethical committee (KINEC: 13/16-17).

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Conflicts of Interest : Nil

References


Analysis of Nurse Personal Factors of Triage Decision-Making in Emergency Installation at University of Muhammadiyah Malang Hospital

Ali Rahmanto¹, Loeki Enggar Fitri², Ika Setyo Rini³
¹Master of Nursing Student, Nursing Department, Faculty of Medicine, Universitas Brawijaya, ²Department of Parasitology, Faculty of Medicine, Universitas Brawijaya, ³Nursing Department, Faculty of Medicine, Universitas Brawijaya

Abstract

The high frequency of patient visits to the ER will disrupt the service process at the Emergency Department (IGD). One solution is the application of a triage system, which is the process of sorting patients according to their level of gravity. The accuracy of triage decisions has a significant impact on patient outcomes so that in this case nurses in the ER must have knowledge, understanding, and skills about triage. Analyze personal factors that influence emergency nurse decision-making. This study aimed to identify personal factors influencing triage decision-making among emergency nurses. Respondents were 30 male and female nurses who worked and were active up to now in the IGD Hospital of the University of Muhammadiyah Malang. Data on work experience, education, and training were taken from data on the characteristics of respondents. The knowledge questionnaire came from the Emergency nurses association/ENA while the skills questionnaire used the Triage Skill Questionnaire/TSQ. The Spearman correlation test shows that there is a significant relationship between skills and education with triage decision making (p=0.000; r=0.626 and p=0.039; r=0.378). Meanwhile, the variables of knowledge, work experience, and training do not correlate with triage decision making (p> 0.05) except that GELS training shows a significant relationship between GELS training and triage decision making (p=0.016; r=0.437). The results of linear regression analysis, skills, and education are factors that influence decision-making in conducting triage in the ER (45.4%). Skills and education are the factors that most influence triage decision-making.

Keywords: triage, decision making, emergency nursing.

Introduction

Emergency Room (IGD) is part of the service in a hospital that provides 24 hours of service every day. The prevalence of emergency patient visits to the ER is increasing, both in patients with stable and unstable conditions. The data on patient visits to the emergency room in Indonesia in 2007 were 4,402,205 patients (13.3%) of the total visits to the hospital¹. Data on patient visits to the ER in East Java province in 2014 were 8,201,606 cases². Data on patient visits to the ER at the University of Muhammadiyah Malang Hospital (UMM Hospital) in 2017 were as many as 25,715 cases³.

The high frequency of patient visits to the ER will disrupt the service process in the ER (National Center for Health Statistics, 2008). Besides, the density of services provided to patients with emergency conditions has an impact on decreasing the quality of service to patients⁴. In this regard, a patient management system or management is needed which will determine the assessment of medical or nursing actions called triage. Triage is a decision-making process in prioritizing the
needs and therapy given to patients in the ER based on the level of emergency. The existence of an optimal and systematic triage system can reduce the waiting time (length of stay) of patients in the emergency room and improve the quality of health services.

Triage is the initial activity performed by nurses when the patient comes to the ER. The accuracy of triage decisions has a significant impact on patient outcomes. There are still many nurses working in the IGD who lack knowledge and skills about triage. In general, two factors affect the accuracy of nurses’ decision-making in implementing triage. The first factor is an internal factor that describes the knowledge, work experience, and training of nurses, while the second factor is an external factor covering matters related to the work environment and workload. Ignoring internal and external factors can cause the implementation of triage to be inaccurate and result in permanent disability in patients. Another study states that the absence of guidelines and socialization regarding filling out the 5-level triage scale raises doubts for triage nurses to determine the patient’s triage scale, thus making the triage assessment undertriage and overtriage. The inaccuracy of filling in the triage scale is the first step in the occurrence of long waiting times and affects the satisfaction rate and patient safety.

In its application, triage guidelines are important for triage officers and medical personnel in determining the success of medical intervention and management in patients because they can be used as a reference in determining patient emergency measures. Failure to determine the right level of priority based on triage will result in delayed intervention in patients with critical conditions so that which will have an impact on the worsening of the condition which can lead to morbidity and mortality. The ability of health workers to carry out triage will affect the interventions that will be carried out on patients. Related to this, there are several factors behind this, including knowledge of triage and work experience. Besides, several other factors also influence, among others, the work environment and personal characteristics. This statement is also supported by research that triage decision making can be influenced by personal factors including experience, assessment skills, training that has been followed, strength in decision making, skills and teamwork, flexibility, knowledge, and the sharpness of the ability to identify problems.

Besides, other research states that to do the correct triage requires a high cognitive process as well as skills, expertise, competence, qualifications, and readiness in making triage decisions. Other results add that sufficient work experience, awareness of signs and symptoms of disease, adequate equipment and resources have a very important role in carrying out the correct triage. In other results, it is said that the accuracy of triage is determined by internal/intrinsic factors which include fear of making mistakes in dangerous situations, insight, clinical qualifications, and the ability of nurses as well as external factors such as work environment stress, high workload, and work environment density.

Based on a preliminary study conducted at UMM Hospital, the triage system used in the last 7 years has undergone 2 changes, namely in the first year at the opening of UMM Hospital using the Simple Triage And Rapid System (START) and in the 5th year using Australian Triage Scale (ATS) which has been modified according to the needs of the hospital and following the existing room in the ER at UMM Hospital. This triage system has only been implemented in UMM Hospital IGD for the last 3 months. ER UMM Hospital often experiences a lot of patient congestion, with the number of patient visits every day around 45-55 patients and the number of patient visits per week around 350-400 patients.

**Materials and Methods**

This research is a type of quantitative research using an analytic observational research design using a cross-sectional approach. The population in this study were all male and female nurses who worked and were active until now in the ER at UMM Hospital, amounting to 30 people. The sampling technique used in this study was the total sampling technique so that this study the samples were all nurses who worked actively, both men...
and women in the ER at UMM Hospital, totaling 30 emergency nurses.

The data used in this study are primary in the form of a questionnaire sheet. The questionnaire sheet consists of a questionnaire, namely a questionnaire that must be filled in by the nurse who conducts the triage, namely knowledge, and the questionnaire sheet which is observed by 2 enumerators who are Triage Course certified. Several statements based on indicators that have been compiled by researchers related to knowledge about triage, work experience, and the skills of nurses in conducting triage, and statements of decision making in the ER in conducting triage to patients who come to the IGD UMM hospital. The work experience, education, and training questionnaires were taken from the characteristic data of respondents, namely emergency room nurses. The knowledge questionnaire came from the Emergency nurses association / ENA. Then modified by researchers. Furthermore, the skills questionnaire uses the Triage Skill Questionnaire/TSQ (Fathoni et al., 2013) modified by researchers. The decision-Making Questionnaire (DMQ) was modified by (French et al., 1993). Both questionnaires were modified and translated into Indonesian. The reliability test was carried out on 10 emergency room nurse respondents at Lawang Medika Hospital on August 26, 2019, with the results of the instrument being reliable because the alpha value was> 0.6.

Analysis of data using univariate analysis includes data on the characteristics of respondents, namely age, gender, education, and length of work of nurses. Univariate analysis on the independent variables includes knowledge, work experience, skills, education, and training. Meanwhile, for bivariate analysis using the Spearman Rank correlation test. Linear regression was used for multivariate analysis.

Results

Table 1 General Characteristics of Respondents (n=30)

<table>
<thead>
<tr>
<th>General Characteristics</th>
<th>Frequency</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>53.3</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>46.7</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diploma</td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td>Bachelor</td>
<td>20</td>
<td>66.7</td>
</tr>
<tr>
<td>Length of Work</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3 months</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>1-1.5 years</td>
<td>9</td>
<td>30.0</td>
</tr>
<tr>
<td>2-2.5 years</td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td>3-4.5 years</td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>Training</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triage</td>
<td>13</td>
<td>43.3</td>
</tr>
<tr>
<td>BLS</td>
<td>18</td>
<td>60.0</td>
</tr>
<tr>
<td>ALS</td>
<td>3</td>
<td>10.0</td>
</tr>
<tr>
<td>BTLS</td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td>ACLS</td>
<td>15</td>
<td>50.0</td>
</tr>
<tr>
<td>Disaster Management</td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td>GELS</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>Others</td>
<td>5</td>
<td>16.7</td>
</tr>
</tbody>
</table>
Based on table 1, explains the characteristics of the respondents based on gender. From the picture above it is known that. The highest number of nurses in the Emergency Room at the General Hospital of the University of Muhammadiyah Malang were male (53.3%). Characteristics of respondents based on the level of education, it is known that the majority of nurses in the Emergency Room Hospital Muhammadiyah University of Malang have S1 education with a total of 20 nurses or 66.7%. Based on the length of work, IGD nurses worked at most for 1-1.5 years with 9 nurses (30%). Based on training experience, it is known that nurses in the Emergency Room at the General Hospital of the University of Muhammadiyah Malang have attended the most BLS training (23%), and others include Advanced Cardiac Life Support / ACLS (19%) and Basic Trauma Life Support / BTLS (13%), ALS (4%), General Emergency Life Support / GELS (4%), disaster management 13% and those who attended triage training (16%).

<table>
<thead>
<tr>
<th>Personal Factors</th>
<th>Triage Decision Making</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td>Knowledge</td>
<td>0,413</td>
</tr>
<tr>
<td>Work experience</td>
<td>0,408</td>
</tr>
<tr>
<td>Skill</td>
<td>&lt;0,000*</td>
</tr>
<tr>
<td>Education</td>
<td>0,039*</td>
</tr>
<tr>
<td>Trainig</td>
<td></td>
</tr>
<tr>
<td>Triage</td>
<td>0,537</td>
</tr>
<tr>
<td>BLS</td>
<td>0,347</td>
</tr>
<tr>
<td>ALS</td>
<td>1,000</td>
</tr>
<tr>
<td>BTLS</td>
<td>0,204</td>
</tr>
<tr>
<td>ACLS</td>
<td>0,871</td>
</tr>
<tr>
<td>Disaster Management</td>
<td>0,897</td>
</tr>
<tr>
<td>GELS</td>
<td>0,016*</td>
</tr>
</tbody>
</table>

* p value < 0,05

Based on table 2, it is found that there is no significant relationship between knowledge about triage, work experience, training which includes triage, BLS, ALS, BTLS, ACLS, and Disaster management with triage decision making. There is a relationship between skills, education, and GELS training and triage decision making.
Table 3. Personal Factors Influencing Triage Decision Making

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variables</th>
<th>Regression Coefficient</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triage decision making (Y)</td>
<td>Constanta</td>
<td>25.723</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Skill</td>
<td>0.288</td>
<td>3.961</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Education</td>
<td>4.416</td>
<td>2.262</td>
<td>0.032</td>
</tr>
<tr>
<td>a</td>
<td>= 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>= 0.674</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted (R2)</td>
<td>= 0.454 (45.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>= 11.211</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on table 3 shows the results of the bivariate test, it is known that 3 variables have a relationship with the dependent variable, namely skills, education, and GELS training. Therefore, the three independent variables will be analyzed using linear regression with the backward method. However, after being entered using the backward method, the results were not significant, so in the second (final) step, it was eliminated from the regression model, leaving skills and education. So that skills and education are the most dominant personal factors in influencing triage decision making in emergency room nurses with a variation of 45.4%.

Discussion

The relationship between knowledge and triage decision making

Based on the results of the data obtained, there is no significant relationship between nurses’ knowledge of triage and decision making. Theoretically, with better knowledge, the better the decision-making should be. However, even though they have been given the same understanding of triage, many nurses still use visual triage according to the understanding of each nurse because it is faster according to the nurse and does not require a long time. Because each nurse had also equipped themselves before entering the ER at UMM Hospital, besides that the nurses felt comfortable with it because several times the triage system in the IGD of UMM Hospital had changed because of hospital needs or because the head of the installation had changed 3 times and triage, too, was revamped. Therefore, the nurse uses each assessment which is considered the same as the other triage, in essence, sorting out the patients.

Research by (Purwoko, 2015)\(^7\) on 50 emergency room nurse respondents was conducted to measure the correlation between knowledge and the accuracy of nurses’ decision-making in the implementation of triage. The results showed that there was a significant correlation between these 2 variables with a p-value of 0.000 and a correlation coefficient of 0.565. While research conducted by Khairina (2018)\(^8\) on factors related to nurse decision making in triage accuracy, it was found that the length of work was the most dominant factor with a p-value of 0.012 and an odds ratio value of 17.856.

A study presented and reported that nurses had insufficient knowledge about hospital triage, 39.94% of the responses to the knowledge level questions were correct in that study. They concluded their findings that in hospital emergency departments in Iran, nurses were
not equipped with triage knowledge. As well as nursing curricula for different nursing programs do not have sufficient triage process content to prepare nurses for these systems in the emergency department. It can be concluded that based on the results obtained, knowledge of triage among nurses is inadequate in Pakistan\textsuperscript{19}.

Another study mentioned 100 nurses in three teaching hospitals in Pakistan, 69\% of nurses were found to have poor knowledge of triage. The correct response to the entire questionnaire was 43.22\%. With these findings, it is considered important to establish a knowledge improvement program through training that will help to build and improve the knowledge of nurses working in the ER\textsuperscript{20}.

The relationship between work experience and triage decision making

The Emergency Nurses Association (ENA) in the United States suggests that qualifications are stated to place triage nurses and may need more consideration, one of which is clinical experience, requiring them to have at least 6 months of experience in emergency resuscitation. However, it is recommended that you have at least 2 years of work experience in the ER to improve competence and triage skills\textsuperscript{21}.

From this research, it is known that the nurses who work in the ER of UMM Hospital are very diverse, ranging from those who have worked for 5 to 6 years, and some who are new, namely 3 months. Even though before becoming IGD nurses, they had been briefed on the triage used in the ER, there are still many nurses who still use their respective understanding so that the triage assessment is following the understanding of each nurse. Nurses who have longer work experience are expected to be more skilled in conducting objective and subjective assessments, as well as in determining triage priorities for patients who enter the ER at UMM Hospital.

The results of this study are in line with the research of Considine et al. (2007)\textsuperscript{22} which states that there is no significant relationship between experience (length of work) and nurses’ decision-making in conducting triage. Experienced nurses as well as nurses with little or less clinical experience may have the same ability to perform triage.

The relationship skills with triage decision making

Based on the results of research on the skills of nurses in making triage decisions, significant results were obtained. From this research, it is known that nurses who work in the ER UMM Hospital are very skilled in terms of assessment and anamnesis, meaning that some respondents have good skills. So the relationship between nurse skills and triage decision making has a positive correlation, indicating that the higher the skills of nurses in conducting triage, the higher the level of success in making appropriate decisions on patients.

Several factors have been identified as having contributed to the skills of nurses in triage. Among them, Chung (2005)\textsuperscript{23} stated that training experience can improve nurses’ skills about triage to identify patient priority scales, diagnose patients, and provide nursing interventions in the ER. To become skilled in triage, ER nurses are required to participate in training programs for triage and other related topics and renew every one to three years. Correct clinical assessment of the patient’s condition requires thinking and insight, which is achieved based on knowledge and skills. The relationship between work experience and the achievement of work skills has been explained by the banner, it is explained that a nurse develops a process of skills and education through clinical experience, and after going through this their decision-making abilities will change and be correct.

Research conducted by Fathoni., Et al (2010)\textsuperscript{17} on the relationship between knowledge about triage, training, work experience, and triage skills in nurses in several hospitals in East Java showed that the level of skills of nurses was moderate, possibly due to emergency room nurses. have a lot of experience, and 82\% of them have worked in triage. Besides, it was also stated that the majority of IGD nurses collaborated with doctors in conducting the triage process, so that this collaboration
could help nurses to have triage skills in making the right decisions in several circumstances. The research also resulted that training experience and knowledge had a positive relationship with triage skills, which reflected that the higher the knowledge and training that nurses participated in, the more skills about triage would also develop.

**The relationship between education and triage decision making**

Based on the results of research on nurse education in triage decision making, significant results were obtained. From this research, it is known that nurses who work in the IGD of UMM Hospital have an average education level of D3 and Nurse, meaning that some respondents have a good education. The positive correlation in the results of this study shows that the higher the education possessed by nurses, the higher the score for triage decision making.

For a nurse who works in an emergency service unit is very important to follow training, especially related to applied triage. CENA in 2007 provided guidelines that were a core component in the choice of triage for nurses consisting of history, knowledge, and applied triage practices, for example, using triage that was appropriate to hospital conditions, communication skills, primary and secondary surveys, assessment, and related triage decision making, with the type of patient condition such as trauma patients, pediatric patients, obstetrics, and gynecology.

Nursing education and high competence will increase the knowledge and skills of nurses in conducting triage assessments quickly and accurately. nurses who have high education and have competencies related to triage can improve nurses in making triage decisions in the emergency room. This high education and competence of nurses is the main basis for nurses to be able to assess the appropriate triage category according to the patient’s condition to improve nurse performance and better outcomes.

The relationship between training and triage decision making

Based on the results of the data obtained, there is a significant relationship between GELS training for nurses on triage and decision making, however, in several other pieces of training, there are no significant results. After the GELS training was entered using the backward method, the results were not significant, so in the second (final) step it was eliminated from the regression model. Theoretically, the better the training, the better the decision-making. However, even though nurses have been given the same understanding of triage and each of them has attended outside training to support personal abilities, many nurses still use visual triage according to their understanding because it is faster according to the nurse and does not require a long time, besides The nurse felt comfortable with it because several times the triage system in the IGD of UMM Hospital had changed because of the needs of the hospital or because the head of the installation had changed 3 times and the triage was also changed. Therefore, the nurse uses each assessment which is considered the same as the other triage, in essence, sorting out the patients.

For a nurse who works in an emergency service unit is very important to take part in training, especially related to applied triage. CENA in 2007 provided guidelines that were a core component in the choice of triage for nurses consisting of the history, knowledge, and practice of applied triage, for example using triage that was appropriate to hospital conditions, communication skills, primary and secondary surveys, assessment and related triage decision making, with the type of patient condition such as trauma patients, pediatric patients, obstetrics, and gynecology.

**Personal factors influencing triage decision making**

The results of the multivariate analysis showed that the personal emergency nurse factors that most influenced triage decision-making were skills and education. These factors explain 45.4% of the variation in emergency nurse decision-making. Triage skills in emergency
nursing are defined as the ability of emergency nurses to use their decision-making skills to prioritize patients into appropriate categories in a fast time. Nurses’ skills in conducting triage include rapid assessment, patient categorization, and patient allocation\textsuperscript{17}. Yuliastuti, 2007\textsuperscript{26} states that the higher the skills possessed by the workforce, the more efficient the body, energy, and thoughts are in carrying out work.

Education is a process of awareness that occurs because of the interaction of various factors concerning humans, the environment, and their potential. Education in the field of nursing is a process of awareness and self-discovery as nursing personnel who have maturity in thinking, acting, and acting as professional nurses, so that they can answer various challenges in their personal and professional life. Higher education is related to triage decision-making (Kusnanto, 2003)\textsuperscript{27}.

**Conclusion**

There is a relationship between GELS skills, education and training, and triage decision-making. There is no relationship between the knowledge and work experience of nurses with triage decision-making. Skills and education are personal emergency nurse factors that significantly influence triage decision-making in the Emergency Room at the University of Muhammadiyah Malang Hospital.

**Ethical Approval:** This study has received the approval of a certificate of ethical review board from the ethical committee of the Faculty of Medicine, University of Brawijaya Malang.

**Conflict of Interest:** There is no conflict interest declare in this study

**Funding:** There is no research funding in this study

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Articaine: Opening up a New Vista for Pediatric Dentists

Ananthu H, Ashwin P Rao, Suprabha B S

1 Post Graduate Student, 2 Associate Professor, 3 Professor and Head, Department of Pediatric and Preventive Dentistry, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education (Mahe), Manipal

Abstract

Lidocaine has remained the gold standard local anaesthetic agent to perform dental procedures both in the adult as well as in the pediatric patients. However, the invent of articaine has offered the clinicians with a newer and more potent local anaesthetic agent, which causes minimal side effects. Articaine is 1.5 times more potent and 0.6 times less toxic than lidocaine. Also, adequate anaesthesia achieved through infiltration route of administration of the drug, almost eliminates the need for the painful and difficult inferior alveolar nerve block in children, thereby minimising the side effects. Thus, achieving adequate anaesthesia through the administration of a small volume of the drug has opened up a new vista for pediatric dentists in managing pain in children, although manufacturers do not recommend the usage of articaine in children less than 4 years of age due to paucity of evidence. So, this review article tries to throw light on the use of articaine in pediatric patients, citing evidence from literature and also tries to portray the recent advances in the research on articaine use in pediatric patients less than 4 years of age.

Key words: articaine, local anesthesia, pain, children

Introduction

Pain management plays a crucial role in the success of any dental treatment in a pediatric patient (1). Minimising the fear and anxiety, a proper management protocol employed, can help develop a positive attitude towards dental treatment in the patient. Though considered a painful and challenging procedure, the administration of local anaesthesia continues to be one of the most commonly used methods for managing pain in the pediatric age group (2). Maximum efficacy through minimum number of injections with negligible adverse events effected, should be the objective of the local anaesthetic agent used (3).

The discovery of the first amide local anaesthetic agent Lidocaine (Proprietary name: Xylocaine), also known as Lignocaine, in the 1940s, marked a revolution in the arena of pain management in dentistry as it offered better potency and triggered less allergic reactions when compared to the then commonly used local anaesthetic Novocain (Procaine) (4). Since its inception, Lidocaine has remained the “gold standard” local anaesthetic agent to perform dental procedures, all around the globe. However, the search for more effective local anaesthetic agents led to the discovery of newer local anaesthetic agents in the subsequent years. Articaine was one among the newer local anaesthetic drugs developed (5). It was originally developed and synthesised as ‘Carticaine HCl’ by H. Rusching et al in 1969 (6). It was introduced into clinical practice first

Article type: Review article

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in 1976 in Germany and Switzerland followed by the other European countries and Canada \(^{(7)(8)}\). In the United States, the US Food and Drug Administration approved the use of articaine in April 2000 \(^{(9)}\). Gradually, articaine started to gain attention and clinicians began employing articaine in adult dentistry. However, the use of the drug in the pediatric age group has raised concern over times, though literature shows that the efficacy and safety of articaine is comparable to the other commercially available local anaesthetic including lignocaine \(^{(10,11)}\) or even superior \(^{(12,13)}\). A survey among pediatric dentists in India concluded that the majority of the respondents still preferred lidocaine with epinephrine as local anaesthetic for use in children \(^{(14)}\).

**Uniqueness of Articaine**

Articaine or Articaine HCl ("4-methyl-3-[2-(propylamino)-propionamido]-2-thiophene-carboxylic acid, methyl ester hydrochloride") is an amide type local anaesthetic. The molecular weight of articaine HCl is 320.84 and it contains a thiophene group instead of a benzene ring, a peculiar characteristic that differentiates it from the other local anaesthetic agents. The greater lipid solubility and potency offered by the thiophene ring helps in the entry of a greater amount of the administered anaesthetic solution into the neurons. It is also able to diffuse more easily through soft tissue surfaces and has a high degree of plasma protein binding of 95%, when compared with the other local anaesthetics. Though classified as an amide, owing to its linkage of intermediate chain, articaine also contains an ester side chain, which triggers the plasma esterases and the hepatic microsomal enzymes to bring about the biotransformation of articaine in plasma and liver respectively. This imparts articaine a half-life of 20 minutes and the primary metabolite obtained is articainic acid which is pharmacologically inactive. This accounts to the decreased systemic toxicity effected by articaine, thereby rendering itself as a safer alternative\(^{(15)}\). Articainic acid (64.2 ± 14.4%), articainic acid glucuronide (13.4 ± 5.0%) and the parent drug (1.45 ± 0.77%) are excreted via urine after the metabolism of articaine \(^{(16)}\).

4% solution with 1:100,000 or 1: 200,000 adrenals are the commonly manufactured forms of articaine and they are dispensed in 2.2ml and 1.7ml glass dental cartridges. The two articaine formulations have been used in both adults as well as in children.

**Safety of Articaine in Children**

Articaine is well tolerated and safe to use in children above 4 years of age. The potency of articaine is 1.5 times that of lidocaine, which enables the administration of a smaller volume of the drug. The reduced volume may help decrease the discomfort felt by the child during anaesthesia administration, especially in cases where an inadequate co-operation of the child is experienced. It is also 0.6 times less toxic as compared to lidocaine \(^{(17)}\). There is also a lesser need for supplemental injections in articaine. Articaine can be given via buccal infiltration to children requiring pulp therapy and extractions and can thereby be used to replace inferior alveolar nerve block (IANB). The high risk of inferior alveolar and lingual nerve damage can be avoided by replacing IANB. The extent of soft tissue analgesia experienced by the patient can be reduced and therefore the incidence of complications like lip biting can be minimized \(^{(18,19)}\). When buccal and palatal infiltration with other local anaesthetic agents was replaced by a single buccal infiltration using articaine, it yielded favourable results during the extraction of primary molars in the maxillary arch. Also, articaine delivered via intraligamentary injection can be used to replace IANB to extract primary molars \(^{(20)}\).

According to Lemay et al, the onset of anaesthesia by articaine was 168±131 sec (nerve block) and 85±60 sec (infiltration) in children; for adults it was 170±131 sec (nerve block) and 119±84 sec (infiltration) \(^{(21)}\). For maxillary infiltration, the duration of action on soft tissues ranges from 2.6 to 4.5 hours whereas for nerve block, it ranges from 4.3 to 5.3 hours \(^{(6,8)}\). The volume of distribution of articaine is inversely proportional to the patient’s age. For children between ages 4-12 years, the dosage of articaine ranges from 5-7 mg/kg body weight, however a lower limit of ≤5mg/kg was advocated in
children aged 4-12 years by authors who performed studies using articaine in conjunction with sedative agents. In any case, it should always be remembered that the concentration of articaine can be considered double as that of lidocaine, and thus will require half the safe number of cartridges.

**Comparison with Other Local Anesthetic Agents in Pediatric Dentistry**

Multiple investigators have reported their findings and conclusions on the use of articaine as a local anaesthetic agent in pediatric population.

Malamed in 2000 compared the pain control property of “4% Articaine HCl (with 1: 100,000 epinephrine)” with “2% Lidocaine HCl (with 1: 100,000 epinephrine)” in children under the age of 13 years, by employing the Visual Analogue Scale (VAS). He found Articaine to be comparable to Lidocaine and gave a green signal to its use in the branch of pediatric dentistry citing its safety and efficacy.

Ram and Amir in 2006 compared Articaine and Lidocaine during operative procedures in children aged 5-13 years. They stated that “Articaine 4% with 1:200 000 epinephrine is as effective as lidocaine 2% with 1:100 000 epinephrine”. However, articaine produced a longer lasting anaesthetic effect on the soft tissues as compared to lidocaine.

Yilmaz et al in 2011 compared Articaine 4% with (1:100000 epinephrine) and Prilocaine 3% (with felypressin) in 162 children belonging to the 6 to 8 age group, undergoing primary molar pulpotomy. Similar intensity of pain was reported during administration of both the anaesthetic solutions. However, coronal pulp amputation, prilocaine gave a pain score which was 1.5 times higher than that of articaine. The adverse events reported were also comparable.

In a review by Leith et al in 2012, it was also reported that 4% articaine showed better pain control than 2% lidocaine for both simple and complex dental procedures.

In 2012, Odabaş compared 4% articaine with 3% mepivacaine, to study the pain reaction and duration of soft tissue numbness during the administration of local anaesthesia in children. A “randomized, double-blind, split-mouth” design was adopted for the study which included a sample size of 50 children (25 girls and 25 boys) belonging to the 7- to 13-year-old age group, who required comparable operative treatment needs in symmetric primary teeth. The objective evaluation of the children was carried out by the modified behaviour rating scale and the subjective evaluation by using the Wong-Baker FACES pain rating scale which helped assess the post injection and post treatment experience. The duration of numbness felt was recorded by the parents by asking the children. Articaine (140.69±49.76 minutes) presented with a prolonged time period of soft tissue anaesthesia than mepivacaine (117.52±42.99 minutes). Neither did the efficacy of the anesthesia nor did the heart rate, blood pressure, or oxygen saturation show any significant differences during all evaluation spans for both anaesthetic solutions. The two anaesthetic solutions also showed similar post treatment experience. It was concluded that same efficacy and identical child behaviour was observed with 4% articaine and 3% mepivacaine, though articaine reported longer soft tissue numbness.

In 2012, Arrow compared “4% Articaine (with 1: 100,000 epinephrine)” administered via buccal infiltration to “2% Lignocaine (with 1: 80,000 epinephrine)” administered via buccal infiltration (BI) or inferior alveolar nerve block (IANB) during standard restorative procedures in the posterior teeth of the mandibular arch among school children. The study assessed pain response via the Faces Pain Scale – Revised (IASP) and found no statistical difference in pain response between both the solutions. The study declared that both Articaine and Lignocaine, were declared equally effective in pain management in children for routine restorative procedures.

In 2014, Thakare evaluated and compared 4% Articaine and 0.5% Bupivacaine through his randomized controlled crossover clinical trial, in patients aged 10-18.
years who had their premolars indicated for orthodontic extractions. The study showed that 4% Articaine presented with a quicker onset of action and lesser Visual Analog Scale scores as compared to Bupivacaine. However, Bupivacaine presented with a longer time period of analgesia and time to the first uptake of medication for rescue analgesia. Articaine was declared more potent and effective considering its quicker onset of action and low pain scale scores.

In 2015, Arali and Mytri P (27) studied the anaesthetic efficacy of “4% articaine (with 1: 100,000 epinephrine)” delivered via buccal infiltration and “2% lignocaine (with 1: 100,000 epinephrine)” delivered via inferior alveolar nerve block (IANB) by employing a “randomized, double-blind, cross over” study design in children aged 5-8 years, presenting with the clinical condition of irreversible pulpitis. The study also assessed the necessity for the administration of supplemental injections. The trial reported that the anaesthetic onset was quicker with 4% articaine than 2% lignocaine. A shorter span of anaesthesia was observed with articaine infiltration and with a lesser requirement for the administration of supplemental injections. The study concluded that 4% articaine can be given via buccal infiltration to children undergoing management for irreversible pulpitis.

A study by Zurfluh et al in 2015 (28) assessed whether an articaine solution could reduce the period of soft tissue anaesthesia and thereby reduce the risk of self-inflicted soft tissue lesions, while still providing an adequate anaesthesia. The study reported that owing to its high efficacy, tolerance, and reduced soft tissue anaesthesia, 4% articaine (with 1: 400,000 epinephrine) was considered safe for treatment in paediatric population.

In 2015, a study was performed by Mittal (29) in 112 children, to compare the anaesthetic efficacy of articaine with lidocaine during the extraction of primary molars in the maxillary arch and assess whether a buccal infiltration injection could achieve sufficient palatal anaesthesia as well. The study reported that buccal infiltration with articaine can be considered a potential substitute for lidocaine in achieving local anesthesia in children, though it did not succeed in achieving sufficient palatal anaesthesia.

In 2016, Chopra et al (30) compared the efficacy of buccal infiltration with articaine against inferior alveolar nerve block with lignocaine for primary molars in children aged 4-8 years, indicated for pulpectomy or pulpotomy. The efficacy of both the anaesthetic agents were assessed using the Pain Scores, the Facial Image score, Sound Eye Motor (SEM) scores and Heft- Parker Visual Analogue Score (HP-VAS). Buccal infiltration with articaine showed significant lower pain scores as compared to IANB (p<0.001). SEM scores at the time of pulp extirpation were also higher for IANB than infiltration (p<0.001).

Kolli et al in 2017 (31) stated that Articaine buccal infiltration can be used as an alternate to conventional local anaesthetic delivered via buccal and palatal infiltration, for extracting primary molars in the maxillary arch.

In 2018, Sharan et al (32) assessed the effectiveness of “2% lidocaine (with 1:80,000 epinephrine)” and “4% articaine (with 1:100,000 epinephrine)” to extract primary mandibular molars in children aged 6-10 years, by employing the intraligamentary injection technique. The study utilised the Sound eye motor scale to assess pain perception. The study showed 80% success rate in Articaine group and 30% success rate in lidocaine group. The study concluded that the intraligamentary injection technique using articaine might serve as an alternative to IANB during extraction of primary molars in the mandibular arch.

Rathi conducted a study in 2019 (33) among 100 children to compare the anaesthetic efficacy of articaine with that of lignocaine. It was concluded that a single buccal infiltration of 4% articaine with 1:100,000 epinephrine is more effective when compared to 2% lignocaine with 1:80,000 epinephrine for extracting primary molars in children aged 7 to 12 years.
Massignan in 2020\(^{(34)}\) stated that though articaine reported a higher pain incidence during the injection, no difference in efficacy was observed when the anaesthetic administration of articaine was compared to lidocaine in the extraction of primary molars. In the study, the efficacy and the adverse events of “4% articaine with epinephrine 1:100 000” was compared with that of “2% lidocaine with epinephrine 1:100 000” during the extraction of primary molars, by employing anaesthesia via buccal infiltration in forty-three children belonging to the 6-10 age group. 21 children received local buccal infiltration with 4% articaine whereas 22 children received lidocaine 2%. The pain experienced during injection of the anaesthetic solution and during extraction of the tooth was the major outcome assessed. The anaesthetic efficacy of both the solutions was observed to be identical though a higher pain incidence was reported by children belonging to the articaine group. Similar adverse effects were observed in both the groups which included post-operative pain, edema and nausea.

It can be summarised that there is evidence to conclude that 4% articaine is comparable to or more efficacious than the other local anaesthetic agents used in children. However, the parents should be cautioned about self-inflicted soft tissue injuries that may arise due to the prolonged numbness, commonly reported with articaine administration. However, the evidence suggests that articaine is safe in children, as the administration of the drug triggers few adverse events, which are commonly noticed with lidocaine as well.

**Evidence For Use in Children Below 4 Years of Age**

Some studies have specifically reported the use of articaine in paediatric dental patients less than 4 years of age with positive results, however the manufacturers still do not recommend it for children younger than 4 years.

Wright et al in 1989\(^{(22)}\) conducted a retrospective research which analysed the usage of articaine hydrochloride as an anaesthetic agent in children aged less than 4 years of age. Records of articaine administration on 211 patients, with additional administrations of the agent on 29 patients was retrieved from two pediatric dentistry offices by a record audit. Some cases even reported administration of higher doses of the drug than recommended for older children. The clinicians have not noted the occurrence of any systemic adverse reactions. The report provided initial evidence for the use of articaine in children under 4 years of age.

Jakobs et al in 1995\(^{(35)}\) studied the pharmacokinetics of articaine on 27 children, 3 to 12 years of age, who underwent dental procedures under intubation anesthesia. They determined the serum concentrations after local anesthesia with 2% and 4% articaine solutions in this age group of children. They concluded that articaine shows age-related differences in the pharmacokinetics and hence there is no need to fix a lower mg/kg dose limit while administration in children.

A study was conducted in young children below four years of age by Elheeny in 2020\(^{(36)}\) to assess the efficacy and safety of 4 % articaine as a local anesthetic agent by comparing it with 2 % lidocaine hydrochloride. The study had an equivalent randomized control trial design, with two parallel arms including 184 young children (92 per group) aged from 36 to 47 months, in whom, pulpotomy of mandibular primary molars was indicated after administering anesthesia by buccal infiltration injection. The results of the study indicated that during pulpotomy, children who received articaine showed less pain as compared to their counterparts in the lidocaine group and no statistically significant differences were observed between both the drugs, considering the post-operative complications. It was concluded that the findings supported the efficient and safe use of articaine hydrochloride 4% with epinephrine 1:100 000 as a local anaesthetic agent during dental procedures in children aged between 3 and 4 years.

The evidence supports the use of articaine in children less than 4 years of age. However, limited research demands clinical trials on a larger sample size to be performed before employing articaine as a local anaesthetic agent in children less than 4 years of age.
Conclusion

Articaine is a safe and effective local anesthetic drug which can be employed in pediatric patients to help achieve dental analgesia during invasive procedures. The evidence that articaine administered via infiltration technique can replace Inferior Alveolar Nerve Block makes it even more interesting. The ability of articaine to penetrate into deeper spaces by diffusing through bone and soft tissue should be understood by the clinicians as it helps to achieve excellent depth of anesthesia, while avoiding block and palatal anesthesia for dental treatment in children. However, advanced research and clinical trials is a necessity, as articaine is not recommended by manufacturers in children below 4 years of age.

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Conflict of Interest - Nil.

References


Amniotic Band Syndrome with Unique Clinical Presentations: A Case Report

Donel Suhaimi1,2, Maya Savira3, ShintaPuja Tilusari4
1Associate Professor, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Riau, Pekanbaru, Indonesia, 2Associate Professor, Department of Obstetrics and Gynecology, Arifin Achmad Hospital, Pekanbaru, Indonesia, 3Assistant Professor, Department of Microbiology, Faculty of Medicine, University of Riau, Pekanbaru, Indonesia, 4Resident, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Riau, Pekanbaru, Indonesia, 4Resident, Obstetrics and Gynecology, Arifin Achmad Hospital, Pekanbaru, Indonesia

Abstract

Amniotic band syndrome is a rare congenital disorder which associated with physical abnormalities such as disruption, deformation, and malformations of organs. The most common abnormalities usually involve the limbs that could range from simple constriction rings to complete amputation. In this case report, we report an amniotic band syndrome with unique clinical presentations. The case was a newborn male baby who was normally delivered and presented with a ring-like constriction at over middle right lower limb and fusion (syndactyly) of left lower limb and right arm. In this report we also discuss different diagnostic modalities which could be used in diagnosing amniotic band syndrome, the risk factors, type of amniotic band syndrome, post-natal diagnose, as well as the therapy.

Keywords: Amniotic band syndrome, ABS, constriction rings, syndactyly, case report

Introduction

Amniotic band syndrome (ABS) is a group of congenital birth defects believed to be caused by entrapment of fetal parts (usually a limb or digits) in fibrous amniotic bands while in utero.1,2 ABS should be called a sequence rather than a syndrome because ABS is related in wide range of physical abnormalities, which are significantly disabling and disfiguring in nature.1-5

Most of infants with ABS have multiple deformity of the limbs and arms and could range from simple constriction rings to complete amputation. ABS has known as amnion rupture sequence, amniotic deformities/adhesions/mutilations (ADAM complex), amniotic band disruption complex, congenital constricting bands, terminal transverse defects, or Streeter anomaly.1,2 Various studies estimate the incidence of ABS to be between 1 in 1,200 to 1 in 15,000 living births.3-5,6 There is no known clear etiology of ABS.3-15 In this case report we report an ABS case with unique clinical presentations.

Case Report

A 22 years old female, 28-29-week gestation, presented to Arifin Achmad Hospital with first stage of labor and preterm premature rupture of membranes (PPROM) and oligohydramnios. The patient was referred from a clinic with placenta previa. The patient complained the rupture of membranes wet in the cloths about one day before admitted to the hospital; however, the patient felt this about a month already. The patient...
also had regular contraction followed by blood mucus.

At the hospital, the patient was thoroughly examined including general as well as obstetric and gynecologic examinations. Patient vital signs were stable and no associated comorbidities such as maternal trauma or drug intake. Physical examinations suggested minimal fluxus from ostium urethra externa, nitrazine test was difficult to interpret due to blood mucus. Ultrasonography showed oligohydramnios with maximum vertical pocket (MVP) of 0.75 cm.

Patient was managed conservatively and was planned for cerclage and amnioinfusion. However, the patient was in labor and delivered a single alive newborn male baby with 1.4 kg, APGAR score was 7/6 and amniotic fluid was clear. On gross examination, the baby had ring-like constriction at over middle right lower limb and fusion (syndactyly) of left lower limb and right arm (Figure 1A-C). The baby was admitted to Neonatology Unit as sepsis and asphyxia and was thoroughly evaluated. Karyotyping and histopathology examination were ordered and returned with normal results (Figure 1D).

![Figure 1](image)

**Figure 1.** Clinical manifestation of the ABS case and its karyotyping. (A) Constriction rings at over middle right lower limb. (B) Syndactyly of the right arm. (C) Syndactyly of left lower limb. (D) Karyotyping showing normal result.

**Discussion**

ABS is a rare disorder with the estimated incidence between 1 in 1,200 to 1 in 15,000 living births. ABS can be diagnosed prenatally by ultrasound and the most important ultrasound diagnostic features are visible amniotic bands, constriction rings, irregular amputations of fingers and/or toes with a terminal syndactyly, or deformation of major anatomic. In a rare case, a strand of amniotic fibrous tissue could be seen attached to tissues and restricting the free movement of fetus in-utero. In this case, patient ultrasound showed no significant deformity since it was difficult to figure the extremities. Some studies found mild defects during ultrasound.
examination; however, ABS is less likely to be diagnosed prenatally and most of defects were seen after birth.6,16-20

ABS etiopathogenesis is still unknown, but there are four main theories.12 In this case, karyotyping result was normal (46 XY), the possibility cause is oligohydramnios. One of the well-known theories is rupture of the amnion in early pregnancy, with formation of amniotic band and liquid loss, or multiple loose strands (amniotic bands), followed by extrusion of all or parts of the fetus into the chorionic cavity. Bands entrap the parts of the growing fetus or limbs or other body parts become entangled and are subject to compression.

The abnormalities such as constriction rings and in some severe cases leading to vascular disruption and could potentially result in amputation of the involved anatomic structures of the body parts. Adherence, even without constriction can have adverse mechanical effect that result in malformation or deformation. In this case, the baby was classified as group IV of ABS (isolated defect).13 Other theories suggest that the amniotic band or vascular disruption due to genetic mutation.8-10,13-17

In such condition, all patients should receive counseling about the fetal abnormalities detected. Consulting with subspecialists is ideally recommended. There is no standard guideline of management for pregnancy complication with fetal ABS. Recently, some treatments for prenatal with ABS have been tried such as fetoscopic laser cutting of amniotic bands before their compression on the fetus.19-20 However, the efficacy of the procedure is not well-documented.

Conclusion

ABS is a rare disorder caused by entrapment of fetal parts usually limbs or arms in fibrous amniotic bands while in utero leading to complex multisystem anomalies. The prenatal diagnosis is difficult although could be diagnosed by ultrasound which indirectly showing the ABS. The treatment and follow-up of ABS children require a team of specialists accordingly.

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Ethical Clearance: Written informed consent for publication was sought from the parent.

Source of Funding: Self

Conflict of Interest: Nil

References


The Effect of Exposure Qari and Qariah Recitation During Pregnancy to the Number of Astrocyte Glia Cells in the Cerebrum Newborn Rattus Norvegicus

Dwi Nurdi Puspita Sari¹, Widjiati², Hermanto Tri Joewono³
¹Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, ²Lecturer, Department of Embryology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, ³Lecturer, Department of Obstetry and Gynecology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

The quality of human resources should be prepared since in early stage. Pregnancy is one of a significant period which can take advantage to by providing proper nutrition and adequate stimulation. For instance, sound and music, the most harmonious combination and easily accepted by the fetus (1). Experimental analytic type with post-test only control group design was employed on 30 pregnant Rattus norvegicus which were divided randomly into 3 groups, namely the control group, qari group, and qariah group. Each of the groups had stimulation starting 6th to 17th days of pregnancy for 60 minutes. At 18 days of pregnancy, the mother of Rattus Norvegicus was sacrificed and bring forth with Sectio Caesarea (SC). As a result, the number of astrocyte glia cells in the cerebrum was higher in the qariah recitation stimulation group (22.62 ± 3.75), compared with the group that was listened to qari recitation stimulation group (19.84 ± 2.48), while the control group (16.54 ± 2.78). A significant difference found in the number of astrocyte glia cells in the Cerebrum Rattus norvegicus newborn that were listened to the qari and qariah recitation stimulation group and the control group. The highest number of Glia cells is obtained from the stimulation of qariah recitation.

Keywords: Stimulation, Pregnancy, Recitation, Astrocyte glia cells, Cerebrum, Rattus norvegicus

Introduction

The quality of human resources should be prepared since the early stage. The important process to generate qualified human resources is by ensuring that every baby is born from a physically, mentally, and socially healthy mother. Providing proper nutrition and adequate stimulation during pregnancy is one of the ways to generate qualified human resources. For instance, stimulating by sound and music is proven to change the structure and function of the fetal brain in the uterus (1).

Glia is the most plenty of cells in the human brain which always increase following the development of the human brain. Glial cells provide lactate for energy sources in neurons and support energy for axons (2). Astrocytes have functions in the physical arrangement of the brain, correlate with neuron synapses, and the assistance of electrical impulses in the brain (16). Damaged or missing astrocytes can be used to function and structure the brain (15). Here, the process to ensure the quality of the fetus is by increasing glial cells with reducing apoptosis and increasing the glial cell ratio (1,3).
The ear starts growing at 18 weeks of age. Then, at 22 to 24 weeks, the fetus will begin to hear low-frequency sounds from outside the uterus \cite{4}. The stimulation of the Mozart instrument influenced the number of neuron cells more than to dangdut and gamelan songs \cite{1}. Mozart’s instrument played with a frequency of 75-10,000 Hz and an intensity of 70-130 dB which provides a lower apoptotic index than other music \cite{5}. A good response came up after playing music for the fetus in the uterus which showed that the heartbeat may often increase, causing it to move faster \cite{17}. Even though the fetus is inside the uterus, it has an environment with sound, vibration, and movement \cite{18}. The heart of the fetus can be seen from the changes in fetal heartbeat and its movements after giving sound stimulation. The escalation response from the fetal heartbeat occurs in ± 20 weeks pregnancy and mostly occurs in ± 26 weeks pregnancy \cite{19}.

The human voice is divided into two major groups, namely the type of voice in men and women. The voice type in men is divided into tenor, baritone, and bass, while in women it is a soprano, mezzo-soprano, and alto \cite{6}. The fundamental frequency (f0) difference between men and women is clear, the fundamental frequency of human voices for men and women is two normal distributed curves with a little overlapping \cite{9}. Qari is a person who recites the Qur’an by restricting to the correct rules. A qari is usually used for a male who recites the Qur’an, and qariah is used for a female who recites the Qur’an \cite{20}.

**Material and Methods**

This study conducted analytical experimental research with a post-test-only control group design by using the *Rattus norvegicus* as a model by giving stimulation as the pregnant mother. The research was carried out in January-March 2021 at the Pathology Laboratory and Experimental Animal Cages of the Faculty of Veterinary Medicine, Campus C Universitas Airlangga, Surabaya, Indonesia.

The population of this study was 2-3 months old white mice Wistar strain (*Rattus norvegicus*) with an initial weight of 120-160 grams, with 30 *Rattus norvegicus* according to the inclusion and drop-out criteria as the sample. They were divided into 3 groups random, namely, the control group, the qari group, and the qariah group. Each of the groups will be listened to recitation for 60 minutes, with an intensity of 65 dB. Here, the mice were inside a soundproof cage so that it is adjusted and arranged to focus on the recitation which will be played through the speakers that had been installed.

In the initial process of fertilization of *Rattus norvegicus* was injected with *Pregnant Mare Serum Gonadotrophin* (PMSG) 10 IU and 10 IU hCG injection. Then, the mating process was done 48 hours later, with 1 male mouse and 1 female mouse, Manomatting. After 17 hours, a copulatory plug will be evaluated as a marker of pregnancy day 0. The treatment was started on day 6th -17th of pregnancy. At the age of 18th days of pregnancy, the mother of *Rattus norvegicus* was sacrificed and the neonatal *Rattus norvegicus* was brought forth by *Sectio Caesarea*. Here, the researcher selected 2 each, starting from the heaviest and the lowest weights.

CUBASE 5 software was utilized to analyze the highest and lowest frequency of qari and qariah recitation to determine the sound intensity using a sound analyzer application and a sound meter application.
Figure 1. Analysis of qari recitation frequency with the farthest frequency, around 15.400 Hz

Figure 2. Analysis of qariah recitation frequency with the farthest frequency, around 13.000 Hz
To measure the number of Glia Astrocyte cells in the cerebrum newborn’s Rattus norvegicus between the treatment group and the control group, employed normality test used the Saphiro-Wilk test, followed by the homogeneity test using the Levene variety. The test to differ each group was by employing the Analysis of Variance (ANOVA) with the Least Significant Difference (LSD) further test. If the p-value <0.05 means that there is a significant difference. Then, computer software SPSS 21.0 was used as a tool to simplify the data processing. At last, the study ethics are considered during the research process to include a replacement, reduction, and refinement.

Results

The number of Astrocyte Glia Cells in the cerebrum was determined and calculated by Hematoxylin-Eosin staining technique with a magnification of 5 fields of view, at 400x magnification.

Table 1. Mean and Standard Deviation the Number of Astrocyte Glia Cells in the cerebrum and cerebellum of Rattus norvegicus offspring from mothers was listened to the recitation of surah Ar-Rahman by qari, qariah, and the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Astrocyte Glia Cell Count at Cerebrum Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>10</td>
<td>16.54 ± 2.78</td>
</tr>
<tr>
<td>K2</td>
<td>10</td>
<td>19.84 ± 2.48</td>
</tr>
<tr>
<td>K3</td>
<td>10</td>
<td>22.62 ± 3.75</td>
</tr>
</tbody>
</table>

Information:

K1: Control group
K2: Stimulation qari recitation
K3: Stimulation qariah recitation

Based on table 1, it was known that the highest average value of the number of Glia Astrocytes cells in the Cerebrum Rattus norvegicus newborn was in the group that listened to the recitation of surah Ar-Rahman by qariah with Astroosit (22.62 ± 3.75).

Table 2. The results of the data normality test used the Shapiro-Wilk test, the Levene Variety homogeneity test, and the data on the number of astrocyte cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Statistic</th>
<th>p-value</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>0.947</td>
<td>0.637</td>
<td>Normal data</td>
</tr>
<tr>
<td>K2</td>
<td>0.913</td>
<td>0.304</td>
<td>Normal data</td>
</tr>
<tr>
<td>K3</td>
<td>0.907</td>
<td>0.259</td>
<td>Normal data</td>
</tr>
<tr>
<td>Levene test</td>
<td>0.985</td>
<td>0.386</td>
<td>Homogeneous between groups</td>
</tr>
</tbody>
</table>

Information:

K1: Control group
K2: Stimulation qari recitation
K3: Stimulation qariah recitation

The results of the normality test using the Shapiro-Wilk test on the number of glia astrocyte cells in the Cerebrum Rattus norvegicus newborn were all normally distributed, with the Astrocyte values each group was normal. It continued with the homogeneity test in the cerebrum of 0.386 which indicated that the variance between groups was homogeneous.
Table 3. ANOVA test results for the number of Astrocyte cells in the cerebrum of Rattus norvegicus

<table>
<thead>
<tr>
<th>Variabel</th>
<th>Statistic</th>
<th>p-value</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrocyte cells in Cerebrum</td>
<td>9.947</td>
<td>0.001</td>
<td>Significant</td>
</tr>
</tbody>
</table>

*p < 0.05 = there are significant differences

Based on table 3, there was a difference number of astrocyte cells in the Cerebrum Rattus norvegicus newborn with a calculated F value of 6.504 and significance value of 0.005, it indicated that there was a significant difference between treatment groups on the number of astrocyte cells in the cerebrum of Rattus norvegicus.

Based on Figure 3, yellow arrow showed the difference number of Glia cells at Cerebrum Rattus norvegicus newborn in the control group (K1), Qari grup (K2) and Qariah grup (K3).

Discussions

In this study, the elements of dB intensity and frequency range are analyzed by utilizing the Sound Analyzer application so that the two groups get almost the same dB value, with qariah recitation with 65.2 dB and qari recitation with 65.3 dB. The sound frequency is processed by employing CUBASE 5 software with resulted in the qariah recitation obtained the highest frequency, around 13.000 Hz, and the lowest frequency of 167 Hz. For qari, the highest frequency is around 15.400 Hz and the lowest frequency is 20 Hz. The difference in frequency generated from the two sounds of qari and qariah can cause a difference in the signal that is picked up by the stereocilia inner hair cell in the cochlea and causes an influx of Ca2 + and stimulates the release of neurotransmitters in the ear nerves. Here, the neurotransmitters will release a potential action and transmitted by the vestibulocochlear nerve then reach the Cerebrum, so that the message captured by the brain is different.

There are many different types of sound based on various classification systems. In music theory, humans have different types of voices, both male and female. Types of voice in men are divided into tenor, baritone, and bass. Whereas women are divided into soprano, mezzo-soprano, and alto(6). Furthermore, men’s and women’s voices have a different frequency, it was found that there was a difference in fundamental frequency (f0) between men and women. The value of f0 women is higher than men. Because of that, the human voice f0 for men and women is two normally distributed curves with slight overlapping. Here, the average female voice is above 200 Hz, while the male voice is below 170 Hz(9).
Producing human voices require three elements, namely, resources, sound sources, and sound modifiers. The power source of the normal sound signal resulted from the compression motion of the lung muscles. A sound modifier is an articulator that changes the shape of the vocal tract so that the frequency characteristics of the acoustic cavity pass through what the sound passes so that the resulting frequency will be different. The accuracy results for the testing process on female voices obtained 100% accuracy results, while men obtained 95.47% accuracy results. The system’s ability to detect the female gender was better than male. From each group, there was a difference in the pitch value of each individual. This is influenced by the shape of the vocal cords, mostly, women’s voices have a higher pitch value than men’s.

Due to the mass and tension of the vocal cords, men have longer and heavier vocal cords than women. Men have a baseline frequency prevalent at 125 Hz, while women have an octave (twice) higher baseline frequency than men at around 250 Hz. The lowest frequency that can be achieved by a bass singer is around 64 Hz. The basic tone of men is lower than that of women, where the average basic tone of men and women is, 129Hz to 167Hz with a peak of 152Hz for men and 207Hz to 269 Hz, and a peak of 239Hz for women.

As a result, there was a significant difference in the average number of glial cells in the cerebrum of Rattus norvegicus newborn, between the control group and the group of exposure to the surah Ar-Rahman recitation by qariah. The highest mean number of glia astrocyte cells is in the group listened to the surah Ar-Rahman recitation by qariah. Here, it can be seen that there are differences in the mean number of astrocyte glial cells in the group listened to surah Ar-Rahman recitation by qari, qariah, and the control group. It occurs due to the difference in dB intensity and the range of the highest and lowest frequencies generated from the voices of reciter and qariah, where the voice of qariah produced the highest average number of glial cells compared to qari and control groups.

**Conclusion**

The number of glial astrocyte cells in the *Cerebrum Rattus norvegicus* newborn in qariah recitation group had the highest value compared to the qari and control groups. Moreover, there was a significant difference in the number of glial astrocyte cells.

**Conflict of Interest:** The author states that they didn’t find any conflict of interest in completing this study.

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**Ethical Approval:** This study has obtained ethical eligibility permission based on the Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University Number: 2.KE.006.01.2021

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Clinical Profile of Scabies in Children in the Outpatient Installation of Dr. Moewardi General Hospital Surakarta, the Period of January 2015- December 2019

Eka Devinta Novi Diana¹, Alfina Rahma¹, Frieda¹, Indah Julianto², Moerbono Mochtar², Suci Widhiati³
¹Recidency Program, ²Professor, ³Associate Professor in Department of Dermatology and Venereology, Faculty of Medicine, Sebelas Maret University/ Dr. Moewardi General Hospital, Surakarta, Indonesia

Abstract

Background: Scabies is caused by parasite, called Sarcoptes scabiei, infestation into the skin. Scabies is generally found in children who live in crowded environments and poor hygiene.

Methods: This is a retrospective descriptive study with secondary data collection from medical record data in the Outpatient Installation of RSDM for the period January 2015-December 2019. The subjects were infants to children aged 14 years with a diagnosis of scabies. Data variables used included age, gender, family history of scabies, diagnosis, comorbidities, supporting examinations and, therapy in scabies patients.

Results: There were 88 pediatric patients with scabies. The most age group that experienced child scabies was 11-14 years (33%) with the most sex being male (55%). The largest source of scabies transmission was from the family (39%). The most common lesion morphology was papules and excoriations (49%). The lesion location was found mostly between the fingers (24%). Examination of skin scrapings using NaCl 0.9% was positive only in 5 patients (6%) with the most diagnosis was scabies (77%)

Conclusion: This study shows that most of pediatric patients with scabies in the 11-14 years range are dominated by males. The most common sources of infection were families with papule morphology and excoriation, whereas the most lesions were found between the fingers. Skin scrapings are only positive 6% of cases. The most commonly used topical therapies are 5% permethrin and 2% ointment mupirocin while the systemic therapies are cetirizine and cefadroxil.

Key words: Scabies, Sarcoptes scabiei, permethrine, cetirizine, cefadroxil

Introduction

Scabies is caused by parasite infestation (Sarcoptes scabiei) into the skin. Scabies is generally found in children who live in crowded environments and poor hygiene.¹ The prevalence of scabies is affecting 200 million population worldwide, 5-10% of children and 10.4% of adults with the same proportion in both sexes.² Pruksachatkunakorn et al reported that scabies is mostly found in 1-7 years old childrens (87.3%).² The prevalence of scabies in Indonesia based on data from the Ministry of Health of the Republic of Indonesia (Depkes RI) in 2008 was 5.6-12.95% and is the third most reported skin disease. Paramita et al reported that the prevalence of scabies was 282 cases with the highest age being 5-14 years old (63.8%).⁴

Cardinal sign of scabies can manifest as nocturnal pruritus. On physical examination, excoriations were seen in the interdigital area, wrist, volar, axilla, areola mammae, scrotum, penis, gluteus and in areas with...
thin skin. The diagnosis is confirmed by finding pathognomonic signs in the form of tunnels in the skin and identification of *Sarcoptes scabiei* mites, eggs or scabies on microscopic examination.\(^1\)

Scabies is also called a great imitator disease. Some of differential diagnosis of scabies include atopic dermatitis, dyshidrotic dermatitis, pyoderma, and prurigo.\(^1\) In atopic dermatitis, the appearance of polymorphic lesions found on face and extensor (infants and children) and flexor areas in adults. Scabies accompanied by atopic dermatitis will give symptoms of itching and skin lesions like papules, pustules or vesicles. Severe pruritus causes patient to scratch the skin excessively, causing erosion and excoriation that facilitating secondary infections like pyoderma.\(^5,6\) In dyshidrotic dermatitis, clinical features are found in the form of papules and vesicles which are mainly found on the lateral fingers.\(^7\) Another complication that can occur in children is crustous impetigo with a clinical picture of multiple vesicles which then break down into crusts (mainly found in the face and extremities).\(^8\)

Some of the therapeutic modalities that can be used in scabies are pyrethroid scabicide drug, namely 5% permethrin cream, 1% gamma hexachlorocyclohexane lotion, 10% crotamiton cream, 5-10% precipitum sulfur, 10% benzyl benzoate lotion and oral ivermectin. These scabiei drugs are neurotoxic to *Sarcoptes scabiei* neurons, causing late repolarization, paralysis and death of adult *Sarcoptes scabiei*.\(^1,9\)

**Materials and Methods**

This research is a retrospective descriptive study with secondary data collection from medical record data in the Outpatient Installation of RSDM in January 2015 until December 2019. The subjects were all infants to children aged 14 years with a diagnosis of scabies. Data variables used were age, gender, family history of scabies, diagnosis, comorbidities, supporting examinations and therapy. The inclusion criteria were children aged 0-14 years, no other skin disorders and no other systemic diseases. The exclusion criteria were patients over 14 years of age, having skin diseases other than scabies and atopic dermatitis.

**Study area and population**

In this research, there were 88 pediatric patients with scabies. The age group that experienced child scabies the most was 11-14 years as many as 29 patients (33%) and most of them is male with 48 patients (55%). The largest source of scabies transmission was from the family in 34 patients (39%), with the most common lesion morphology was papules and excoriations in 43 patients (49%), the lesions location were mostly between the fingers in 21 patients (24%). Skin scrapings using NaCl 0.9% was positive only in 5 patients (6%) with the most common diagnosis being scabies in 68 patients (77%) (Table 1).
Table 1. Data on child scabies dermographism in the outpatient installation of Dr. Moewardi General Hospital for the period January 2015-December 2019

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of cases (n=88)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (year)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>1-4</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>5-10</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>11-14</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>48</td>
<td>55</td>
</tr>
<tr>
<td>Female</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td><strong>History of Transmission</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family</td>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td>Schoolmate</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Islamic Boarding School</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Neighbors</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Unknown</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td><strong>Morphology of the lesion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papules and excoriatio</td>
<td>43</td>
<td>49</td>
</tr>
<tr>
<td>Papules dan macula</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Papules dan pustule</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Papules dan erosion</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td><strong>Location of the lesion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Body</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Extremity :</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between the fingers</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>Between the toes</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Genital</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Gluteus</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td><strong>Scabies examination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl 0,9%</td>
<td>76</td>
<td>86</td>
</tr>
<tr>
<td>Burrow ink test</td>
<td>47</td>
<td>41</td>
</tr>
<tr>
<td><strong>Positive examination result</strong></td>
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<td></td>
</tr>
<tr>
<td>NaCl 0,9%</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Burrow ink test</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Diagnose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scabies without secondary infection</td>
<td>68</td>
<td>77</td>
</tr>
<tr>
<td>Scabies and secondary infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyoderma</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Impetigo</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Scabies and comorbidities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Leukemia</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Hashimoto thyroiditis</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Down Syndrome</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

HIV : Human immunodeficiency virus

Topical therapy using permethrin 5% was administered to all scabies patients, the most used topical antibiotic was mupirocin ointment 2% in 16 patients (18%). Systemic therapy was administered using antihistamine cetirizine in 51 patients (58%) and cefadroxil in 4 patients (5%) (Table 2).
Table 2. Management of child scabies in the outpatient installation of Dr. Moewardi General Hospital for the period January 2015-December 2019

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Amount (n=88)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Topical Therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scabicide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permethrin 5%</td>
<td>88</td>
<td>100</td>
</tr>
<tr>
<td><strong>Topical Antibiotic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without therapy</td>
<td>69</td>
<td>78</td>
</tr>
<tr>
<td>Mupirocin</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Systemic therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotik oral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without therapy</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>Cefadroxil</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cefixime</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Oral antihistamines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cetirizine</td>
<td>85</td>
<td>97</td>
</tr>
<tr>
<td>Desloratadine</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CTM</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bepotastin besilate</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Description: CTM: Chlorpeniramine maleate

There were 67 patients who did not return for control (76%). The duration of therapy in most cases was 8-14 days in 11 patients (13%), with improvement in clinical symptoms such as itching and skin lesions in 21 patients (24%), and recurrence was found in 3 patients (3%) (Table 3).

Table 3. Evaluation of pediatric scabies therapy in the outpatient installation of Dr. Moewardi General Hospital Surakarta for the period January 2015-December 2019

<table>
<thead>
<tr>
<th>Evaluation of therapy</th>
<th>Number of cases (n=88)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Back to control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>1-7 days</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>8-14 days</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>&gt;14 days</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>No duration of therapy (days)</td>
<td>67</td>
<td>76</td>
</tr>
<tr>
<td>1-7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8-14</td>
<td>86</td>
<td>97</td>
</tr>
<tr>
<td>&gt;15</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Improvement in clinical symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itchy</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>Skin rash</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>Unknown</td>
<td>67</td>
<td>76</td>
</tr>
<tr>
<td><strong>Recurrence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>No</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Unknown</td>
<td>67</td>
<td>76</td>
</tr>
</tbody>
</table>
Discussion

Scabies is a parasitic infestation caused by Sarcoptes scabiei on the skin which often affects children. Scabies is a problem in developing countries due to poor hygiene, low socioeconomic conditions, and crowded environment. Dagne et al. reported that scabies commonly affects children aged 11-14 years of 46%, males more often than females. The higher prevalence of 10-14 years old children suffering from scabies is due to the more frequent close contact of children in that age range and most of them do not know how to maintain proper personal hygiene, especially in boys.

Several factors related to scabies transmission include close contact with the sufferer, especially among household members. Paramita et al. reported that the most common source of scabies transmission was through family members (51%). This study is in accordance with Paramita et al. with the most common source of scabies transmission between family members being 39%. Family members have sufficient time together which allows the scabies transmission through direct or indirect contact, such as using clothes, sheets, towels, and family tools together.

The morphology of scabies lesions are papules, pustules, erosions, and excoriations. The distribution of scabies lesions mostly on interdigital area of manus and pedis, volar, wrist, lateral palmar, elbow, axilla, scrotum, penis, glutea, and also in the areola mammae and labia in women. Paramita et al reported that most scabies morphology lesions are papules and pustules (35%) and was most commonly found between fingers (37%). This study reported different results with Paramita et al. that the most common morphology lesions are papules and excoriation by 49% and 24% between the fingers.

Simple investigations to diagnose scabies include skin scrapings taken from unexcoriated papules. Skin scrapings are placed on a slide and then examined under a microscope in dim light. Abdel-latif et al. reported that skin scrapings were positive in 10% of cases. This study reported 6% positive skin scrapings and is in accordance with Abdel-latif et al. Positive results on skin scrapings have low accuracy and are influenced by the low number of mites found due to errors during specimen collection and improper collection time (daytime).

The diagnosis of scabies can be made based on the discovery of 2 out of 4 cardinal symptoms, including nocturnal pruritus, infection attacking humans in groups, found cuniculus, mites, and egg. Paramita et al reported that the diagnosis of scabies was found in 73% of cases. This study reported the results consistent with Paramita et al. which the diagnosis of scabies without secondary infection was found in 77% of cases.

Permethrin cream 5% is the first line recommendation for scabies therapy because high effectiveness with low toxicity risk. Anderson et al. reported that 69% of scabies patients received 5% permethrin therapy. This study is in accordance with Anderson et al., that all pediatric scabies patients (100%) were treated with 5% permethrin cream. Permethrin is a synthetic pyrethroid which works by damaging the cell membrane of mites, disrupting the balance of the sodium channels which results in depolarization and paralysis of the respiratory tract in Sarcoptes scabiei.

Scabies is often accompanied by secondary infestations for example pyoderma which is most commonly caused by gram-positive coccus bacteria. Mupirocin is a broad-spectrum topical antibiotic that’s recommended especially for gram-positive bacterial. Vasani et al. reported that the topical antibiotic used in secondary infestation of scabies was mupirocin by 30%. This study is in accordance with Vasani et al. that the topical antibiotic used in secondary infestation of scabies was mupirocin by 18%. The action mechanism of mupirocin is to interfere with the synthesis of isoleucyl-tRNA synthetase, thereby inhibiting bacterial protein synthesis. Mupirocin has been shown to have excellent activity against Staphylococcus and most Streptococci but less effective against gram-negative bacterial infestations. Mupirocin has good skin penetration, can be absorbed through the circulation and metabolized into an inactive derivative, namely monic acid, and there is rarely a side effect of irritation on the skin.
Systemic therapy for secondary infestations of scabies that can be considered is the first generation cephalosporins (cefadroxil), macrolides (azithromycin) and clindamycin. This study is inconsistent with Mark et al. which reported that the most common systemic antibiotic used in cases of secondary infestation of scabies was cefadroxil by 5%. Cefadroxil is a broad-spectrum beta-lactam ring antibiotic used in both gram-positive and gram-negative bacteria. The beta-lactam ring binds to protein binding protein (PBP) to play a role in inhibiting the activity of cell wall synthesis. Cephalosporins are also thought to play a role in autolysis activation of bacterial cells which causes bacterial cells to become lysis.

Cetirizine is a second generation non-sedative antihistamine which is effective in reducing itching. Paramita et al. reported that the most used antihistamine in scabies was mephidrolin napadisylate at 64%. This study the antihistamine given was cetirizine (97%) which is not in accordance with Paramita et al. Cetirizine is a fast-onset antihistamine and selective antagonist against histamine 1 (H1) receptors on smooth muscle cells, vascular endothelial cells, and gastrointestinal tract. Cetirizine does not cross the blood brain barrier and provides less sedation than first generation sedative antihistamines. Cetirizine reduces pruritus by inhibits histamine production, neutrophil and eosinophil migration. Cetirizine is rapidly absorbed through the gastrointestinal tract and excreted through kidneys.

Scabies patients are advised to return for control within 7-14 days after receiving the first treatment. Paramita et al. reported that 61% did not return for control. This study’s results were consistent with Paramita et al. That 67% of patients did not return for control. It is probably because the patient feels that the disease already recovered or the existance of secondary infections causing patient to go to other medical personnel.

Scabies therapy using 5% permethrin can be repeated 7 days after receiving the first therapy with an average length of treatment for 14 days. Sungkar et al. reported that scabies therapy using 5% permethrin was administered for 21 days in 69 patients (74 %). This study reported results inconsistent with Sungkar et al. which scabies therapy using 5% permethrin was administered for 14 days in 86 patients (97%). Evaluation for 14 days is necessary because the larvae will become adult stage Sarcoptes scabiei within 10-14 days. Permethrin 5% cannot kill Sarcoptes scabiei eggs so that repeated therapy after 7 days is needed to kill Sarcoptes scabiei eggs that have hatched into adult mites.

Improvement of clinical symptoms in the form of reduced itching, skin rash and, the absence of new papules in scabies patients are indicators of successful therapy. Musthaq et al. reported that pruritus and skin rash decreased during the 14-day evaluation were found in 45% of cases. This study reported the results according to Mushtaq et al., that pruritus and skin rashes were reduced in 21 (24%) cases. The correct application of scabicide, for example 5% permethrin, is very important in the success of therapy.

Scabies recurs if the individual still shows clinical manifestations after 6 weeks of initial therapy. Sungkar et al. reported that scabies recurrence was 15.9% of cases. This study is not in accordance with Sungkar et al who reported that scabies recurrence was found in 3% of cases. Symptoms that persist for more than 6 weeks should be re-evaluated because the possibility of treatment failure leading to recurrence in cases of scabies.

**Conclusion**

In conclusion, there were 88 pediatric scabies patients in January 2014-December 2019. The age group experiencing child scabies the most is 11-14 years (33%), more males than females. The most common source of scabies transmission is the family (39%), with the morphology of papules and excoriations (49%), the lesions location was found mostly between fingers (24%). Only 6% positive skin scrapings were diagnosed with scabies in 77% of patients. Topical therapy using permethrin 5% was given to all scabies patients, the
most used topical antibiotic was mupirocin ointment 2% (18%). Systemic therapy was given in the form of antihistamines cetiricine (58%) and cefadroxil (5%).

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References


Role of Mathematics Instructors in Enhancing Student’s Self-Confidence in Distance Learning During Coronavirus Disease

Eman Almuhur1, Manal Al-labadi2, Amani Shatarah3, Tala Sasa4, Raeesa Bashir5, Nazneen Khan6

1Assistant Professor, Department of Basic Science and Humanities, Faculty of Arts and Science, Applied Science Private University, Amman, Jordan, 2Assistant Professor, Department of Basic Science and Humanities, Faculty of Arts and Science, University of Petra, 3Assistant Professor Department of Mathematics, Faculty of Science and Arts, Taibah University, Kingdom of Saudi Arabia, 4Teacher, Department of Basic Science and Humanities, Faculty of Arts and Science, Applied Science Private University, Amman, Jordan, 5Assistant Professor, Department of Mathematics, Faculty of Science and Arts, Taibah University, Kingdom of Saudi Arabia

Abstract

Background: This article aims to discuss and investigate forms of pedagogical knowledge of mathematics instructors and influence of their educational beliefs on their students during Coronavirus disease where progress in learning is typically influenced by self-confidence and anxiety.

Materials and Methods: A study survey was carried out to find out the extent of anxiety and self-efficiency students have before and during mathematics exams in distance learning.

Results: Out of 252 students, 88% are females and 12% are males, majority of students of the second level from different faculties filled the survey. The study reveals student’s responses to the levels of the variables where confident is of ‘always’ attitude with mean 2.3714 (SD=.37764). On the other side, anxiety is of ‘sometimes’ attitude with mean 1.8595(SD=0.4196). Those results are good and indicates that no big problems in mathematics either inside or outside university faced by sample we dealt with, this result refers to the simplicity and good characteristics of instructors of the university. Moreover, lots of programs that help in understanding basics and advanced mathematics.

Conclusion: Successful pedagogies instructors of mathematic used give the best explanation of results we have got in this study especially in distance learning. Detailed study plan that clarifies the course objectives, topics, evaluation method, books and resources must be given to students.

Key words: Mathematics learning, pedagogy, Self-confidence, Anxiety.

Introduction

Pedagogy is a Latin word that means the art, methods, strategies and procedures used in teaching. These methods or procedures include the targets of education which focus on creating an interactive environment between the teachers and their students. The procedures include many aims beside creating an interactive environment, exploring the ways that knowledge and skill grow and training instructors before starting teaching throughout educational programs by social and educational experts. In order to increase the efficiency of learning process, monitor learning and fulfill targets of learning process, experts and novices must collaborate and strike horizons of conversation.

Scientific theoretical frameworks the shape of learning process and instructional decisions, hence pedagogy depends essentially on the scientific theories of learning that are considered as basis of how learning works, theories are behavioral, cognitive and constructivist. Many of those theories imply the roles of both of the learner and teachers. In addition, professional educators are guided by these theories.
Behavioral theories are inadequate, unsupported but no alternative explanation instead them where they are recently having a widespread acceptance. Typically, behavioral education must be done within a reasonable framework rather than within scientific human theories. We must focus on reasonable justification perspectives on individual’s behaviors to enrich our reasoning and thinking of behavioral theories.

Decisions made by teachers are influenced by the strategies they acquired from their experience in teaching where they use many techniques such as activities, assignments, learning groups,…, etc. The importance of pedagogy comes from its ability to deal with events occur during teaching, thus without this terminology, teachers cannot explore, criticize, analyze and investigate the credence about the behavior of students, so the behaviorist pedagogical tactic focuses on teachers, so behaviorism is considered as a traditional teaching model.

It is primarily outcome-based, approaching defined and preordained subject matter and standardized basic skills that adult decision-makers (not learners) want teachers to transfer to learners, employing instructional models that include the use of conditioning through incentives, coercion, manipulation and other more forceful means used to achieve goals that were selected for the learner instead of by or with the learner. The theory of interaction describes learner’s behaviors during the learning process where those behaviors can be imitated or repeated for many purposes. However, behavioral theories have an uneasy relationship with theories of learning. Using pedagogy depends on basic skills, instructional models, conditioning via incentives, coercion, manipulation, ... etc.

Constructive theories are acquired via experiences and thinking where it is based on projects, inquiries and brain storms. Piaget initiated ‘schemas’ based on the fact that learners are ready to learn, whereas teacher construct activities that simplify thoughts and ideas for learners. For learners with little ages, learning must be done through physical activities, however older learners outline symbolic and abstract ideas.

Contradicting constructivism that Piaget put some of its basics, social constructivism pedagogy considers learning as an interactive participatory process between students and teachers like group works, in addition it is considered as a mixture of two priorities: teacher is guiding learning process and student is under his concentration. Typically, interactive activities between teachers and students are vital methods for creating such a common context of experience within the school/university especially when teachers and the students are not of the same background.

For effective pedagogies, clear outcomes must be taken in consideration as necessity either long-term goals or short-term goals where depending lecturer’s knowledge, beliefs, skills and behaviors is a priority.

Lots of learning strategies fit different situations instructors meet while teaching either online or in a class. Such strategies reinforce skills of instructors besides knowledge and behaviors of learners. In addition, they develop higher thinking proficiency, meta-cognition and improve dexterity of dialogue for both of instructors and learners. Embedding variety of ways of assessment helps in understanding what a learner needs exactly, but some instructors lack how to assess in a valid, reliable and efficient way. We carried out a survey on our students to assess what are problems they have in dealing with mathematics. We analyzed results statistically and described it. Next section shows what we got and what are strategies we will take in consideration to come over our student’s problems.

Our survey titled “Self-Confidence and Anxiety of mathematics in distance learning” is designed to measure the range of fear of mathematics especially before exams. The survey includes a sample of 252 students of Applied Science Private University in Amman, Jordan. The students a survey includes are from different faculties and different levels where the levels are first, second, third…and seventh year. Each participant filled an online questionnaire including demographic information, information concerning their
ways to deal with mathematics in and outside university and how they prepare to mathematics exams. [5] For the validity and reliability of the results we have got, inductive and statistical analysis are used. Moreover, we calculated correlation coefficients between many items and determined their type and the strength of them.

2. Sample description

A sample of 252 students of Applied Science Private University were recruited to the study titled “Self-Confidence and Anxiety of mathematics in Coronavirus disease”. Approximately 88% of them are females and the rest are males. We found that 90 students in their second year, that is 35.7% of the sample.

<table>
<thead>
<tr>
<th>Academic Year</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>57</td>
<td>22.6</td>
</tr>
<tr>
<td>second</td>
<td>90</td>
<td>35.7</td>
</tr>
<tr>
<td>Third</td>
<td>61</td>
<td>24.2</td>
</tr>
<tr>
<td>fourth</td>
<td>16</td>
<td>6.3</td>
</tr>
<tr>
<td>Fifth</td>
<td>17</td>
<td>6.7</td>
</tr>
<tr>
<td>Sixth</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>seventh</td>
<td>10</td>
<td>4.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>30</td>
<td>11.9</td>
</tr>
<tr>
<td>female</td>
<td>222</td>
<td>88.1</td>
</tr>
</tbody>
</table>

Study of items’ means

The following part of our study reveals student’s responses to the levels of the variables. Here, mean and standard deviation are calculated for each item, then they are ranked in descending order according to mean. Higher mean value indicates more agreement on that item.

A. Confident

<table>
<thead>
<tr>
<th>Rank</th>
<th>Item No.</th>
<th>Item</th>
<th>Mean</th>
<th>SD</th>
<th>Attitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>3</td>
<td>I think I can complete all mathematics assignments.</td>
<td>3.0000</td>
<td>.00000</td>
<td>always</td>
</tr>
<tr>
<td>1.5</td>
<td>4</td>
<td>I think I’m a persons who knows mathematics.</td>
<td>3.0000</td>
<td>.00000</td>
<td>always</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>I hope that I know mathematics well</td>
<td>2.4167</td>
<td>.60952</td>
<td>always</td>
</tr>
</tbody>
</table>
From table 2, item 3 “I think I can complete all mathematics assignments” and item 4” I think I’m a person who knows mathematics” have the highest mean value of 3(SD=0.000) with ‘always’ attitude.

In the second rank is item 7 “I hope that I know mathematics “ with mean 2.4167 (SD=.60952) with ‘always’ attitude. Item 8 “I feel confident when taking a mathematics test “ has the lowest mean with 1.9841 (SD=.64350) with ‘sometimes’ attitude. In general, confident is of ‘always’ attitude with mean 2.3714 (SD=.37764).

**B. anxiety**

Table (3): Mean, standard deviation and attitude for items of anxiety

<table>
<thead>
<tr>
<th>Rank</th>
<th>Item No.</th>
<th>Item</th>
<th>Mean</th>
<th>SD</th>
<th>Attitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>I feel nervous when preparing for a mathematics test.</td>
<td>2.2262</td>
<td>.65018</td>
<td>sometimes</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>I am afraid I will not be able to get good grades in mathematics subjects</td>
<td>2.1667</td>
<td>.68274</td>
<td>sometimes</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>I am afraid I will not be able to get an “A” in mathematics</td>
<td>2.1508</td>
<td>.70363</td>
<td>sometimes</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>I’m afraid to answer incorrectly in a mathematics class</td>
<td>2.0000</td>
<td>.68565</td>
<td>sometimes</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>I’m afraid I won’t be able to complete mathematics assignments</td>
<td>1.9841</td>
<td>.68547</td>
<td>sometimes</td>
</tr>
</tbody>
</table>
Cont... Table (3): Mean, standard deviation and attitude for items of anxiety

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Attitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>7</td>
<td>Working on mathematics assignments bothers me</td>
<td>1.9603</td>
<td>.73502</td>
<td>sometimes</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>I am concerned that I do not know enough mathematics to</td>
<td>1.9167</td>
<td>.72312</td>
<td>sometimes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>be good in future</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>I fear I will not be able to understand mathematics</td>
<td>1.8492</td>
<td>.70363</td>
<td>sometimes</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>I am afraid I cannot use mathematics in my work in future</td>
<td>1.8333</td>
<td>.73338</td>
<td>sometimes</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>I feel nervous when asking questions in the lecture</td>
<td>1.7540</td>
<td>.69358</td>
<td>sometimes</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>I get nervous when I have to use mathematics outside of</td>
<td>1.4722</td>
<td>.66442</td>
<td>Never</td>
</tr>
<tr>
<td></td>
<td></td>
<td>university</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>I feel nervous when listening to the math lecturer at the</td>
<td>1.0000</td>
<td>.00000</td>
<td>Never</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lecture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anxiety Cronbach’s Alpha (0.861)</td>
<td>1.8595</td>
<td>.41962</td>
<td>sometimes</td>
</tr>
</tbody>
</table>

From table 3, item 1 “I feel nervous when preparing for a math test “ has the highest mean of 2.2262(SD= 0.650) with attitude of ‘sometimes’. In the second rank is item 4 “ I am afraid I will not be able to get good grades in math subjects “ with mean 2.1667(SD=.6827) with attitude of ‘sometimes’. The minimum mean is for item 5 “ I feel nervous when listening to the math lecturer at the lecture “ with only mean 1.000(SD=.0000) with attitude of ‘never’. In general, anxiety is of ‘sometimes’ attitude with mean 1.8595(SD=0.4196).

Correlation analysis

Table (4): Pearson’s correlation coefficients

<table>
<thead>
<tr>
<th></th>
<th>Confident</th>
<th>Anxiety</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pearson Correlation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confident</td>
<td>1</td>
<td>-0.742</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>-0.742**</td>
<td>1</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

From table 4, the relationship between confidence and anxiety is strongly negative.
3. Tests and Analysis of variance

Are there significant differences in the levels of the study constructs that can be attributed to gender, class? Independent samples t-test will be used to test for gender while, analysis of variance (ANOVA) will be used to test for other personal variables.

Table (5): Mean and standard deviation for males and females

<table>
<thead>
<tr>
<th>Group Statistics</th>
<th>gender</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confident</td>
<td>male</td>
<td>30</td>
<td>2.4250</td>
<td>.39231</td>
<td>.07162</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>222</td>
<td>2.3641</td>
<td>.37594</td>
<td>.02523</td>
</tr>
<tr>
<td>Anxiety</td>
<td>male</td>
<td>30</td>
<td>1.7500</td>
<td>.37842</td>
<td>.06909</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>222</td>
<td>1.8742</td>
<td>.42349</td>
<td>.02842</td>
</tr>
</tbody>
</table>

Table (6): Independent samples T tests

<table>
<thead>
<tr>
<th>Variable</th>
<th>t</th>
<th>Df</th>
<th>Sig. (2-tailed)</th>
<th>Mean Difference</th>
<th>Std. Error Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Confident</td>
<td>.828</td>
<td>250</td>
<td>.408</td>
<td>.06089</td>
<td>.07350</td>
<td>-.08388</td>
</tr>
<tr>
<td>Anxiety</td>
<td>-1.526</td>
<td>250</td>
<td>.128</td>
<td>-.12425</td>
<td>.08141</td>
<td>-.28458</td>
</tr>
</tbody>
</table>

Actually, from table 6, There are no significant differences between confident and anxiety that can be attributed to gender.

Recommendations

Provide a weekly summary containing a reminder of the assignments and tasks that are supposed to be achieved and delivered during the week, as well as lectures and topics that will be studied during it. Some applications and videos on a websites help students who feel shy or cannot ask freely during mathematics lectures to understand well because of their flexibility, availability and simplicity. Moreover, these application reinforce student’s self-confidence. Lots of cartoon films and games support axioms, theories and facts students learnt and contribute to the stability of information in their memory. Investing time has a vital in raising student’s awareness and responsibility and helps them to realize the need of every hour and minute, you can imagine the storm in his brain thinking what he must do to be better?
Typically, giving an immediate stimulating comment to the student’s performance has a great effect on the student’s self-confidence, provides students reassurance and creates a positive relationship between them and their instructors. [7] Comments might be written by massages, e-mails, audio, or through some applications. Definitely, rapid replies to students’ inquiries and questions makes them feel trusted, not tense and comfortable. If we overcome socially accepted dominant ideas thought “I’m not good at mathematics”, [9] we will enhance a student self-confidence and it will be a great job we ever done. Encourage each student especially those who feel that they can’t do mathematics well to get rid of their fears.

For exams, [8] it is good to persuade student to practice some previous exams at home before their exams. Practicing gives them the ability to prioritize questions from easiest to hard ones, they also get used to the atmosphere of the exams, hence mitigating their fears of exams, pacing and knowing how exam looks like. Connections between ideas, theorems and axioms of mathematics with objects students use in their daily lives easiest thoughts stocking in a mindset, we can use expressions like “All Students Take Calculus” to illustrate in which quadrant a trigonometric function has a positive sign by relating the first letter of each word with a first letter of a trigonometric function. We tried to easiest the irrational number $\pi = 3.1415926$ by letting a student counts words of “May I Have a Great Container of Coffee”. [10] This strategy leads us to emphasis on the importance of embedding games in teaching mathematics, the way that make mathematics fun and engage student’s attention, like using a box filled with letter noodles and asking each student to draw number of those noodles and calculate probabilities of getting a certain letter or number of ways of getting letters of a word a student guess. We may use a die while talking about axioms of probability.

Finally, trying to make distance learning during Coronavirus useful via letting students imagine the world after Coronavirus disappearance and how can this affect their future plans.

Acknowledgement: All thanks and appreciation to the administrative and academic staffs of Applied Science Private University and everyone who contributed to the completion of this scientific study.

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Ethical Clearance: Taken from Applied Science Privet University

References


Effect of Aqueous and Alcoholic Extract of *Hydrastiscanadensis* Plant on Bacteria Isolated from Otitis Media

Eman Fadal Abbas Al-Lahibi¹, Hadeel MizherYounis Al-Hadeethy²

¹Research Scholar, Biology Department, Faculty of Science, University of Tikrit, Iraq, ²Professor, Basic Science Department, Faculty of Dentistry, University of Tikrit, Iraq

Abstract

The results of the current study showed that the effect of the alcoholic extract of the *Hydrastiscanadensis* plant was more effective than the aqueous extract on the bacteria isolated from otitis media at the same concentrations. As the alcoholic extract had the highest effect on *proteusmirabilis, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa* and *Klepsiella pneumoniae* with an inhibition diameter ranging (20, 18, 17, 16, 16, 15) mm respectively at a concentration of 200 mg/ml. The study also showed that the higher the concentration of the extract, the greater the inhibitory activity against the bacterial species, with significant differences in the probability level 0.05 of the two extracts on the bacterial species, except *Pseudomonas aeruginosa, Staphylococcus aureus* there was no significant difference in the effect.

Keywords - *Hydrastiscanadensis, alcoholic extract, aqueous, Bacteria, Otitis media.*

Introduction

Otitis media is one of the main reasons for visiting health centers all over the world, as it causes serious complications, including hearing loss(¹), meningitis and brain abscesses(²), if not treated properly. It is an inflammatory disease that affects the lining of the mucous membrane of the middle ear and the associated incision(³), which is either acute(⁴) or chronic(⁵). The causative agent may be bacterial, viral or fungal(⁶), but more causes of inflammatory middle ear common are infections with bacteria such as *Pseudomonas aeruginosa, Staphylococcus aureus, Prokusspp, Staphylococcus epidermidis, Escherichiacoli, Klepsiella pneumoniae*⁷. The increase in bacterial resistance to antibiotics in recent times has become one of the most serious threats facing the entire world, as some strains have acquired resistance to most antibiotics, which calls for seeking to find new antibacterial agents that cause disease to overcome bacterial resistance(⁸). Medicinal plants with antimicrobial properties are alternatives to the increasing problem of bacterial resistance to antibiotics⁹, including plant *HydrastisCanadensis*, which also known by other names including Goldenseal, Orange root, Yellow puccon, India eye, eye root, Ground raspberry(¹⁰). It is a small perennial herb found in the humid forests of Canada and the eastern United States and belongs to the Ranunculaceae family(¹¹), the effectiveness of alcoholic extract due to the alkaloids such as berberine, canadine, hydrstine, canadline and others, which have anti-bacterial, antifungal, protozoal and tuberculosis effectiveness as well(¹²). The efficacy of alkaloids against bacteria has been proven by many studies, where it was found that they have high inhibitory efficacy against both Gram positive and Gram negative bacteria(¹³). They also play an important role in treating a number of diseases such as asthma, analgesic and anti-cancer conditions(¹⁴). It's

Corresponding Author:
Eman Fadal Abbas Al-Lahibi
Research Scholar,
Biology Department, Faculty of Science, University of Tikrit, Iraq, E-mail: abbaseman300@yahoo.com

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mechanism of action is to inhibit bacterial cell division by interfering with DNA replication, targeting enzymes RNA polymerase, gyrase, topoisomerase IV and stopping bacterial replication\(^{15}\). Berberine has been found to be effective against Vibrio cholera, Staph. aureus, Klebsiellaspp, Shigella, Streptococcus pyogenes\(^{16}\).

**Materials and Methods**

The aqueous extract of the plant in this study were prepared by mixing 40 g of the plant model in 160 ml of sterile distilled water (4:1 weight/volume), then the plant model was placed with the Blander crushing device inside an ice bath, and the plant samples were stirred by the electro-magnetic stirrer for 60 minutes, in order to disintegrate and tear the cell wall of the samples, leave the mixture in the refrigerator for 24 hours for the purpose of soaking, filter the mixture through several layers of gauze, then filter again using a Buchner bowl funnel and using Whatman No.1 filter papers with vacuum. By means of a vacuum, to get rid of (the non-milled parts and the remnants of the fibers), after that the extracts were poured into sterile glass containers and placed in an electric oven at a temperature of 40 °C until all the water evaporated and to preserve the active substances and after drying the extracts, they were placed in airtight glass containers. Close, labeled, and refrigerated until use\(^{17}\). In the same way previously mentioned, the alcohol extract was prepared by adding ethyl alcohol at a concentration of 95% instead of distilled water.

The method of researchers Shinkafi and Dauda 2013 was adopted to test the effectiveness of both the aqueous and alcoholic extract of the Golden Seal against the bacterial species isolated from otitis media (Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis) after being diagnosed with biochemical tests and confirming the diagnosis with the VITEK2 system device. The plates containing Muller Hinton agar medium were prepared for the purpose of implanting a bacterial suspension on them after comparing them with a McFarland tube. The disc diffusion method was used in which disks of Whatman No.1 filter paper with a diameter of 6 mm were prepared and then sterilized with oven at 160 °C for two full hours. Then the tablets were saturated with concentrations (200, 100, 50, 25, 12.5, 6.25 mg/ml) aiming to test their effectiveness against bacterial isolates, and using sterile forceps, they were placed on the surface of the medium and then incubated at 37 °C for 24 hours to observe the effectiveness or not, and measure the inhibition diameters in mm compared to chloramphenicol antibiotics as positive control\(^{18}\).

**Results**

The results of the current study showed, that the effect of alcoholic extract of goldenseal plant was higher than that of aqueous extract as showed in table (1), the effect of the alcoholic extract had the largest effect on P. mirabilis with an inhibition diameter of 20 mm. The least effect was on bacteria. pneumonia, where the inhibition diameter was 15 mm, while the effect of the aqueous extract was almost non-existent at the same concentration of 200 mg/ml where the maximum effect of the aqueous extract was on isolates with an inhibition diameter of 7 mm for each of P. aeruginosa, Staphylococcus epidermis, k. pneumonia, in which there are statistically significant differences, at a probability level of 0.05.
Table (1): The inhibition diameters of both the alcoholic and aqueous extract of the *Hydrastis Canadensis* on bacteria isolated from otitis media.

<table>
<thead>
<tr>
<th>Cont.</th>
<th>Extract concentrations in mg/ml</th>
<th>Bacterial isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>23</td>
<td>0 g</td>
<td>0 g</td>
</tr>
<tr>
<td>24</td>
<td>0 f</td>
<td>0 f</td>
</tr>
<tr>
<td>27</td>
<td>0 h</td>
<td>9 e</td>
</tr>
<tr>
<td>22</td>
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<td>8 e</td>
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<td>25</td>
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<td>0 e</td>
</tr>
<tr>
<td>26</td>
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Similar letters mean there is no significant difference at the level of probability 0.05

**Discussion**

The study showed that the extract of the golden seal plant had inhibitory activity against the isolated bacterial species of otitis media, especially the alcoholic extract, which was more effective than its aqueous counterpart. This is due to the solubility of the active substances in alcohol more than water, and as the study showed that the lower the concentration of the extract, the less inhibitory activity against bacteria. Udvardy and his colleagues (2015), found that the medicinal effect of golden seal plant is mainly due to the alkaloids such as berberine - hydrastine - which are present mainly in the roots and other alkaloids. In another study, it was found that the extracts of this plant were effective against germs that cause periodontitis. Junio and his colleagues (2011) also found that extracts dissolved with ethanolic alcohol from the aerial parts of the plant and roots have efficacy against *Staph. aureus* by influencing flow inhibition of ethidium bromide. The aqueous and alcoholic extracts of herbs plants, especially the golden seal, are composed of complex active ingredients with bioactivity that can limit the increase in bacterial species gaining resistance. The mechanism by which bacteria are targeted by using plant extracts in general has not yet been clarified, but there is proposed mechanisms include influencing the genetic synthesis of the bacterial cell, especially DNA and RNA, disrupting the biological membranes of the microorganism, interfering with the nutritional pathways of the microorganism, stimulating clotting of cytoplasmic contents and influencing the phenomenon of quorum sensing (QS) which means cutting off the normal cellular communication of germs.
Conclusions

Golden seal (Hydrastis Canadensis) plant extract has inhibitory efficacy against bacteria isolated from otitis media and can be considered as antibacterial agents.

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References


The Construction of Legal Protection For Aesthetic Patients

Endang Sri Sarastri
Faculty of Law, University of 17 Agustus 1945 Semarang, Indonesia

Abstract

Plastic surgery, primarily aesthetic plastic surgery, is increasingly becoming one of the beauty procedures that is increasingly considered commonplace to be carried out by people in various countries in the world. When viewed from the sociological aspect of this aesthetic operation, it is solely aimed at improving the quality of life and utilizing science and technology, both of which, when linked from a legal perspective in Indonesia, is in line with the formulation contained in Article 28 C paragraph (1) of The 1945 Constitution of the Republic of Indonesia as the state constitution of Indonesia. However, this aesthetic plastic surgery encountered various obstacles; this can be proven from the problems that occurred with Mrs. P and Dr. W, where this case is related to the actions of Doctor W, who performed reconstructive and aesthetic surgery on Mrs. P’s nose. For this case, Dr. W was threatened with unlawful acts for the medical actions he took. Therefore, this article will analyze the legal construction in Indonesia that regulates legal protection for plastic surgery patients. This article aims to find out and analyze how legal construction in Indonesia regulates aesthetic plastic surgery. This is indicated to provide an understanding and test whether the medical action of aesthetic plastic surgery can be said to be part of the scope of regulation regarding consumer protection which is accommodated in Law No. 8 of 1999 or not. This research uses normative legal research methods. That is research conducted by examining library materials. The data used is secondary data, which includes primary legal materials in statutory regulations, then also uses secondary legal materials that explain primary legal custody. Construction of Legal Protection for Aesthetic Plastic Surgery consists of laws and regulations relating to the health/medical field that apply in Indonesia and the laws that apply in Indonesia, namely Law no. 29 of 2004 concerning Medical Practice, Law No. 36 of 2009 concerning Health, Law Number 44 of 2009 concerning Hospitals, Law Number 36 of 2014 concerning Health Workers, and the Civil Code: Article 1313; 1319; 1320; 1330; 1333; 1337; 1338 and 1454, so that the provisions governing consumer protection are not included in the scope for medical treatment.

Kata Kunci: Construction, Legal protection, Aesthetic Patients

Introduction

Aesthetic plastic surgery is increasingly becoming one of the beauty procedures that is increasingly considered to be commonly carried out by people in various countries globally, including Indonesia. Such a statement is based on the fact that social media in Indonesia has recently reported many appearance enhancements carried out using aesthetic plastic surgery. The term plastic surgery in medicine is called “aesthetic plastic surgery” because, in the medical world, the field is included in the specialist in reconstructive and aesthetic plastic surgery. A doctor carries out aesthetic plastic surgery with particular expertise in Reconstructive Plastic Surgery Specialist and Aesthetics, commonly abbreviated as dr.SpBP-RE. In recent times, many social media have reported improving appearance using plastic surgery. Indeed, plastic surgery is increasingly becoming one of the most common cosmetic procedures to be performed. Actually, what is meant by social media in the medical world, is called “aesthetic plastic surgery” because, in the medical world, this field is included in the specialty of reconstructive and aesthetic plastic surgery. The doctor who performs the medical procedure is called the Reconstructive and Aesthetic Surgeon, abbreviated as dr.SpBP-RE.
The tendency to be beautiful is the natural desire of every woman. However, according to Hamid Reza Salehi, the significant doubts regarding the legality of aesthetic plastic surgery have been removed by considering the purpose of the medical procedure of this type of surgery and also contemporary theories in the authorization of surgery which aims to improve one’s appearance so that aesthetic plastic surgery is now legal under the law. However, the law in Islamic countries does not agree with this.

In Indonesia, from the sociological aspect, aesthetic operations to improve the quality of life and utilize science and technology are under Article 28 C paragraph (1) of the 1945 Constitution of the Republic of Indonesia based on Pancasila. Then from a philosophical point of view, the decision to perform aesthetic plastic surgery is a human right following Article 13 of the Republic of Indonesia Law No. 39 of 1999, based on considering how physical and psychological suffering that has been carried so far, it is regarded as the best treatment is aesthetic plastic surgery because it can increase self-confidence.

From the patient’s side, they assume that they are service consumers, so they want Laws of the Republic Indonesia no. 8 of 1999 concerning Consumer Protection, also applies to consumers of health services. So that they have the right to comfort, security and safety, this is reinforced by the issuance of the Decree of the Minister of Health of the Republic of Indonesia No: 756/Menkes/SK/VI/2004 concerning Preparations for Trade Liberalization and Services in the field of Health. However, medical procedures for aesthetic plastic surgery include surgery using invasive “anesthesia” where a person’s condition is “unpredictable,” while the Protection Act applies to goods and services. What is meant by goods here are inanimate objects or non-humans, and what is meant by services are services whose results can be predicted/predicted, while a person’s condition cannot be predicted.

Aesthetic plastic surgery was initially a taboo subject to be carried out, primarily if it was related to religious issues and also the legality of its implementation. But over time, many women tend to be beautiful (something that is a natural desire of every woman), especially if a woman who has problems on her face, such as burns, will need aesthetic plastic surgery to overcome these problems. So, Hamid Reza Salehi stated that the legality of implementing aesthetic plastic surgery in Indonesia has now undergone developments that have implications for aesthetic plastic surgery, which is now legal according to Indonesia’s legal construction in force.

In Indonesia, aesthetic plastic surgery, viewed from a sociological perspective, can be said to be a series of efforts to improve the community’s quality of life and utilize science and technology. The development of the quality of life and the use of information technology has a constitutional basis in Article 28 C paragraph (1) of the 1945 Constitution of the Republic of Indonesia which states, “Everyone has the right to develop themselves through the fulfillment of their basic needs, the right to education, and to benefit from science and technology, arts and culture to improve the quality of their lives and for the welfare of mankind.”

The implementation of aesthetic plastic surgery in practice does not always go according to the desired expectations. In practice, there are many cases of failure of aesthetic plastic surgery caused by several factors, which can be proven from the problems that occurred Mrs. P and dr. W in Bandung. This case is related to the actions of doctor W, who performed reconstructive and aesthetic surgery on Mrs. P’s nose. Although, for this case, dr. W was threatened with unlawful acts for the medical actions he took, fortunately in this case, and peace was carried out.

Based on the case above, the patients always assume that they are consumers of services, so they want the Law of the Republic of Indonesia no. 8 of 1999 concerning Consumer Protection and applies to consumers of health services. So that they have the right to comfort, security and safety, this is reinforced by the issuance of the Decree of the Minister of Health of the
Republic of Indonesia No: 756/Menkes/SK/VI/2004 concerning Preparations for Trade Liberalization and Services in the field of Health. However, medical procedures for aesthetic plastic surgery include surgery using invasive “anesthesia” where a person’s condition is “unpredictable,” while the Protection Act applies to goods and services. What is meant by goods here are inanimate objects or non-humans, and what is meant by services are services whose results can be predicted, while a person’s condition cannot be predicted.

Based on the problems that arise related to the implementation of aesthetic plastic surgery, the writing in this article will try to discuss and analyze the legal basis for the performance of aesthetic plastic surgery, especially the legality of the law regarding the protection of patients for all actions and practices of this aesthetic plastic surgery.

**Research Method**

This research uses normative legal research methods. That is research conducted by examining library materials. The data used is secondary data, which includes primary legal materials in the form of statutory regulations. Also, it uses secondary legal materials that explain primary legal prisoners, for example, research results and works from legal circles, as well as tertiary legal materials in the form of language dictionaries and legal dictionaries. Data collection is carried out using a literature study, namely collecting, reviewing, processing literature, legislation and articles, journals, and scientific works to support the theory. The data obtained were described, then analyzed qualitatively, namely by studying, analyzing, and interpreting the collected data. The results are described in the form of sentences.

**Observation and Results**

According to the author, in this case, what is meant by construction is an arrangement consisting of several elements into a single unit, which is the main force that can be used to build a particular thing. Thus the Construction of Legal Protection for Aesthetic Plastic Surgery Patients in this study consists of several legal elements, which are the main forces used to build legal protection for aesthetic plastic surgery patients. So that in this study, several aspects of legal protection are combined into a single unit to create a guideline that provides legal protection for aesthetic plastic surgery patients.

People, especially patients, are now increasingly aware of their rights and obligations as patients in health services. Often critically question the disease, examination, treatment, and medical action that will be carried out. Often they even seek a second opinion. In this case, the patient must be seen as a subject that significantly influences the final service outcome, not just an object. Patient rights must be fulfilled considering that patient satisfaction is one of the barometers of service quality, while patient dissatisfaction can be the basis of lawsuits.

According to the author, service quality assurance should be prioritized along with the development of medical technology and the patient’s way of thinking, especially aesthetic plastic surgery patients who demand results from a medical action. However, it must be realized that in the provision of services, health services have differences with other services, namely the existence of patient ignorance or patient ignorance, so that special skills are needed in handling medical actions. Then there is the supplier-induced demand. In this case, the aesthetic plastic surgery patient does not know what medical action is being taken and how to deal with it if things are not desirable so that the patient does not have bargaining power and choice. In addition, health care products are not a homogeneous concept, limitations on competence, uncertainty about illness due to the different conditions of each patient, and healthy conditions are human rights.

In this case, providers are dominant in meeting the needs of patients as recipients of health services. So, if the provider recommends health services or medical actions and the types of drugs to be consumed, for example: what kinds of medical activities taken and
what medications and how many doses to take and others, usually the patient will follow suit.

Thus, the Construction of Legal Protection for Aesthetic Plastic Surgery Patients, especially in Indonesia, consists of several pillars of legislation as guidelines for providing legal protection. The laws and regulations are:

1. **Civil Code.**

The term therapeutic agreement is not known in the Civil Code. Still, the elements in a therapeutic agreement can be categorized as an agreement as mentioned in Article 1313. An agreement is that one person binds himself to one or more other people. At the same time, Article 1319 of the Civil Code reads all agreements, both those that have a particular name, or those that are not known by a specific name, are subject to general rules regarding engagements in general.[17] In addition, in general, provisions regarding arrangements based on the principle of freedom of contract as regulated in Article 1338. This principle relates to the agreement’s content, namely the freedom to determine what is to be agreed upon and with whom the agreement will be carried out legally by the parties is a rule for those who make it.[18]

A valid agreement is an agreement that fulfills the conditions determined by law. According to Article 1320 of the Civil Code, if it is associated with aesthetic plastic surgery patients, the requirements for a valid agreement are:

a. In this case, there is an agreement (consensus) or will between the parties who agree, in this case, between the patient and dr. SpBP-RE. The agreement in question is an agreement between the patient and dr. SpBP-RE regarding the subject of the agreement.

b. The existence of the ability between the parties (capacity) to agree. People who carry out legal actions are adults, namely 21 (twenty-one) years old or before 21 (twenty-one) years of marriage. As stated in Article 1330 of the Civil Code, people who are incapable of making agreements are people who are not yet adults, people under guardianship, women who have husbands. They must be represented when carrying out legal actions. Most plastic surgery patients are more than 21 (twenty-one) years old because plastic surgery is usually done to increase self-confidence. Therefore it is generally done by people who have worked a lot who socialize with the community. If they have not reached the age of 21 (twenty-one) years, there must be a representative, while if a married woman and her husband are still present, it must be with the husband’s approval, meanwhile for dr. SpBP-RE is clearly more than 21 (twenty-one) years old. It is because to study to become dr. SpBP-RE takes a long time. Children start attending elementary school. Usually, they are more than 6 (six) years old, then the travel time from elementary school to dr. SpBP-RE takes 21 (twenty-one) years or more. Therefore for dr. SpBP-RE is of sufficient age.

c. The existence of a particular thing (object), which is the subject of the agreement, the object of the agreement, and the achievements must be fulfilled. An elucidation in Article 1333 of the Civil Code, what is meant by a particular thing is that the object of the agreement must be specific. Clarity of the principal agreement or entity of the agreement to enable the implementation of rights and obligations. In this case, it is aesthetic plastic surgery, not reconstructive surgery or other surgery. In this aesthetic plastic surgery, because it involves results, it includes the *resultaat verbintennis*. However, because this requires surgery using anesthesia, which is an invasive medical procedure, where the condition of different people/patients cannot be predicted, the agreement also includes *inspanning verbintennis*. [19]

d. The existence of a legal cause (causa) is the agreement’s content that describes the goals to be achieved by the parties agreeing, whether prohibited by law or not, contrary to public order and morality or not following the sound of Article 1337 of the Civil Code. For aesthetic plastic surgery, does it have a purpose as included in the prohibition stated in Article 69 paragraph (2) of the Republic of Indonesia Law Number 36 of 2009, concerning Health, which reads: reconstructive plastic
surgery must not conflict with the norms prevailing in society and not intended to change identity. Changing identity is prohibited because there is a possibility to commit a crime after it.

The first and second conditions are Article 1320 of the Civil Code, personal requirements regarding the subject of the agreement. If the agreement is not fulfilled, it can be cancelled. But if the judge does not ask for cancellation, this agreement remains binding even though it is threatened with the cancellation article, namely Article 1454 of the Civil Code. The third and fourth conditions of Article 1320 of the Civil Code are objective conditions, namely regarding the object of the agreement. If these conditions are not met, the agreement is void, that is, if one of the parties does not fulfill its obligations.[20]

In this study, in patients with aesthetic plastic surgery, the first requirement is the agreement or will of the parties, namely between the patient and dr. SpBP-RE. Initially, patients with aesthetic plastic surgery came to dr. SpBP-RE to ask for help in fulfilling their wishes, namely in the form of aesthetic plastic surgery, for example, “sharp nose (rhinoplasty).” Then dr. SpBP-RE conducts examinations and interviews, where the patient expresses her/his wishes or desires. Next dr. SpBP-RE explains everything related to the medical action carried out, from preparation, treatment during surgery and after surgery. Legally dr. SpBP-RE guarantees the patient’s authority. This is reflected in the talks/interviews and the approval that aesthetic plastic surgery patients will give. Aesthetic plastic surgery patients usually have no medical indications, so the patient concerned can think clearly. If you agree, then between dr. SpBP-RE and the patient agree to agree that the contents follow the parties’ wishes. Thus this has fulfilled the legal requirements of the agreement Article 1320 of the Civil Code.[21]

Furthermore, for the second condition, the therapeutic agreement has an exceptional nature, so that not all provisions of the Civil Code can be applied. Under Article 1 point (7), the Minister of Health of the Republic of Indonesia Number: 290/MENKES/PER/III/2008, concerning Approval of Medical Actions, it is explained that the patient concerned is an adult patient or not a child or had been married, is not physically disturbed, can communicate naturally, does not experience mental retardation and does not suffer from mental illness, can make independent decisions independently. Who came to dr. SpBP-RE is a competent patient. If the patient is not qualified, then dr. The SpBP-RE can refuse because the aesthetic plastic surgery patient described was a healthy patient with no medical indications, so that medical action could be postponed. According to Bayu Wijanarko, to anticipate this, unwritten legal provisions can be used, namely customary law, which states that someone is considered an adult if he/she is already working.

Based on the therapeutic agreement described above, the basis for medical liability is the default (Article 1234 of the Civil Code) and onrechtmatige daad as stated in Article 1365 of the Civil Code. There is a difference between the notion of default (broken promise) and onrechtmatige daad (acts against the law).

The definition of default in this study is dr. SpBP-RE did not carry out its obligations, not because of overmacht conditions. Dr.SpBP-RE who deviates in the form of breaking a promise or breach of commitment on the therapeutic agreement he made, will be subject to rules or must be held civilly responsible according to Article 1239 of the Civil Code, which essentially reads: Each engagement to do something, or not to do something, if the debtor does not fulfill his obligations, gets a settlement in the responsibility by providing costs, losses and interest. Aesthetic plastic surgery patients who feel aggrieved because they think dr. SpBP-RE does not perform its contractual obligations. It can file a claim on the grounds of default and demands material and immaterial compensation for the losses it suffers. It’s different when dr. SpBP-RE resulted in harm to the patient. It turned out to be an unlawful act as regulated in Articles 1370 and 1371 of the Civil Code, then dr.SpBP-RE is responsible for repaying the patient even though there is no contractual relationship.
According to civil law, a person can be considered to be in default if he does not do what he can do.

a) Too late to do what was promised to be done.

b) Carry out what was promised, but not as expected.

c) Do something that, according to the agreement, should not be done.

Meanwhile, the elements that can be used as the basis for filing a lawsuit against the law are as follows:

a) there is an unlawful act

b) there is a loss

c) there is a causal relationship between illegal actions and losses

d) there is an error

In the world of medicine, a doctor can be declared to have made a mistake and must pay compensation if there is a close relationship between the losses caused by the errors made by the doctor. In determining a doctor’s error, it must refer to professional standards. So that in the implementation of medical practice, acts against the law can be identified with the actions of doctors that are contrary to or not following professional standards that apply to professional development in the field of medicine.


Following Article 1 point (1) of this Law, what is meant by Medical Practice is a series of activities carried out by doctors and dentists for patients in carrying out health efforts. Then in Article 3, it is stated that the regulation of medical practice aims to provide protection to patients, maintain and improve the quality of medical services provided by doctors, also provide legal certainty to both doctors, patients, and the public. In this article, the purpose of the regulation of medical practice is apparent.

In receiving health services during the implementation of medical practice, the following articles relating to patient protection are:

a) Article 44 states that doctors and dentists carrying out medical practice must comply with medical and dental service standards.

b) Article 45 states that every medical or dental action performed by a doctor or dentist on a patient must obtain approval. The approval is given after the patient has received a complete explanation regarding matters such as the diagnosis and procedure for medical action, the purpose of the medical action taken, alternative actions. Risks and complications that may occur as well as the prognosis of the action taken. In addition, article 45 also states that consent can be given orally or in writing. Any high-risk activity must be approved in writing. As explained above, what is meant by high risk is surgery related to anesthesia because the patient’s physical condition is different and unpredictable. A Ministerial Regulation regulates provisions regarding the procedure for the approval of such medical/dental procedures.

Furthermore, according to Article 45 of the Republic of Indonesia Law Number 29 of 2004, concerning Medical Practice, Minister of Health Regulation Number 290/MenKes/Per/III/2008 and the 2008 KKI Medical Action Approval Manual, Informed Consent is approval for medical action given by the patient or the family after receiving a complete explanation of the medical action to be carried out. Meanwhile, other laws and regulations that are in line with informed consent are Article 37 of the Law of the Republic of Indonesia Number 44 of 2009, concerning Hospitals, which reads:

(1). Every medical action carried out in a hospital must obtain the consent of the patient or his family.

(2). The provisions regarding the approval of the medical action are carried out following the requirements of the legislation.

So basically, informed consent [22] explains until
the patient understands the medical action and the medical risks carried out on him. Informed Consent is more emphasized on the obligation to provide correct information. A new agreement is valid if it has been signed and agreed upon by both parties. The agreement can be cancelled if the information provided is incorrect.

Terms of valid informed consent:

2. Patient competence in giving consent.
3. Voluntary, and there is no element of compulsion.
4. The doctor explains in a language that is easy for the patient to understand.
5. The patient understands what the doctor is explaining.

Several rules must be considered in the preparation and provision of informed consent so that the law of this engagement is not legally flawed, including:

1. Not beguiling (fraud)
2. Not trying to force (force)
3. Do not create fear (fear)

From criminal law, absolute informed consent must be fulfilled not to be included in Article 351 of the Criminal Code concerning persecution. An invasive action (e.g., surgery, radiology) carried out by a medical service provider without permission from the patient. The medical service provider can be prosecuted for committing a criminal act of persecution. In Article 52, patients in receiving services in medical practice have the following rights:

- get a complete explanation of the medical action that will be carried out. In terms of aesthetic plastic surgery, the relation in this research is to explain the advantages and risks that arise as a result of the operation.
- ask another doctor’s opinion, in this case, regarding the surgery to be performed.
- get services according to medical needs, in this case. There is a possibility that the doctor will order other medical actions, which have absolutely nothing to do with the surgery to be performed by the patient, for example, rhinoplasty, the patient is asked for an x-ray, even though this is not something to do with rhinoplasty.
- refuse medical action, since aesthetic plastic surgery is performed on patients who usually do not have medical indications, then if there are things that are considered unsuitable for both the patient and dr. SpBP-RE, then one or both parties can refuse.
- get medical records, which is vital for further medical action if things are not desirable.

In Article 53, patients in receiving services in medical practice have obligations, among others:

- provide complete and honest information about their health problems. In this study, aesthetic plastic surgery patients came to dr.SpBP-RE to change specific body organs according to her wishes. What is expressed is that the desire is not the disease. However, the patient must inform the condition she has suffered (comorbid) so that medical action can be carried out with extra care.
- comply with applicable regulations in health care facilities. In this case, the patient must comply with the rules that apply in health care facilities or hospitals, namely during their stay in the hospital, both before and during post-operative care.
- comply with the doctor’s advice and instructions. These instructions are given by dr.SpBP-RE both before surgery, during surgery (if using local anesthetic), and after surgery or post-surgery. Especially in this study, according to the authors, are post-operatively, where the patient is not under intensive supervision by dr. SpBP-RE, because the patient has been discharged from the health care facility (hospital) where the aesthetic plastic surgery was performed.
- provide compensation for services received. In this case, both dr.SpBP-RE services and hospitalization costs as long as the patient stays at the health care facility where the person concerned is being treated.

This Law, apart from regulating the rights and obligations of patients, also protects users of doctor or dentist services to complain about losses due to negligence or errors on doctors or dentists in carrying out their profession to the Chairperson of the Indonesian Medical Disciplinary Board. This is regulated in Chapter VII, Article 66 number (1).


In this Law, some provisions protect patients. The article is Article 56:

(1). the patient has the right to refuse the doctor’s action after receiving and understanding the complete explanation of the information.

(2). this right does not apply if the patient suffers from an infectious disease, the patient is unconscious, or the patient is suffering from.


The Law of the Republic of Indonesia regarding Hospitals can be related to patient protection, namely in the following articles:

I. Article 29

Number (1) reads that every hospital has an obligation to:

a. provide correct information about hospital services to the community.

b. provide safe, quality, anti-discriminatory, and effective health services by prioritizing the interests of patients following hospital service standards.

c. provide emergency services to patients according to their service capabilities.

d. provide facilities and services for the poor and underprivileged.

e. carry out social functions, including service facilities for indigent patients, emergency services without asking for a down payment, free ambulances, social services in humanitarian missions.

f. maintain service quality standards in serving patients.

g. maintain medical records.

h. provide accurate, clear, honest information about the rights and obligations of patients.

i. respect and protect patient rights.

II. Article 32

Every patient has the right to:

a. obtain information about hospital rules and regulations.

b. obtain information on the patient’s rights and obligations.

c. obtain humane, fair, honest services without discrimination.

d. obtain quality health services following professional standards and standard operating procedures.

e. obtain effective and efficient services to avoid physical and material losses.

f. complain about the quality of service received.

g. choose a doctor and treatment class according to the wishes and hospital regulations.

h. ask for consultation with other doctors, both inside and outside the hospital.

i. get privacy about the confidentiality of his illness.

j. get complete information related to his health
Articles related to patient protection are:

I. Article 68:

This law relates to patient protection. It reads as follows:

(1). Every individual health service activity carried out by a health worker must obtain approval.

(2). The consent is given after getting a proper and sufficient explanation.

(3). The description shall at least include:
   a. service action procedures
   b. the purpose of the service action performed
   c. the alternative course of action
   d. possible risks and complications
   e. the prognosis for the action taken

(4). The consent is given orally or in writing.

(5). Every action of a health worker that contains a high risk must be provided with a written agreement signed by the person who has the right to consent.

(6). A Ministerial Regulation regulates the provisions of this procedure.

II. Article 77:

Every Health Service Recipient who is harmed due to the error or negligence of a Health Worker can request compensation following the provisions of the legislation. Legal protection for Health Service Recipients or other Patients in this Law is as stated in the criminal conditions on:

I. Article 84:

(1). Health workers who commit gross negligence resulting in serious injury to the Health Service Recipient shall be punished with imprisonment for a maximum of 3 (three) years.

(2). If his negligence causes death, the sentence is a
maximum of 5 (five) years.

II. Article 85:

(1). Health workers who practice not having a Registration Certificate are fined a maximum of one hundred million rupiahs.

(2). Foreign Health Workers without a Temporary Registration Certificate fined a maximum of one hundred million rupiahs.

III. Article 86:

(1). Health workers who do not have a Practice License are fined a maximum of one hundred million rupiahs.

(2). Foreign Health Workers without a Temporary Practice License are fined a maximum of one hundred million rupiahs.


The two objectives in this Law are:

1. Empower consumers in their relationship with business owners (public/private), goods, and services.

2. Develop an honest and responsible attitude of business owners.

Some parties argue that this Law is also a provision that regulates patient protection. They consider that the basis of the condition in Article 1 number (2) states: every person who uses goods and/or services available in the community, both for the benefit of themselves, their families, other people, and living creatures. The final consumer is the final consumer in the form of goods, such as medicines, food, tools, or other objects, including services provided by people, such as building construction services, Environmental Impact Analysis making services, doctor/dentist, insurance services, etc. From this understanding, patients can be categorized as consumers. It can be seen that patients are included as users of services available in the community.

In a therapeutic transaction, the engagement is *inspanning verbintenis*, meaning that it is not based on the result but is based on earnest effort. In this case, the doctor or hospital is not required to provide the patient’s desired outcome because, for the patient’s recovery, many things are influential and are factors beyond the reach of the doctor’s ability, namely: the patient’s physical condition, age, body resistance, drug quality, level of illness, patient compliance, health care facilities.

Furthermore, according to Article 1, number (3) of the Consumer Protection Act, a business actor is any individual or business entity, whether in the form of a legal entity or not a legal entity established and domiciled or conducting activities within the jurisdiction of the Republic of Indonesia that conducting business activities in various economic fields. Thus, doctors/health workers include business actors in the area of health services.

Several parties are still debating this explanation. To find out whether the profession of a doctor or health worker or health service facility is a business owner or not, several provisions of the applicable laws and regulations must be considered. Those provisions are:


“Health Worker” is any person who devotes himself to the health sector and has the knowledge and/or skills through education in the health sector which for certain types requires the authority to carry out health efforts.


“Patient” is any person who consults on his health problem to obtain the necessary health services, either directly or indirectly, from a doctor or dentist.

c. Law of the Republic of Indonesia Number 36 of 2014, concerning Health Worker.

States that a “Health Worker” is any person who devotes himself to the health sector and has the
knowledge and/or skills through education in the health sector, which for certain types requires the authority to carry out health efforts, and “Health Service Recipients” are anyone who conducts health consultations to obtain the necessary health services, either directly or indirectly, to health workers.

d. Law of the Republic of Indonesia Number 44 of 2009, concerning Hospital.

States that a “Hospital” is a health service institution that provides complete individual health services that provide outpatient, inpatient, and emergency services. Meanwhile, a “patient” is any person who consults on his health problems to obtain the necessary health services, either directly or indirectly, at the hospital.

The rights and obligations of health service providers for doctors/health workers/hospitals according to the Law of the Republic of Indonesia Number 36 of 2009, regarding health as explained earlier that their right is to obtain legal protection in carrying out their duties following their profession. At the same time, the obligation is to comply with professional standards and respect patients’ rights. Then for patients, their rights are: receive clear information, give consent, medical secrets, ask for opinions from other doctors, while their obligations are: following information and procedures instructions, having good intentions, paying agreed fees, and complying with applicable provisions in health care facilities.

Thus, in this study, the relationship between dr. SpBP-RE with Aesthetic Plastic Surgery Patients based on trust because it was the patient who came to dr. SpBP-RE, so that there is a therapeutic agreement that requires a result (resultaat verbintennis), but due to patient endurance (age, physical condition, level of medical action taken, patient compliance, quality of drugs, and available health care facilities), it is difficult to predict, doctors can only try to achieve the maximum possible results (inspanning verbintennis). Then, the relationship between Hospitals or Health Service Facilities with Aesthetic Plastic Surgery Patients follows the treatment agreement; namely, the Hospital provides treatment rooms, nurses, and dr.SpBP-RE personnel for medical action.

Furthermore, the relationship between dr. SpBP-RE with the Hospital is in providing health services. Hospitals need dr. SpBP-RE as its medical personnel, so that the Hospital is civilly responsible for all activities carried out by its health workers. This is under Article 1367 of the Civil Code, namely “That employers and those who appoint other people for their affairs are responsible for losses issued by their servants or subordinates in carrying out the work for which these people are used.” Therefore, if the patient is not satisfied because of what happened at the hospital, the patient can sue and sue and hold the hospital accountable, then the hospital chief will determine who is at fault.

e. Law of the Republic of Indonesia Number 8 of 1999 concerning Consumer Protection.

As has been explained above regarding what is called a consumer and what is called a business owner, according to the WTO (World Trade Organization) or GATS (General Agreement on Trade Services), the professions of doctors and dentists are as follows:

* Health Sector:
  - Hospital Services
  - Other Human Health Services
  - Other

* Business Services Sector:
  - Professional Services
  - Medical and Dental Services
  - Physiotherapist
  - Nurse and Midwife

The Black Law Dictionary also states that: Business (business activities in various economic fields) includes employment occupation, profession, or commercial activity engaged in/or gain or livelihood (all activities to
earn profit/livelihood).

In addition, the Decree of the Minister of Health of the Republic of Indonesia Number: 756/MENKES/SK/VI/2004 concerning Preparations for the Liberalization of Trade and Services in the Health Sector is a business. So that the position of health service providers and consumers of health services is the same: they both have rights and obligations.

Several parties, namely, have debated the things mentioned above:

1. Parties argue that the patient’s position is as a “customer of health services.” In contrast, doctors/health workers/health service facilities (hospitals) are “business actors in the field of health services,” as described above.

2. Parties who argue that the therapeutic relationship/agreement is an engagement relationship of a unique nature so that in the event of a dispute between the “provider/provider” of health services and the “recipient” of health services, each party is subject to the legal provisions governing it. Especially in the research, according to the author’s opinion, because this medical procedure has “targeted results” as stated before, the agreement includes resulataat verbintennis, while since everyone’s endurance is different, dr.SpBP-RE strives for the maximum possible results, where the agreement consists of inspanning verbintennis. Therefore, this engagement is subject to both, namely inspanning & resulataat verbintenis.

Apart from these differences of opinion, the author believes a conflict or dispute between dr. SpBP-RE/health service providers with their patients, conflict resolution, can be done in 2 (two) ways, namely: litigation method (through the judicial process) and non-litigation method (out of court). To file this lawsuit, the plaintiff must be able to prove 4 (four) criteria, namely:

- there is a duty of care, where the doctor, in this case, dr. SpBP-RE is obliged to provide the best possible care to patients as a medical contract. Dr. SpBP-RE promises to try to take the best possible medical action for the patient. Then the patient will comply with the instructions and treatment given by dr. SpBP-RE as well as possible.

- there is a breach of duty, where dr. SpBP-RE does not perform its proper obligations, while the form of the violation is an error/error in medical action such as misdiagnosis, interpretation of supporting examination results, medication errors. Meanwhile, a violation in the form of negligence is not doing things that should be done according to good medical practice standards.

- There is an injury to the patient in the form of physical, mental, to the heaviest, death.

- there is a direct causal relationship between the occurrence of violations in the form of errors/errors/omissions with physical disabilities suffered by the patient, even to death.

According to the author, since aesthetic plastic surgery patients are “healthy” or not sick patients, this medical action does not have to be done immediately. So to realize the medical activity, both parties need to think again. Dr.SpBP-RE must explain all the risks and complications that may occur, while if the patient disagrees, the operation can be cancelled.

**Conclusion**

Construction of Legal Protection for Aesthetic Plastic Surgery consists of laws and regulations relating to the health/medical field that apply in Indonesia and the laws that apply in Indonesia, namely:

1. Law Number 29 of 2004, concerning Medical Practice.

2. Law Number 36 of 2009, concerning Health.

3. Law Number 44 of 2009, concerning Hospital.

4. Law Number 36 of 2014, concerning Health Worker.

5. Civil Code: Article 1313; 1319; 1320; 1330; 1333; 1337; 1338; and 1454
To avoid things that are not desirable, make a special agreement that is complete and clear in detail, including mentioning the risks and complications that may occur. So that if one or both parties do not agree, it can be cancelled.

**Ethical Clearance : Not applicable**

**Source of Funding : Self**

**Conflict of Interest : Nil**

**References**


Prevalence and Determinants of Hypertension in Indonesia

Eny Dwimawati¹, Fitri Dian Nila Sari², Evamona Sinuraya³, Purwaningsih³

¹Researcher, Faculty of Health Sciences, Universitas Ibn Khaldun Bogor, Indonesia. ²Researcher, Nutrition Science Study Program, Nahdlatul Ulama University of North Sumatera, ³Researcher, Nursing Study Program, Akademi Keperawatan Kesdam I/Bukit Barisan Medan, Indonesia

Abstract

It is estimated that there are 15 million people with Hypertension in Indonesia, but only 4% are controlled. The prevalence of hypertension sufferers is relatively high, 7% to 22%. Prevention of Hypertension can be done by eliminating or minimizing risk factors, one of which causes it, such as reducing salt consumption, if not done, then there is a risk of Hypertension. This study aims to determine the risk factors with the behaviour of Hypertension in Puskesmas. This research is a quantitative study with a cross-sectional method. A sample of 225 respondents who visited/sought treatment at the health centre using Accidental Sampling technique. Data analysis was performed using the chi-square test. The results showed that the factors causing Hypertension were age, exercise habits, smoking habits, alcohol consumption, nutritional status, sodium intake and stress. To reduce people suffering from Hypertension, the Puskesmas should be able to provide information to the public about Hypertension and how to prevent it by doing health promotion or counselling either individually or in groups.

Keywords: Risk factors, Hypertension, prevalence and determinants

Introduction

Hypertension or better known as high blood pressure is a condition in which a person’s blood pressure is above the standard limit, where the systolic pressure is ≥ 140 mmHg. In comparison, the diastolic pressure is ≥ 90 mmHg, the causes of Hypertension are smoking, drinking alcohol, obesity, genetics, stress, excessive salt consumption, consumption of seafood, such as shrimp, fish, shellfish, and others. Symptoms that often appear in Hypertension are dizziness in circles, feeling heavy in the neck, tingling, palpitations, shortness of breath, headaches. Hypertension is not only at high risk of suffering from heart disease but also suffering from other conditions such as diseases of the nerves, kidneys, and blood vessels and the higher the blood pressure, the greater the risk.¹⁴.

Correspondence Author:
Eny Dwimawati,
Faculty of Health Sciences, Universitas Ibn Khaldun Bogor, Indonesiaeny@uika-bogor.ac.id

The increase in cases of Hypertension, especially in developing countries, is estimated to be around 80% in 2025 from a total of 639 million cases in 2019, estimated to be 1.15 billion cases in 2025. Predictions on the current number of hypertension sufferers and the recent population growth. In Indonesia, the number of hypertension sufferers is estimated at 15 million people, but only 4% are controlled. The prevalence of hypertension sufferers is relatively high, 7% to 22%. Based on the results of a survey of patients who ended up in 75% heart disease, 15% stroke, and 10% kidney failure. Research also shows the prevalence of Hypertension also increases with age. From various epidemiological studies conducted in Indonesia, it shows that 1.8% -28.6% of the population aged over 20 years are hypertensive sufferers⁵⁶.

Until now Hypertension is still a problem because there are still many hypertensive patients who have not received treatment or have been treated, but their blood pressure has not reached the target, as well as the
presence of comorbidities and complications that can increase morbidity and mortality.

Hypertension is the third leading cause of death after stroke and tuberculosis in Indonesia. Hypertension will be a severe problem because if it is not treated as early as possible, it will develop and cause dangerous complications such as heart disease, congestive heart failure, stroke, vision problems, and kidney disease.

Prevention is better than cure. Hypertension can be avoided by eliminating or minimizing the risk factors causing it. Risk factors that cannot be changed or eliminated are genetics, age, gender. In contrast, risk factors that can be changed are weight loss for those who are obese, controlling stress, reducing salt consumption, stop smoking, not drinking alcohol, doing regular exercise, limiting consumption of fat, reduce caffeine consumption.

Public knowledge and attitudes about hypertension prevention are deemed necessary because it will affect their behaviour, to prevent it is better than a cure. So you can avoid attacks of Hypertension by eliminating or minimizing the risk factors that cause it. We cannot control risk factors such as genetics, increasing age. But other risk factors can be reduced or even eliminated. If you are overweight to obesity, you can certainly try to reduce your weight to a healthy weight with diet and exercise. Smoking, drinking alcohol and caffeine habits can be reduced or even eliminated. Stress, which can cause blood pressure, can be managed in such a way that it is slightly reduced.

Material and Method

This study used a cross-sectional study and analyzed using chi-square, the population in this study were 225 people who visited the Puskesmas. The variables measured in this study were risk factors for Hypertension such as gender, age, exercise habits, smoking habits, alcohol consumption, BMI, sodium consumption and stress.

This study used a questionnaire which aims to act as a research tool to make it easier to conduct research and obtain accurate data from respondents so that it can describe the research variables which are risk factors for hypertension disease.

Findings

Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Blood Pressure</th>
<th>P-Value OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypertension</td>
<td>Normal</td>
</tr>
<tr>
<td>Sex</td>
<td>27 (24.3%)</td>
<td>84 (75.7%)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>42 (36.8%)</td>
<td>72 (63.2%)</td>
</tr>
<tr>
<td>Age</td>
<td>57 (55.9%)</td>
<td>45 (44.1%)</td>
</tr>
<tr>
<td>≥ 40 years old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 40 years old</td>
<td>12 (9.8%)</td>
<td>111 (90.2%)</td>
</tr>
</tbody>
</table>
Cont.. Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>p-value</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sports Habits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irregular</td>
<td>54 (52.9%)</td>
<td>15 (12.2%)</td>
<td>0.000</td>
<td>56.3 (10.67-230.53)</td>
</tr>
<tr>
<td>Regular</td>
<td>6 (4.5%)</td>
<td>126 (95.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking Habit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>54 (52.9%)</td>
<td>48 (47.1%)</td>
<td>0.000</td>
<td>8.1 (2.55-25.64)</td>
</tr>
<tr>
<td>No</td>
<td>15 (12.2%)</td>
<td>108 (87.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consumption of Alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15 (71.4%)</td>
<td>6 (28.6%)</td>
<td>0.048</td>
<td>7.346 (1.236-40.114)</td>
</tr>
<tr>
<td>No</td>
<td>54 (26.5%)</td>
<td>150 (73.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>60 (76.9%)</td>
<td>18 (23.1%)</td>
<td>0.001</td>
<td>51.1 (11.6-22.49)</td>
</tr>
<tr>
<td>Normal</td>
<td>9 (6.1%)</td>
<td>138 (93.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Often</td>
<td>45 (51.7%)</td>
<td>42 (48.3%)</td>
<td>0.003</td>
<td>5.098 (1.895-14.806)</td>
</tr>
<tr>
<td>No</td>
<td>24 (17.4%)</td>
<td>114 (82.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>57 (61.3%)</td>
<td>36 (38.7%)</td>
<td>0.000</td>
<td>17.45 (4.70-65.67)</td>
</tr>
<tr>
<td>No</td>
<td>12 (9.1%)</td>
<td>120 (90.9%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Gender roles are part of social roles and are not only determined by the sex of the person concerned, but by the environment and other factors. In women, blood pressure generally increases after menopause. Postmenopausal women have a higher risk of hypertension than those who have not menopause. So far, it has been concluded that hormonal and biochemical changes after menopause are the main causes of changes in blood pressure. These hormonal changes make women experience increased sensitivity to salt and weight gain. Both of these have the potential to trigger higher blood pressure3,13.

Generally, people with Hypertension are people aged over 40 years, but at this time, it is possible to suffer from young people. Most of the primary Hypertension occurs at the age of 25-45 years, and only in 20% occurs under the age of 20 years and over 50 years. This is because people at a productive age rarely pay attention to health, such as eating patterns and unhealthy lifestyles such as smoking8,14.

There was a trend toward increasing prevalence with increasing age and usually at ≥ 40 years of age. This is due to the arterial pressure that increases with age, the occurrence of aortic regurgitation, and the presence of a degenerative process, which is more frequent in old
When there is an increase in age until they reach old age, there is also an increased risk of diseases including neurological/psychiatric disorders, heart and blood vessel disorders as well as the reduced function of the five senses and metabolic disorders in the body.\textsuperscript{15,16}.

Based on the results of the statistical test of exercise habits with Hypertension, the irregular exercise proved a significant relationship with Hypertension, with \( p = 0.000; \ OR = 56.3; \ 95\% \ CI = 10.67-230.53 \). This means that people who do not exercise regularly have a risk of developing Hypertension by 56.3 times compared to people who have regular exercise habits.

People who do not regularly exercise will have an increased risk of developing hypertension compared to those who regularly exercise. Exercise is widely associated with the management of Hypertension because regular and isotonic exercise can reduce peripheral resistance which will lower blood pressure. Exercise is also associated with a role for obesity in Hypertension. Lack of exercise will increase the likelihood of obesity, and if salt intake is also increased, it will facilitate hypertension\textsuperscript{17}.

Exercise can reduce blood pressure not only due to weight loss but also how it is generated. Blood pressure is determined by two things, namely the amount of blood pumped by the heart per second and the obstacles faced by the blood in doing its work through the arteries. Exercise can lead to the growth of new capillaries and new blood vessels. Thus things that inhibit blood flow can be avoided or reduced, which means lowering blood pressure. Although the ability of the heart to do its job is increased through exercise, the effect of this reduction in resistance results in a significant reduction in blood pressure\textsuperscript{17–19}.

Lack of physical activity increases the risk of suffering from Hypertension because it increases the risk of being overweight. Inactive people also tend to have a higher heart rate, so the heart muscle has to work harder with each contraction. The harder and often the heart muscle has to pump, and the more pressure is placed on the arteries\textsuperscript{18,19}.

Based on the results of statistical tests between smoking habits and blood pressure, it was found that there was a significant relationship between smoking habits and blood pressure \( p = 0.000 \). The relationship between smoking and Hypertension is not clear. According to literature, nicotine and carbon dioxide contained in cigarettes will damage the endothelial lining of arteries, reduce the elasticity of blood vessels, causing blood pressure to rise. This mechanism explains why respondents who smoke every day have a risk of suffering from hypertension\textsuperscript{12,20}.

Smoking behaviour is an act that has no positive value in all respects, especially in health. Smoking is the beginning that brings various types of deadly degenerative diseases, such as cancer and heart disease. The nicotine in tobacco is the cause of the increase in blood pressure immediately after the first inhale. Like other chemicals in cigarette smoke, nicotine is absorbed by the tiny blood vessels in the lungs and circulated into the bloodstream. In just a few seconds, the nicotine has reached the brain. The brain reacts to nicotine by signalling the adrenal glands to release epinephrine (adrenaline). This powerful hormone constricts blood vessels and forces the heart to work harder due to higher pressure. By smoking, a cigarette will have a big effect on increasing blood pressure. This is because cigarette smoke contains approximately 4000 chemicals, 200 of which are poisonous, and 43 other types can cause cancer in the body\textsuperscript{21–23}.

In the alcohol consumption variable, respondents who consumed alcohol and were exposed to Hypertension were 71.4\%, and those who did not consume alcohol were 26.5\%. The results of statistical tests stated that there was a significant relationship \( p = 0.048 \).

Some theories support this statement, among others, people who drink alcohol too often or who drink too much have a higher blood pressure than individuals who do not drink or drink little. Blood pressure due to alcohol is unclear. However, it is suspected that an increase in cortisol levels and an increase in red blood cell volume and blood viscosity play a role in raising blood pressure.
Several studies have shown a direct relationship between blood pressure and alcohol intake and among them report that the effect on blood pressure is only seen when consuming 2-3 glasses of standard size alcohol per day; 22,24,25.

Overweight (obesity) is a characteristic of the hypertensive population, and it is proven that this factor is closely related to the occurrence of Hypertension in the future. To find out if someone is overweight or not, it can be seen from the calculation Body Mass Index (BMI). The relationship between blood pressure and body weight was more significant for systolic pressure than for diastolic pressure; 2,26.

The results showed that there was a significant relationship between BMI and Hypertension (p<0.05). One of the controllable risk factors for Hypertension is obesity. The risk of Hypertension in someone who is obese is 2 to 6 times higher than someone with normal weight. Based on the results of the study, it is known that there are 76.9% of hypertensive respondents who have a BMI which shows more nutrition (obesity) and 6.1% who have a BMI which shows no more or normal nutrition. This study shows the relationship between body weight and Hypertension. If the body weight increases above the ideal body weight, the risk of Hypertension also increases.

When body weight decreases, total blood volume also decreases, the hormones associated with blood pressure change, and blood pressure decreases; 26. Weight loss will result in a decrease in blood pressure. An experiment shows that a 1% reduction in body weight will result in a decrease of 1 mmHg for systolic pressure and two mmHg for diastolic pressure; 2,26.

The results of the analysis showed that the incidence of Hypertension was more prevalent in respondents who had frequent sodium intake (51.7%) than respondents whose sodium intake was not frequent (17.4%). From the statistical test, it is known that there is a significant relationship between sodium intake and blood pressure with a value of p = 0.000. People who frequently consume foods high in sodium have a greater number of cases of Hypertension who do not frequently consume foods high in sodium.

Several studies have shown that an average decrease in sodium intake by ± 1.8 grams/day can reduce the systolic blood pressure of 4 mmHg and diastolic 2 mmHg in hypertensive patients. Another supporting theory is that a high-salt diet can pump harder to push the increased blood volume through narrower spaces which in turn causes blood pressure to rise; 27,28.

High sodium intake (≥ 2,300 mg), when supported by decreased renal adaptation, can cause sodium retention in the kidneys and also reduce potassium stored in the body. This results in a volume displacement of the extracellular fluid resulting in the release of factors associated with Na⁺ / K⁺. Excess sodium and potassium deficiency in cells result in contraction of blood vessel cells.

The existence of contraction of blood vessels increases peripheral vascular resistance which results in Hypertension. High sodium intake can cause an increase in plasma volume, cardiac output and blood pressure. Sodium causes the body to retain water at levels exceeding the body’s normal threshold, which can increase blood volume and high blood pressure. High sodium intake causes adipocyte cell hypertrophy due to lipogenic processes in white fat tissue. If it continues, it will cause narrowing of the blood vessels by fat and increase blood pressure. Besides, individuals who are overweight and obese are more likely to have salt sensitivity which affects blood pressure; 27,28.

The effect of stress is also still controversial, and the effect is thought to be through sympathetic nerve activity which can increase blood pressure as a physical reaction when someone experiences a threat (fight or flight response). One of the causes of increased blood pressure in hypertensive patients is stress. Stress is an unpleasant physical and psychological stress. Stress can stimulate the child’s kidney glands to release adrenaline and stimulate the heart to beat faster and stronger, so that blood pressure will increase. If it occurs for a long time, it will be dangerous for people who already
suffer from Hypertension, causing complications. These complications can attack various target organs of the body, namely the brain, eyes, heart, arteries, and kidneys. As a result of the complications of Hypertension, the patient’s quality of life is low and the worst possibility is the death of the patient due to complications of hypertension. 29–31.

**Conflicts of Interest:** All authors have no conflicts of interest to declare.

**Source of Funding:** The source of this research costs from self.

**Ethical Clearance:** The study was approved by the institutional Ethical Board of The Ibn Khaldun University.

All subjects were fully informed about the procedures and objectives of this study each subject before the course signed an informed consent form.

**References**

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Correlation between Gestational Weight Gain in the Second and Third Trimester and Preeclampsia Risk: A Study From Indonesia

Ernawati Ernawati\(^1,2\), Lilis Kurniawati\(^3\), Sri Umijati\(^4\)

\(^1\)Department of Obstetrics & Gynaecology, Faculty of Medicine, Universitas Airlangga/ Soetomo Teaching Hospital, Indonesia, \(^2\)Department of Obstetrics & Gynaecology, Airlangga University Academic Hospital, Indonesia, \(^3\)School of Midwifery, Faculty of Medicine, Universitas Airlangga, Indonesia, \(^4\)Department of Public Health Sciences and Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Indonesia

Abstract

Introduction: Many studies have proposed that pre-pregnancy body mass index and higher gestational weight gain (GWG) during pregnancy are risk factors for preeclampsia incidence. However, most of those studies give attention to total GWG during pregnancy to the risk of preeclampsia, not in a specific trimester.

Aim: To examine whether abnormal gestational weight gain in the second and third trimester correlated with the incidence of preeclampsia.

Methods: This cross-sectional study was conducted in a public health centre, in Surabaya, Indonesia, between October 2018 and October 2019. The samples were 63 pregnant women in the second and third trimester. The variable observed was gestational weight gain (GWG) each week during the second and third trimester compared to their pre-pregnancy body mass index (BMI) using the Institute of Medicine (IOM) standards and preeclampsia incidence. Statistical analysis used was Fisher’s Exact Test and Chi-Square Test with a significance level of 5%.

Results: There were 11 and 52 samples in the second and third trimester, respectively. Abnormal weight gain was recorded in 54% of samples in the second trimester and 57% of the preeclampsia third-trimester samples. Moreover, preeclampsia was diagnosed in 83.3% and 60% samples with abnormal weight gain in the second trimester and third trimester. Statistical analysis showed abnormal weight gain in the second and third trimester related to preeclampsia with P-values 0.015 and 0.0001.

Conclusion: Abnormal gestational weight gain in the second and third trimester was correlated to preeclampsia.

Keywords: preeclampsia, abnormal, gestational weight gain

Introduction

Preeclampsia and other pregnancy hypertensive disorders are still significant problems in maternal health services. It can affect 10% of pregnancies and cause maternal morbidity and mortality in the world\(^1,2\). This condition also occurs in Indonesia; preeclampsia/eclampsia cases are still the most significant cause of maternal death and tend to increase from year to year. This can have long-term adverse effects on the health of the mother and the outcome of pregnancy\(^3\).
Many studies have shown that body mass index (BMI) before pregnancy, and a tremendous increase in GWG during pregnancy are risk factors for preeclampsia\(^4,5\). Excessive gestational weight gain (GWG) is associated with increased maternal and neonatal complications, including hypertensive disorders of pregnancy, fetal macrosomia, and increased cesarean delivery rate\(^6,7\).

However, most of the existing studies only looked at total GWG during pregnancy with preeclampsia risk. These results are broad bias because pregnant women with preeclampsia are more likely to develop oedema during pregnancy than normal pregnant women. Of course, this will have an impact on a very significant increase in GWG. Other studies suggest that the correct management of GWG that occurs in early pregnancy can reduce the risk of developing hypertension in pregnancy\(^6\).

Effective treatment remains a considerable challenge\(^8\). GWG management in the second and third trimester of pregnancy is a potential target for interventions to reduce preeclampsia risk. Alternatives that can be taken are to manage in the field of prevention\(^9,10\). Based on the above background, this study aims to determine whether abnormal pregnancy weight gain in the second and third trimesters is associated with preeclampsia incidence. This study’s results are expected to be a reference for improving the quality of maternal health services.

**Methods**

This observational analytical study was done using a cross-sectional approach on 63 pregnant women in the second and third trimester in a public health centre, Surabaya using medical records, the study was conducted between October 2018 and October 2019. The Human Research and Ethics Committee approved the trial for Basic Science and Clinical Research Dr Soetomo Academic Medical centre Hospital, Faculty of Medicine Universitas Airlangga Surabaya.

The sample consisted of all pregnant women in the 2nd and third trimesters who had antenatal care at Public Health Center Surabaya with several inclusion criteria, such as (1) 20 weeks gestational age or above; (2) had an antenatal care at least two times in the 2nd trimester; (3) had an antenatal care at least two times in the 3rd trimester; and (4) there is a record of prepregnancy body mass index (BMI). Multiple pregnancies were excluded from this study. Simple random sampling was applied to the sampling technique.

The variables measured were gestational weight gain in the 2nd and third trimester and the incidence of preeclampsia. The diagnosis of preeclampsia was following the 2014 ISSHP criteria that (1) the blood pressure is by 140/90 mmHg or more and (2) there are one or more organ disorders that previously did not exist but then appeared in or after 20 weeks of pregnancy\(^11\). The increase of body weight was assessed by calculating the average weight gain in one month reported in the medical record when the respondents performed an antenatal care (ANC) examination, then compared to the weight gain based on The Institute of Medicine (IOM) recommendation and prepregnancy BMI\(^9\). The recommended weight gain for pregnant women in the second and third trimester, which is underweight, normal, overweight, and obese BMI, was 0.44-0.58; 0.35-0.50; 0.23-0.3; and 0.17-0.27 kilograms per week.

Statistical analyses were carried out using the Fisher’s Exact Test and Chi-Square Test with a significance level of 5% of the SPSS program. Abnormal GWG was determined to be abnormal, increasing gestational weight gain outside of the IOM recommendation was deficient or excessive weight gain.

**Results**

Out of the 63 respondents, 17.5% were in the 2nd trimester and 82.5% in the 3rd trimester. Forty-five percent of respondents were diagnosed with preeclampsia in the 2nd trimester while 38.5% were diagnosed in the 3rd trimester.

**Characteristics of Respondents**

Characteristics of pregnant women in this study are presented in Table 1. Women aged over 35 years,
Nulliparous women and pre-pregnancy BMI >30 (obese) were more likely to develop HDP.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Preeclampsia Status</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20 years-old</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>20-35 years-old</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>&gt; 35 years-old</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
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<td></td>
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<tr>
<td>Primiparous</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Multiparous</td>
<td>17</td>
<td>29</td>
</tr>
<tr>
<td><strong>Pre pregnancy BMI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Normal</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Overweight</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Obese</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td><strong>Gestational Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤34 weeks</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>&gt;34 weeks</td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>

*) BMI: Body mass Index, Underweight (<18,5 kg/m²), Normal (18,5-24,9 kg/m²), overweight (25,0-29,9 kg/m²), Obese (>30 kg/m²)

The table below presents that out of a total of 63 respondents, 11 respondents were in the 2nd trimester; 83.3% of them had abnormal weight gain and experienced preeclampsia (p = 0.015, CC = 0.64).

**Second Trimester Weight Gain and Preeclampsia Incidence**
Table 2. Second Trimester Weight Gain and Preeclampsia Incidence

<table>
<thead>
<tr>
<th>GWG at Second Trimester</th>
<th>Preeclampsia</th>
<th>Total</th>
<th>P value*</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td>5  83.3</td>
<td>1  16.7</td>
</tr>
</tbody>
</table>
| GWG: Gestational Weight Gain, CC: Correlation Coefficient
*Fisher exact test (p<0.05)

Third Trimester Weight Gain and Preeclampsia Incidence

Likewise, 60% of respondents in the 3rd trimester with abnormal weight gain (deficient and excessive) had preeclampsia (p-value = 0.0001, CC 0.45).

<table>
<thead>
<tr>
<th>GWG at Third Trimester</th>
<th>Preeclampsia</th>
<th>Total</th>
<th>P value*</th>
<th>CC*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
<td>18 60</td>
<td>12  40</td>
</tr>
<tr>
<td>Normal</td>
<td>2  9.1</td>
<td>20 90.9</td>
<td>22 100</td>
<td>0,0001</td>
</tr>
</tbody>
</table>

GWG: Gestational Weight Gain, CC: Correlation Coefficient
* Chi Square test (P <0.05)

Discussion

This study proves that one of the risk factors for preeclampsia is maternal age, where at the age of more than 35 years, there have been changes in tissues and reproductive organs. Previous studies have shown that preeclampsia is more common in women of advanced maternal age. Furthermore, it is also an independent risk factor for adverse outcomes in first-time mothers with preeclampsia\(^1,5\). Sixty-nine percent of respondents who experienced obesity before pregnancy were known to have preeclampsia. Obesity is a risk factor that has been widely investigated for the occurrence of preeclampsia\(^12,13\). Systematically reviewed by Ren et al., it also mentioned that pregnant women who had an excessive BMI before pregnancy had a higher risk factor in getting preeclampsia, high BMI before pregnancy had twice the risk of preeclampsia\(^9\). Excess fat in obese pregnant women increases oxidative stress, stimulating an inflammatory response and accelerating endothelial vascular damage resulting in preeclampsia manifestations. In other words, high BMI before pregnancy can increase oxidative stress, which then will stimulate the inflammatory response and accelerate endothelial damage\(^1,14\).
A previous study recorded GWG could change throughout pregnancy and challenge separate weight gain due to oedema or weight gain due to rising adiposity. Oedema is the normal picture in normotensive pregnancies, but it is more often in preeclampsia, which might have an impact in higher weight gain during pregnancy and is noted as overestimation of excessive GWG on Preeclampsia. So it is hard to see whether increased oedema in patients with preeclampsia causes a higher weight gain or whether higher weight gain due to preeclampsia15. However, patients with gestational hypertension should have less chance to have oedema because there is no protein urine on gestational hypertension, so the weight gain is more likely adiposity16.

Another study on increasing weight gain in early pregnancy stated that oedema is rare to occur in early pregnancy, so excessive weight gain at this stage is correlated with oedema. Thus it suggests that GWG predicts the development of preeclampsia. However, research on weight gain in early pregnancy and the risk of preeclampsia is sparse. Only one study from Macdonald-Wallis C et al. examined weight gain during early pregnancy and proved that GWG in early pregnancy is a risk factor for preeclampsia and gestational hypertension6. They recorded that excessive weight gain during the first 18 weeks of pregnancy was associated with risks of developing preeclampsia and gestational hypertension. More study is needed to prove the relationship between weight gain in early pregnancy and preeclampsia risk10.

Our study proved that abnormal weight gain, excessive and deficient weight gain, increases risk factors for developing preeclampsia in the second or third trimester. Many studies, as discussed above, show that excessive weight gain correlates with preeclampsia. Endothelial activation is the impact of various stimuli are oxidative stress and inflammatory mediators. Elevated lipids, mostly fatty acids, may have a direct effect on endothelial function. These could have a beneficial or detrimental effect. Failure to push non-esterified fatty acids into insulin resistance will reduce endothelium-mediated vasodilatation12.

However, only a few studies discussed the effect of short weight gain on risk of preeclampsia occurrence. One of the old journals in 1976, Davies et al., stated that undernutrition was beginning to be considered more critical than over-nutrition; they published a study of 180 preeclampsia women in Jerusalem using dietary recall to get a diet assessment. The preeclampsia diagnosis included patients with hypertension and proteinuria or oedema. They compared 180 preeclampsia women with 360 concurrent normal women. Lower intake of energy, fats and protein was shown in preeclampsia women than in control women. However, further investigation indicated that it was due to illness, so proposed it would be secondary to the disease rather than causal17. Another study by Clausen et al., examined 3771 Norwegian women on dietary intake using food questionnaires in 17–19 weeks gestation. This study showed that higher energy intake in preeclampsia women and early-onset preeclampsia was the highest18.

Our study results proved that excessive weight gain would increase the risk of preeclampsia occurrence and deficient weight gain and increase preeclampsia risk. This is supported by Clausen and Davies studies. Even though it is challenging to conclude from these studies, increasing energy and carbohydrate intake observed in women who later develop preeclampsia may be due to population differences. Another study showed that low protein intake is correlated with the risk of preeclampsia19, but no studies indicated that lower protein intake increase risk to develop preeclampsia. Trials of protein supplementation recorded no preeclampsia incidence reduction20.

**Conclusion**

Gestational Weight Gain (GWG) is a critical indicator in monitoring pregnant women’s nutritional health. Deficiency or excessive increase can be abnormal and may cause some complications to arise in pregnancy. This study showed that abnormal gestational weight gain (GWG) in the second and third trimester was associated with preeclampsia. GWG management in the second
and third trimester of pregnancy is a potential target for interventions to reduce preeclampsia risk.

**Conflict of Interest:** The authors declared there were no competing interests in the study.

**Ethical Approval:** This research was declared an ethical pass test by the Ethics Committee of Dr. Soetomo General Hospital, Surabaya East Java Indonesia.

**Acknowledgements:** We would like to thank the all staff in a public health center in Surabaya for their support and coordination during data collection.

**Funding:** This study was funded by the authors.

**Availability of Data and Material:** Data will be available on request.

**References**


The Impact of Extra-Weighted Exercises in Improving Physical Abilities Towards Accurate Ace Shots in Volleyball Game

Ethar Hamdi Abdulrahman¹, Adnan Fad’us Omar²

¹PhD Student, The Ministry of Education – General Directorate of Anbar Education, Iraq, ²Professor, College of Sport and Physical Education Sciences, University Of Anbar, Iraq

Abstract

This study aimed to prepare special exercises to develop some of the physical and skill aspects of volleyball players, and to pay attention to the use of additional weights and thus stand on one of the main and important aspects and its money in the role of preparing players in accordance with the requirements of the game and to identify the effect of these exercises using different resistances in some physical abilities and my skills Serving and crushing volleyball players, and the research sample consisted of (12) players in the specialized training center for volleyball aged (15-17) years and in a deliberate manner for the academic year (2019-2020), and the researchers concluded that the training curriculum has a positive effect on developing physical abilities And skill, which indicates the impact of the training curriculum prepared by the researchers in developing these abilities. As the research objectives are achieved, the researchers recommended making use of the prepared training curriculum in building similar approaches to develop some physical and skillful abilities of volleyball players.

Keywords: Volleyball, physical abilities, Fitness, extra-weighted.

Introduction

Proper planning based on solid scientific foundations and principles in the field of training and the use of various tools and resistances leads to rapid development in various games and sporting events, whether individual or collective, so the specialists in the field of training have paid attention to the physical aspects and focus on special approaches that seek to develop them. Physical preparation is one of the most important pillars on which the trainer relies to reach the individual to the optimal performance of sports activity, as it aims to develop the capabilities and improve the level of his physical capabilities to meet the requirements of progress in the methods of practice for sporting activities until the individual reaches the level required to perform in competition to achieve the goal.

Fitness is one of the important and basic elements for volleyball players, as the better the player’s level of physical fitness, the greater his ability to improve his skill and planning level, and the poor physical fitness of volleyball players leads to a weakening of the skill performance of volleyball skills depending on physical fitness. As for how can a player implement offensive skills unless he has the strength in the legs to jump up¹.

Researchers reviewed the training curriculum developed by the school coaches. Notice that the coach does not pay much attention to the use of different resistances, thus this is reflected in some physical characteristics and abilities. The physical aspect of performance has a great influence in raising the level of the players’ skillful performance. This was shown in the intermediate test results for special physical and skill aspects. To address the problem. First preparing exercises using different external resistances for the players of the specialized volleyball training center. Second identify the effect of exercises using different resistances on some physical abilities and the accuracy
of the crushing skill performance of the Volleyball Training Center players.

The importance of this paper lies in preparing special exercises to develop some of the physical and skill aspects of the players of the Specialized Volleyball Training Center and paying attention to the use of additional weights. Thus standing on one of the basic and important aspects and its role in preparing the players in accordance with the requirements of the game, the player who is well-prepared physically and skillfully is in a good condition to perform and implement technical skills and economy in an effort, and then the achievement is towards the best.

**Areas of Research:**

1. The human field: the players of the Specialized Volleyball Training Center-aged (15-17) years in the district of Hit - Anbar province.


3. The place field: Hit middle school for boys.

**Methodology**

The researchers used the experimental method and the experimental design method for one group with two pre and post-tests.

**The Research Sample:**

The researchers selected the research sample from the players of the Specialized Volleyball Training Center at the ages of (15-17) years, according to the deliberate method for the academic year (2019-2020). The research sample included (12) players, and (Libero) players were excluded from the research sample, as the research sample became (10). Thus, the percentage of the sample represented (83.33%) of the research community.

**Devices:**

Electronic Stopwatch - Video Camera - Digital Electronic Calculator - Pulse Meter.

**Tools:**

Volleyball arena - ball holder - volleyballs - tape measure (30) m - extra weights - colored adhesive tape - various medical balls (2-3-4) kg - chalk - whistle - different height surfaces - different height barriers (30-40-50) cm.

**Means of gathering Information:**

Arab and foreign sources - tests and measurements - questionnaire and data collection form - exploratory experience - the Internet.

**Procedures:**

Determine the research variables (physical and skill)

Determining the research variables is one of the important and basic matters in the subject of the research, which is to be developed for the players of the specialized volleyball training center, as the physical and skill variables were determined by the researchers and their reliance on previous studies in this aspect. And on Tuesday, 6/8/2019, as well as their reliance on the research problem that was extracted as a result of the researchers observing the tracer (intermediate) tests performed by the trainers of the training center.

**Table (1) Shows the research variables that have been identified**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ability</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical aspects</td>
<td>Distinctive power as speedily of arms</td>
<td></td>
</tr>
<tr>
<td>of selected</td>
<td>The explosive power of the arms</td>
<td></td>
</tr>
<tr>
<td>Artistic skills</td>
<td>Beating overwhelming</td>
<td></td>
</tr>
</tbody>
</table>

**Tests Used:**

After determining the research variables by the two researchers, the following tests were used:
A- the test of the force of characteristic velocity of the muscles of the arms. 

Title of the test: - Flexing and extending the arms continuously for a period of (10) seconds.

The purpose of the test: - To measure the velocity force of the muscles of the arms.

Used tools: - Electronic stopwatch.

Description of the performance: - The laboratory takes the front support position on the ground so that the body is in an upright position. At the start signal, the tester bends and extends the arms completely and continues to repeat the performance to the largest number of iterations without stopping for a period of (10) seconds.

The conditions: - Are not allowed to stop. To note the straightness of the laboratory body during performance. The necessity of touching the chest to the ground when performing. Notice the bending and extension of the arms completely.

Scoring method: The laboratory score is the number of correct repetitions during a period of (10) seconds, as shown in the following figure:

B- Second: Throwing a medical ball weighing (3 kg) with two hands.

The purpose of the test: to measure the explosive force of the muscles of the arms and shoulders.

Devices and tools: medicine ball weighing (2 kg), chair, trunk fixation belt, tape measure, flat space area, airtight.

Performance specifications: The laboratory sits on the chair and the medical ball is carried by hands over the head, with the torso adjacent to the edge of the chair.

The conditions: The tester is given three attempts to score the best. When the chair vibrates or moves during the performance, the result is not counted.

Scoring method: The distance between the front edge of the chair and the nearest point the ball places on the ground is calculated, as shown in Figure (2).

C- Third: Sargent’s vertical jump test of stability.

The aim of the test: to measure the muscular capacity of the two men.

Devices and tools used: a wall height (3.50 m), a tape measure, and a blackboard fixed to a wall of (0.5 m) width and length (1.50 m), on which lines are drawn in white and the distance from one line to another is (2 cm), and pieces of chalk and pieces of cloth are also used. To wipe the blackboard after reading every attempt made by the laboratory, the blackboard can also be used so that it is fixed on the wall and its lower edge is higher than the ground (1.50 m), and the board can be movable and fixed according to the length of the laboratory with the arm, and then the laboratory is jumped.

How to perform the test: The tester holds a piece of chalk and stands facing the blackboard. Knees and push the feet together to jump up with the arms swinging strongly forward and up.

Test conditions: The upward jump is done with the feet together from a steady position and not by taking a step. Measurements should be taken to the nearest centimeter. Do not extend the piece of chalk outside the fingers of the hand.

Scoring method: The recording is made by the number of centimeters reached by the laboratory from the standing position. The distance between the first mark and the second mark is calculated for the amount
of muscle capacity. See figure 3.

**Figure 3: Shown the explosive force test of the legs.**

- **Fourth:** the crushing multiplication test 1.

   The purpose of the test: to measure the accuracy of the crushing attack in the inner triangle of the opponent’s court.

   Tools: five volleyballs, dividing the playing field as shown in Figure (4). So that divides the pitch into two triangles (half of the pitch opposite) and then divides the inner triangle (grid point) into three regions each display area (3 m).

   Performance specifications: After preparation, the tester makes a crushing attack towards the inner triangle of the network side.

   The conditions: Each laboratory has five attempts. Good preparation must be done in each attempt.

   Scores are calculated according to where the ball fell as follows: (In the first region (3) degrees - In the second region (1) a degree - In the third region (5) degrees - Outside these areas the laboratory gets a (zero)).

   Scoring: The laboratory records the scores he obtained in the five attempts, meaning that the final score for this test is (25) marks.

**Exploratory Experience**

The two researchers conducted the pilot experiment on a sample of (4) players on Thursday, 5/9/2019 at 05:00 PM. And they were randomly selected by drawing lots from the research sample. The researchers were keen to fulfill the same conditions and circumstances in which the pre-tests are possible, and the aim of them was the following:

1. Verify suitability and ease of application of tests used for the sample.
2. Verify the safety of devices and tools used in the research.
3. Knowing the time required to carry out the tests and the suitability of the place.

**Pre-Examinations (Physical and Skill):**

The two researchers applied the pre-tests of the variables under study for the research sample on Saturday (14/9/2019) at 05:00 PM. The two researchers took care as much as possible to control the variables in terms of time, place, and auxiliary work team.

**Main Research Experience:**

Exercises have been used to develop the physical capabilities of the arms and legs. And the skills of serving and spiking. These exercises were aimed at:

1. Trying to develop physical capabilities.
2. Development of the skills side of the research sample.
3. Trying to reach the player to a high training state in terms of physical and skill.
4. Achieve the goals that have been set through the use of exercises.

Two researchers developed special exercises using extra weights to develop some physical aspects and skills of serving and spoiling with volleyball.

The exercises were applied on Thursday 19/9/2019.
until Thursday 15/11/2019 on the research sample (Specialized Volleyball Training Center players). The exercises were applied by the center’s coaches.

Researchers used the circular method in carrying out the exercises with the intensity of interval training. The researchers used the additional weights that were determined according to the relative weights of each player in the implementation of the selected exercises, and the use of the fixed ball carrier device. Exercises were carried out circularly in the form of the stations of each station in which a different exercise was carried out. The choice of exercises in the one training unit were exercises of close and equal time to carry out exercises in all stations at one time because of the presence of another player in the station. It also helps to control the time to move from one station to another. The difference is in the number of repetitions or the distance over which the selected exercise is carried out in one training unit. The components of the exercise load for the exercises used are stability in intensity, comfort, and change in exercise volume.

* The curriculum included (24) training units with (3) training units per week on Saturdays, Tuesdays, and Thursdays, and the application of the used curriculum took (8) weeks for special preparation.

* The two researchers used the calculation of the total intensity of the training units that were contained in the following equivalent exercises.

**Dimensional tests:**

The two researchers applied the dimensional tests for the research sample on Thursday (28-11-2019). The researchers took care as much as possible to control the variables.

**Statistical means:**

The statistical data were processed by using the (SPSS) program to process the results.

Table (2) shows the arithmetic mean, standard deviations, and the two calculated and tabular (T) values of the research variables.

<table>
<thead>
<tr>
<th>Variables</th>
<th>measuring unit</th>
<th>Pre-tests</th>
<th>Posteriori tests</th>
<th>Value (T) calculated</th>
<th>The value of arithmetic mean</th>
<th>Indication of differences</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The power of the arms with speed</strong></td>
<td>Repetition</td>
<td>8.25</td>
<td>12.8</td>
<td>11.12</td>
<td>1.45</td>
<td>9.48</td>
</tr>
<tr>
<td><strong>The explosive power of the arms</strong></td>
<td>M</td>
<td>4.44</td>
<td>0.94</td>
<td>5.26</td>
<td>0.82</td>
<td>7.06</td>
</tr>
<tr>
<td><strong>The explosive power of the legs</strong></td>
<td>M</td>
<td>48.75</td>
<td>9.69</td>
<td>53.62</td>
<td>27.14</td>
<td>9.56</td>
</tr>
<tr>
<td><strong>Crushing beating</strong></td>
<td>Degree</td>
<td>10.5</td>
<td>5.92</td>
<td>17.37</td>
<td>2.38</td>
<td>6.93</td>
</tr>
</tbody>
</table>
Discussion

So, the table showed (the results of the search sample in the variables of physical abilities for the pre-tests, the arithmetic means were respectively (8.25, 4.44, 48.75, and 10.5) and standard deviations (1.28, 0.94, 9.69, and 5.92). Where the dimensional arithmetic mean (11.12, 5.26, 53.62, 17.37) and standard deviations (1.45, 0.82, 27.14, 2.38) and by extracting the value of (T) calculated of (9.48, 7.06, 9.56, 6.93), and It was greater than the tabular (T) value of (2.26) and at (9) temperature and with an error level (0.05). This indicates that there is a significant difference between the pre and post-tests.

There is an evolution in all physical abilities understudy in the post-tests when compared with the results of the pre-tests of the second experimental group. The researchers attribute the effectiveness of the exercises used in the program, which used times, to the development of the characteristic strength, speed, and muscle strength. The development of muscle strength as well as the raising of the degree of neuromuscular compatibility.

(Hussein and Ahmad) stated, “Weight training has a fast and effective effect on the growth of muscle strength on the one hand and the increase in muscle size on the other hand” 2. And (Hammad) states, “The use of weights aims to increase the intensity of exercise performance and thus decrease the volume and increase the positive comfort, but it remains incomplete. Also, one of its main goals is to develop the characteristic of strength distinguished by speed” 3. In the explosive force variable of arms and legs to the effectiveness of interval training using strength exercises represented in jumping and throwing exercises, using additional weights, and the use of medical balls in the throwing exercise, the effect on developing this trait. “Exercises in which resistance is used are an appropriate means for developing the components of explosive force. 4. As strength training increases the muscle’s ability to excite the largest possible number of fibers, as (Allawi and Abdel Fattah) mentioned that “the more muscle fibers participate, the more muscle fibers are involved in, the greater the strength that the muscle can produce” 5.

The development in the post-test was due to the effectiveness of exercises used with additional weights, according to the researchers. This led to the development of strength characteristic of speed for the muscles of the arms and legs. And speed is possible with accuracy in directing the ball to the opponent’s court, as (Wadih) states, “The development of special kinetic abilities or characteristics enables the athlete to perform the kinetic performance of the skill in the best possible way” 1, and that the volleyball player is constantly moving on the field, whether he is in Defensive or offensive condition. Thus, in addition to possessing the main physical qualities, he needs compatibility when performing skills. Thus, when performing the serving skill, he needs neuromuscular compatibility, as well as accuracy when hitting the ball, and this is in agreement with (Abdul Khaleq). Special mobility “2).

Conclusions

The training curriculum has a positive effect on developing physical and skill abilities, which indicates the effect of the training curriculum prepared by the researchers on developing these capabilities. Benefiting from the training curriculum prepared by the researchers in building similar curricula to develop some physical and skillful abilities of volleyball players. Generalizing the proposed training curriculum in training volleyball juniors. The need for coaches to pay attention to the tests adopted by the researcher to measure physical abilities and spiking skills.

Source of Funding: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: The scientific research ethics committee at Al-Anbar university has approved the research by letter No. 19 date 29-09-2019. All experiments were conducted at a specialized center at Hit city.

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Correlation of HIF-1α, CXCR4 and MMP13 Expression in Laryngeal Squamous Cell Carcinoma with Cervical Nodal Status

Etty Hary Kusumastuti1,4, Anny Setijo Rahaju1,4, Alphania Rahniayu1,4, Sjahjenny Mustokoweni1,4, Rovi Anggoro1, Muhtarum Yusuf2,4, I Ketut Sudiana3

1Researcher, Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, 2Associate Professor, Department of Otolaryngology-Head and Neck Surgery, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, 3Professor, Department of Anatomical Pathology, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia, 4Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

Abstract

Background: Laryngeal carcinoma is the most common malignancy of the upper respiratory tract, more than 98% are squamous cell carcinoma (SCC). New method is necessary for identifying and predicting nodal metastasis in laryngeal SCC. Overproliferating tumor cells will induce hypoxia and release HIF-1α, which in turn will upregulate CXCR4. CXCR4 then will induce MMP13, a protein that degrade extracellular matrix (ECM), thus promoting metastatic process.

Methods: A cross sectional study, using 30 samples of laryngeal SCC, divided into two groups: based on cervical lymph node status. All samples were stained immunohistochemically against HIF-1α, CXCR4 and MMP13 antibody. The expressions were evaluated using immunoreactive score (IRS).

Result: There were significant difference among HIF1α, CXCR4, and MMP13 in laryngeal SCC with positive nodal metastasis group compared to negative nodal metastasis group (p<0,05). There was no significant correlation between HIF-1α expression and CXCR4 expression (p = 0,403) (p>0,05). There were significant correlation between HIF-1α and MMP13 expression (r = 0,499), and between CXCR4 and MMP13 expression (r = 0,409). Conclusion: There were significant differences in HIF-1α, CXCR4 and MMP13 immunexpression in laryngeal SCC with cervical nodal metastasis compared to laryngeal SCC without cervical nodal metastasis. There was a positive correlation between HIF-1α and MMP13 expression, positive correlation between CXCR4 and MMP13 expression, but there was no correlation between HIF-1α and CXCR4 expression.

Keywords: Laryngeal carcinoma, lymph node metastasis, HIF-1α, CXCR4, MMP13

Background

Laryngeal carcinoma is the most common malignancy of the upper respiratory tract. Globocan estimates that there will be 177,422 new cases and 94,771 deaths due to laryngeal carcinoma worldwide in 2018.1 More than 98% of laryngeal malignancies are well differentiated SCC.2

Prognosis of laryngeal carcinoma depends greatly by tumor stadium. Laryngeal carcinoma confined to glottis has good 5 years survival rate (YSR), up to 85-95%, while presence of nodal metastasis will reduce 5 YSR significantly to 50%. The important significant single prognostic indicator of laryngeal carcinoma is the cervical nodal status.3,4

Identification of nodal metastasis in laryngeal carcinoma patients is not always easy. Probability of nodal metastasis without palpable nodal enlargement is about 13,7-37%, and better identification methods is indispensable.5
In SCC there will be changes in microenvironment of the tumor caused by proliferation of tumor cells. Up to certain tumor volume, blood and nutrients supply become inadequate and lead to tissue hypoxia. Hypoxia will induce HIF-1α, which is a major regulator of cellular response to changes in oxygen concentration supporting the adaptation of tumor cells to hypoxia in an oxygen-deficient tumor microenvironment. Overexpression of HIF-1α which in turn upregulates CXCR4.

Chemokine is a family of chemotactic that modulate cell movement and positioning, and act by coupling to G-protein coupled receptor. CXCR4 is a unique chemokine receptor because it has exclusive interaction with its ligand, CXCL12. The binding of CXCR4 and CXCL12 will initiates various downstream signaling pathways, resulting in various responses such as rising intracellular calcium, genetic transcription, cell proliferation, migration, adhesion, and invasion. CXCR4 is a critical tumor marker with a proven role in cancer progressivity and metastasis, but its role in laryngeal cancer is not yet well studied.

Infiltration of cancerous cell to the surrounding tissue is an important behavior in cancer progressivity. Proteolytic enzymes such as matrix metalloproteases (MMPs) contribute to tumor expansion by degrading extracellular matrix (ECM) components. Some studies showed expression of MMP in SCC. MMP13 has a vital role in MMP activation, and it’s also expressed in some head and neck SCC. Meanwhile, its role in nodal metastasis is not yet well known.

This study aims to analyse the expression of HIF-1α, CXCR4, and MMP13 in laryngeal SCC to unveil the roles of those markers in nodal metastasis process, which is expected to be an invaluable prognostic biomarker.

**Material and Methods**

This is an observational analytic study with cross-sectional approach. The population consists of all laryngeal SCC tissues with and without cervical nodal metastasis in paraffin blocks, archived in Anatomical Pathology Laboratory of Dr. Soetomo General Academic Hospital in Surabaya, during 2013 – 2016 period. The samples are 30 laryngeal SCC tissues in paraffin blocks from laryngectomy specimens, taken by random sampling methods.

In this study, immunoeexpressions of these three proteins were evaluated by immunohistochemistry examination. We used monoclonal antibody against HIF-1α (NB100-131, Novusbio) with 1:100 dilution, monoclonal antibody against CXCR4 (sc-53534, Santa Cruz Biotechnology, Inc., Texas, USA) with 1:50 dilution, and monoclonal antibody against MMP13 (sc-101564, Santa Cruz Biotechnology, Inc., Texas, USA) with 1:50 dilution. All antigen retrievals were done using decloaking chamber (heat-induced epitope retrieval) with DIVA solution in 110°C for 30 minutes.

HIF-1α was positive if expressed in nucleus or cytoplasm of tumor cells. CXCR4 was positive if expressed in the nucleus, cell membrane or cytoplasm. MMP13 was positive if expressed in cytoplasm of tumor cells.

HIF-1α, CXCR4, and MMP13 immunohistochemical staining were assessed using semiquantitative Immunoreactivity Score (IRS) refferancing to Remmele and Stegner. To determine the IRS, the percentage of positive tumor cells was classified into five grade (no positive cells = 0, <10% of positive cells = 1, 10-50% positive cells = 2, 51-80% positive cells = 3, and > 80% positive cells = 4). Then the grade of positive cells percentage is multiplied by the grade of staining intensity which is divided into 4 grades (no staining = 0, weak staining = 1, moderate staining = 2, and strong staining = 3). The final IRS score ranges from 0-12. The assessment was performed by two pathologists.

All datas were analyzed statistically using SPSS program. Analysis of expression differences of HIF-1α, CXCR4, and MMP13 was done with Mann-Whitney test. Analysis of correlation was done with Spearman correlation test. Statistical result is significant if p < 0.05.

**Result**

Mean age of all 30 patients was 55.9±8.36 years
The youngest was 49 years old and the oldest was 75. Patients were grouped into 4 groups with 10 years interval. As many as 53.33% patients were in the 51-60 years old group. Most patients were male. Out of 30 cases, there were only 2 female patients.

As many as 80% or 24 out of 30 cases of laryngeal SCC in this study were well differentiated. Four cases (12.33%) were moderately differentiated, and only 2 cases (6.67%) were poorly differentiated. Only 1 case (3.33%) was diagnosed in early stage (T1). Most cases, 43.33% or 13 cases of these cases were resected in stadium T3. There were 15 cases with cervical nodal metastasis; 8 cases (26.67%) in stadium N1 (metastasis to one ipsilateral lymph node with diameter ≤ 3 cm), and 7 cases with stadium N2.

Statistical analysis with Mann-Whitney showed that there was a significant difference among median scores of HIF1α, CXCR4 and MMP13 expression in laryngeal SCC without nodal metastasis group compared to laryngeal SCC with nodal metastasis group (p<0.05), as depicted in table 5.2.

In this study, Spearman correlation test showed no significant correlation between HIF-1α and CXCR4 expression, with p = 0.403 (p>0.05). Meanwhile, the test showed positive correlation between HIF-1α and MMP13, and between CXCR4 and MMP13 with rs = 0.499 and rs = 0.409 (p < 0.05), respectively.

Table 1: Sample Characteristics (n=30)

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Table 2: HIF1α, CXCR4 and MMP13 Expression in Laryngeal SCC with and without Nodal Metastasis

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Figure 1. HIF1α expression, stained positively in cytoplasm of tumor cells. A, with weak intensity. Internal positive control was inflammatory cells (arrow) (HE, 200x). B, with strong intensity (HE, 200x).
Discussion

In this study, statistical analysis showed that there was a significant difference in HIF-1α expression in laryngeal SCC with nodal metastasis compared to laryngeal SCC without nodal metastasis (p<0.05). HIF-1α was expressed stronger in metastasis group. This finding is in concordance with previous studies, such as studies by Wu et al and Popov et al who studied HIF-1α expression in laryngeal SCC\textsuperscript{13,14} and also in oral SCC\textsuperscript{15}. Zhou et al in a meta-analysis study showed that HIF-1α associated with not only lymph node metastasis, but also tumor size, tumor stage and overall survival\textsuperscript{16}.

Figure 2. CXCR4 expression, stained positively in cytoplasm and nucleus of tumor cells. A, with weak intensity (HE, 200x). B, with strong intensity (HE, 200x).

Figure 3. MMP13 expression, stained positively in cytoplasm of tumor cells. A, with weak intensity (HE, 200x). B, with moderate intensity (HE, 200x).

HIF-1 is an important factor regulating cell adaptation to oxygen deprivation. In a normoxic condition, the proline and lysine residues of HIF-1α subunits are hydroxylated by oxygen-dependent prolyl-4-hydroxylases (PHDs). Von Hippel-Lindau E3 ubiquitin ligase binds to hydroxylated HIF-1α and acts as a substrate recognition component of E3 ubiquitin ligase complex, which leads to proteasomal degradation of HIF protein. In solid tumors like laryngeal SCC, hypoxia is commonly found since there is a higher proliferation rate and intratumoral oxygen will be rapidly depleted. In hypoxic condition, the activity of PHDs and FIHs are suppressed, and HIF-1α translocates to the nucleus to bind with HIF-1β. The heterodimeric HIF-1α with HIF-
1β with the help of coactivators such as cyclic adenosine monophosphate response element-binding protein (CBP) and acetyltransferase (p300), then bind to the target gene hypoxia response element (HRE), resulting in their transcriptional upregulation. HIF-1α will then induce various pathways which influence survival, proliferation, apoptosis, intratumoral angiogenesis, and many other processes.6,17

Statistical analysis showed that there were significant differences of in CXCR4 expression in laryngeal SCC with nodal metastasis compared to laryngeal SCC without nodal metastasis (p<0,05). Previous studies on laryngeal and head and neck SCC were also found similar results.18-20 A study by Toyoma et al even proved that inactivation of CXCR4 will inhibit invasion and migration of tumor cells in SCC hypopharynx.21

Overexpression of CXCR4 is found in many malignancies. This is caused by CXCR4 upregulation by HIF-1α and HIF-2 secreted by malignant cells as a response to their increased oxygen demand.7 CXCR4 upregulation can also be caused by DNA demethylation of CXCR4 promotor, a particular process detected in various types of cancer.22 CXCR4-CXCL12 binding can increase MMP2 expression and inhibit tissue inhibitors of MMP. In addition, CXCL12 can promote the separation of tumor cells from tumor tissue and activated several cell adhesion molecules, and induce them to secrete more MMP and VEGF to dissolve ECM. These mechanisms contribute to tumor growth, angiogenesis, and metastasis.23 Biasci et al, proved that inhibition of CXCR4 combined with blockade of PD-1/PD-L1 T cell check points can induce T lymphocyte infiltration, resulting in an anti-cancer response.24 A study conducted by Tulotta et al showed that CXCR signaling supports the interaction between tumor cells and host neutrophils in developing tumor metastasis.25

This study also showed a significant difference in MMP13 expression in laryngeal SCC with nodal metastasis compared to laryngeal SCC without nodal metastasis (p<0,05). In this study, the median MMP13 expressions were stronger in laryngeal SCC with nodal metastasis group. Previous studies in laryngeal SCC.26,27 The study conducted by Huang showed that siMMP13 knockdown not only reduced the tumor invasion and migration, but also decreased the adhesion abilities of oral cancer cells. It is supported that MMP13 promotes invasion and metastasis in oral cancer cells.28

MMP13 has a wide range proteolytic capacity. It is produced and secreted from many cell types as a precursor form (pro MMP13), which can be activated by plasmin, MMP2, MMP3, and MMP14. MMP13 or collagenase-3 has the ability to degrade not only fibrillary collagen (type I, II, III, V, and XI), but also basal membrane, cartilage, collagen type IV, IX, X and XIX, gelatin, fibrillin-1, tenascin, aggrecan, perlecan, fibronectin, and osteonectin. MMP13 is expressed physiologically in conditions where rapid and effective remodelling of ECM are necessary, such as in fetal bone development and adult bone remodelling, and gingival and foetal skin wound healing. MMP13 is expressed pathologically in conditions involving overdegradation of ECM such as in rheumatoid arthitis, atherosclerosis, osteoarthritis and malignancy.29-31

In laryngeal SCC, tumor cells proliferate overwhelmingly. Tumor cells will secrete proteolytic enzymes and induce stromal cells, fibroblasts and inflammatory cells, in particular tumor associated macrophage (TAM), to produce MMP. MMP13 is a central key for other MMP cascade activation like MMP2, MMP3 and MMP9. All MMPs will then degrade ECM and basal membrane components together, facilitating tumor cells to invade stromal tissue and metastasize to lymph nodes and distant organs.32,33

Spearman correlation test showed no significant correlation between HIF-1α and CXCR4 expression, p = 0,403 (p>0,05). This finding is inconsistent with previous studies that showed a positive correlation between HIF-1α and CXCR4 expression in many types of malignancy, and also in laryngeal SCC.14,34

HIF-1α expression of laryngeal SCC tumor cells in this study tends to be low, while CXCR4 tends to be overexpressed. HIF-1α is known to be a major regulator
for CXCR4 upregulation, hence a low expression of HIF-1α should be followed by low expression of CXCR4. This contradictory finding could be caused by other molecular mechanisms which can also alter both protein levels, since molecules and biomarkers interactions in carcinogenesis are very complex and dynamic processes. As mentioned before, CXCR4 is not regulated solely by HIF-1α. Many malignancies had DNA demethylation of CXCR4 promoter, this will also upregulate CXCR4. Changes in multiple growth factors and transcription factors were also upregulate of CXCR4. A recent study from Izumi et al also found that CXCR4 could be secreted by stromal myofibroblasts, known as cancer-associated fibroblasts (CAFs). In this study, Spearman correlation test showed positive correlation between HIF-1α and MMP13 expression in laryngeal SCC, \( r_s = 0.499 \) (\( p < 0.05 \)). The stronger HIF-1α expression, the stronger MMP13 expression.

Hypoxia condition with high HIF-1α level will influence many mechanisms for tumor cells survival. HIF-1α regulates some proteins such as protein Twist, MMP-2, MMP-9, VEGF, and CXCR4/SDF-1. Matrix metalloproteinase like MMP-2, MMP-9 and MMP13 will degrade ECM surrounding tumor cells, eventually facilitate invasion of tumor cells through basal membrane into lymph and blood vessels to metastasize to lymph node and distant organs.

In this study, Spearman correlation test also found positive correlation between CXCR4 and MMP13 expression, \( r_s = 0.409 \) (\( p < 0.05 \)). The stronger CXCR4 expression, the stronger MMP13 expression. Previous studies also found similar results, not only in laryngeal SCC (Tan et al., 2008), but also in other malignancies such as basal cell carcinoma and colorectal cancer.

CXCR4 activation will induce various downstream signaling pathways, induce EMT (epithelial to mesenchymal transition), and promote metastasis. Activated CXCR4 will dissociate to α and βγ subunit. By β γ subunit will activate phospholipase C-β (PLC-β) and PI3K. PLC-β will then divide phosphatidylinositol into IP3 (inositol (1,4,5) triphosphate and diacylglycerol (DAG). IP3 will induce intracellular calcium release and then together with DAG will activate protein kinase C and MAPK/ERK pathway. MAPK/ERK then will relay the signal to induce MMP activity for ECM degradation by promoting transcription and translation of proMMP13 to MMP13.

Regulation of MMP13 expression by CXCR4 has been proven by Bu et al. They analyzed silencing effect of CXCR4 gene in some signaling pathways, and found a significant decrease in ERK phosphorylation level, mRNA and MMP13 protein level. This proved that CXCR4 could regulate MMP13 transcription level through ERK/NFκβ pathway.

### Conclusion

There were significant differences among HIF-1α, CXCR4, and MMP13 expression in laryngeal SCC with nodal metastasis compared to laryngeal SCC without nodal metastasis. All three were expressed stronger in laryngeal SCC with nodal metastasis group. There was a positive correlation between HIF-1α and MMP13 expression, positive correlation between CXCR4 and MMP13 expression, but there was no correlation between HIF-1α and CXCR4 expression.

### Conflict of Interest:
The authors declare that they have no competing interest

### Ethical Clearance:
This study had been approved by RSUD Dr. Soetomo, Surabaya, Indonesia No. 474/Panke.KKE/VIII/2017

### Source of Funding:
Independent

### Acknowledgements:
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### References


11. Tan C., Chu C., Lu Y., Chang C., Lin B., Wu H.,


Effectiveness of Video Assisted Teaching (VAT) on Jacobson Progressive Muscle Relaxation (JPMR) Exercises to Reduce the Stress Level among B.Sc. Nursing Students

Deepak Kumar Shandily¹, Tapti Bhattacharjee², Ramandeep Kaur Dhillon³

¹Ph.D. (Nursing) Scholar, Nims University Jaipur, Rajasthan, ²Professor, Department of Nursing, Nims University Jaipur, Rajasthan, ³Professor & Principal, Ajit Nursing Institute, Sunam, Punjab

Abstract

Stress is the mileage that life causes on the body. Every individual is facing one or another stressful events in life, in which students are also not an exception. Present study aimed to examine the effectiveness of Jacobson Progressive Muscle Relaxation (JPMR) Exercises to Reduce the Stress Level among B.Sc. Nursing Students at selected nursing institutes of Punjab and Haryana State. This was a pre-experimental one group pre-test and post-test study, in which 500 B.Sc. Nursing students were recruited by using non-probability convenient sampling technique. The study tools socio-demographic variables and Modified Perceived Stress Scale (MPSS) was used to gather the information’s. After pre-test the JPMR were introduced to each participant through video-assisted teaching and after 7th day of JPMR the post-test conducted. Analysis of study noted that majority of participants 51.2% have shown that they don’t have previous knowledge about JPMR, while highest number of participants 50% reported that they have once seen JPMR but not performed ever. The paired t test value of total academic score was 213.518 at calculated p value of <0.001 which concluded that the administered JPMR is effective to reduce the stress level among B.Sc. Nursing students.

Key words: Nursing students, Jacobson Progressive Muscle Relaxation, stress, video-assisted teaching

Introduction

The World Health Organization (WHO) has characterized youth as the movement from the presence of auxiliary sex attributes (pubescence) to sexual and regenerative development, the advancement of mental cycles and grown-up personality, and the changes from complete financial reliance to relative freedom. Stress is related with physical, mental and social encounters of life.¹

Stress is the mileage that life causes on the body. It happens when an individual experiences issues managing life circumstances, issues, and objectives.² Stress is an ordinary unavoidable truth. We can’t stay away from it. Stress results from any change we adjust to, running from the negative extraordinary of real physical risk to the thrill of becoming hopelessly enamoured or making some since quite a while ago wanted progress.³ Stress isn’t new understanding to us all, it’s viewed as present day society’s sickness, as the vast majority, regardless of how composed, will endure at any rate transient trouble while they arrange times of change, injury, difficulties and misfortune. Stress is something that we all experience every once in a while yet experience issues characterizing it.⁴

WHO report described that college students are facing stress which further leads to many other mental health disorders. Researcher have personal experience that during his professional journey, he come across
with many of nursing professional students who are facing many of stressful situations which are not just pressure of academic grades but there are many of such issues which creates pressure and stressful situations to nursing students. Being a healthcare professional and in that when we talk about nurses then we are speaking about center workforce of any healthcare setup. Getting an important and center member of healthcare team, nurse workload begins from their study onward, which further make their study structure different with other professional. Their extensive engagement in patient care made them to acquire theoretical, clinical (practical) and updated (research) knowledge of each and every side of human health which can be at institutionalized or in public health.5

In one investigation on nursing students at Greece pointed with to decide the commonness of sorrow and tension and the relationship between melancholy, nervousness and mental manifestations among nursing students.6 Stress for longer time prompts extreme emotional wellness issues in which the downturn is one of driving issue. One surveyed the downturn prevalence among undergrad clinical students at clinical school of Nepal and conveyed that the commonness of discouragement is high from the outset year students, so endeavors ought to be taken from starting. Since scholarly stress end up being one of the central point, measures to make the scholastic educational program more understudy inviting is proposed.7

Inevitably, when individual feel stressed, the muscles straighten out. At the point when muscles straighten out an excess of then individual can’t have open to feeling or to state get undesirable sentiments, for example.8 It alteration may be settle down by the help of some of muscle relaxation technique, hence among all the Jacobson Progressive Muscle Relaxation is widely used technique. Researcher gathered enough evidence that this technique is unique and helped to relax the muscle at comfortable level. As the use of such technique also promoted by WHO by stating utilizing of non-pharmacological therapies.9

Subhash M Khatri et al referenced in their randomized clinical trial with an intention to discover the viability of Jacobson’s relaxation technique in hypertension. The dissected information expressed that the normal decrease in circulatory strain was fundamentally more prominent in subjects treated with Jacobson’s relaxation technique alongside prescriptions when contrasted with just drugs. Investigators reasoned that Jacobson’s relaxation technique can be utilized as an adjunctive intercession in the treatment of hypertension.10 In a research by Wen-Chun Chen et al referenced their investigation to look at the adequacy of progressive muscle relaxation training on anxiety in patients with intense schizophrenia. They have found its effective to reduce anxiety level of schizophrenic patients.11

These evidences guided the researcher to assess the effectiveness of Jacobson Progressive Muscle Relaxation on stress of nursing students but reaching out to maximum students at different location was questionable, which was taken care seriously by researcher and adopted a video-assisted technique, so through video-assisted technique investigator can able to reach all participants.

**Study Objectives:**

1. To assess the pre-test stress level score among B.Sc. Nursing students.

2. To assess the post-test stress level score among B.Sc. nursing students.


4. To determine association of post-test stress level scores with selected demographic variables of B.Sc. Nursing students.

**Materials and Methods**

Researcher attempted a quantitative research approach with a pre-experimental research design to achieve the objectives and test the hypothesis of...
this research. Study participants were included B.Sc. Nursing students of selected nursing institutes of Punjab and Haryana institute. The samples were recruited by non-probability convenient sampling technique and total 500 samples recruited based on eligibility criteria.

**Inclusion criteria:**

The B.Sc. nursing students who are:

- Providing informed consent to participate in study.
- Any genders
- Any age
- Having any device to see video such as mobile, laptop, PC

**Exclusion Criteria:**

The B.Sc. nursing students who are:

- Physical handicapped.
- Having physical illness in which physical exercise is prohibited.
- Having vision related injury.
- Diagnosed with any mental health illness in last one year.

The data collected by validated tools. Data were collected in between December 2019 to January 2020 from all 500 participants. The tools were grouped in two sections, Section-I were leading to collect socio-demographic information’s whereas in section-II intended to measure stress level among B.Sc. Nursing students and it named as Modified Perceived Stress Scale (MPSS). Tool’s reliability assessed by split half method and found reliable with r=0.86. These tools were introduced first in from of collect pre-test data, then after researcher administered Jacobson Progressive Muscle Relaxation (JPMR) as a video to each participant, followed to it after 7 days the post-test data were collected. The gathered data were arranged in excel format as a master sheet. Researcher used 21 version of SPSS to analyse the data.

**Results and Discussion**

Relevant data were arranged and analysis completed. Table 1 reflecting the frequency and percentage distribution of B.Sc. nursing students.

<table>
<thead>
<tr>
<th>Table 1: Frequency and Percentage Distribution of Socio-Demographic Variables among B.Sc. Nursing Students:</th>
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Table 1: Frequency and Percentage Distribution of Socio-Demographic Variables among B.Sc. Nursing Students:  N=50

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<td>38.</td>
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<td>48.0</td>
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Table mentioned that maximum number of participants 51.8% belongs to age of 20 years, majority of participants 90% are males, maximum number of participants 68.4% are staying in nuclear family, maximum number of participant’s father 41% are under-graduated, the majority of participant’s mother 23% acquired primary education, majority of participant’s father 34% are farmer by their occupation, the highest number of mothers 72.8% are housewife by occupation, majority of participants 61% have their family monthly income in between 30000 to 50000/- Rs., majority of participants 51.2% have shown that they don’t have previous knowledge about JPMR, while highest number of participants 50% reported that they have once seen JPMR but not performed ever.

Table 2: Effectiveness of Jacobson Progressive Muscle Relaxation  N=500

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<td>&lt;0.001</td>
<td>S</td>
</tr>
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<td>1.32</td>
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<td>113.789</td>
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<td>Total stress score</td>
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<td>213.518</td>
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</table>

P<0.05 level of significance  S- Significant
Table-2 presented the paired t test value and it divulged that not only total stress level but also each category of stress found strongly significant, as mentioned the calculated p value in each category is <0.001 which is much lesser than then the pre-determined level of significance p<0.05. These findings suggested that the provided JPMR is very effective method to reduce or manage the stress among B.Sc. Nursing students.

Discussion

Sneha Pitre et al has supported present research findings of frequency and distribution of socio-demographic variables. They have revealed that maximum number of their study participants 80% are belongs to age of 18 to 20 years and same percentage 80% of female gender were participated. A similar finding was suggested by a study by Wafaa Yousif Abdel Wahed and Safaa Khamis Hassan has stated their findings that majority of participants 66.1% were in age group of 20 to 22 years, highest number of participants 61.1% were female. Majority 92.3% were unmarried, more than half of participants 56.6% were residents of villages, majority of participants 27.1% were studied at first year followed by 26.7% in third year, 24% in second year and 22.2% were in final year of their study. But this differs in present study finding in case of socio-economic standards, which is in this study is high among maximum participants 40.3%. One study by Timothy Baghurst and Betty C. Kelley also stated that majority of participants were belongs to female 58.56% gender than male, the mean age of participants were 21.4 years.

Prameelarani Bommareddi et al has mentioned significant effectiveness of Jacobson progressive muscle relaxation exercise on anxiety and depression which was found effective at the p level of <0.001. G S Tak et al has mentioned in their study in which they attempted to see effectiveness of Jacobson progressive muscle relaxation exercise on anxiety and the findings suggested that the intervention is highly effective to reduce the anxiety level of participants at the level of <0.05. These findings are supporting our study findings. Archana Khanna et al has divulged in their study that out of three group intervention among stressed females, in which second group received Jacobson progressive muscle relaxation exercise has shown mean and standard deviation as 65.9±6.33, in compare with the pre-test which was 73.60±4.19.

Conclusion

B.Sc. nursing students found stressed at each level such as academic, personal or social which cumulatively stressed upon the fact that nursing students found stressed. Here, investigators would like to conclude the study based on study findings that administered Jacobson progressive muscle relaxation through video-assisted technique found effective to reduce the stress level of B.Sc. nursing students. Investigators recommended to institutes to offer such relaxation technique to nursing students with objective to keep them relax and focused.

Acknowledgement: Researcher would like to acknowledge untried support and guidance of NIMS university along with My PhD Supervisor and co-supervisor.

Ethical Clearance: Taken from Institutional Ethical Committee, National Institute of Medical Science and Research, NIMS University, Jaipur, Rajasthan with Ref. No.: NIMSUNI/IEC/2018/PHD/122 dated on 28th August, 2018.

Source of Funding: Self-funded project

Conflict of Interest: Nil

References

Mandibular Foramen –The Peephole

Deepali P Mohite¹, Prakash M Mohite², Devendra Palve³, Alka H Hande⁴
¹Associate Professor, Department of Oral and Maxillofacial Pathology, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur; ²Professor, Department of Forensic Medicine and Toxicology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha; ³Professor and Head, Department of Oral and Maxillofacial Pathology, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur; ⁴Professor and Head, Department of Oral and Maxillofacial Pathology, Sharad Pawar Dental College and Hospital, Sawangi (Meghe), Wardha

Abstract

Background: Forensic Odontology is a rapidly evolving branch of dentistry which deals with the identification of an individual. In instances of deceased individual, establishing identity may still pose difficulties depending upon the time that has elapsed since death. The oral structures can reliably be used for establishing identity of the deceased as they are preserved for long after death. The mandibular foramen is amongst the core structures & has been used for age estimation with reliable degree of accuracy using radiographs which is a non-invasive and easily reproducible technique. Hence we decided to assess its importance in establishing gender. Methods: Digital Orthopantomograph (OPG) were used for the study as they are taken for all dental procedures and can be preserved as records and may facilitate comparison of ante-mortem and post-mortem records. Various dimensions from the mandibular foramen were recorded. Conclusion: We found statistically significant correlation of Anteroposterior and Posteroanterior dimensions in position of mandibular foramen to gender.

Keywords Mandibular Foramen, OPG, gender, dimensions, Superoinferior, AnteroPosterior.

Introduction

Identification of the individual followed by identification of gender is a very important and necessary tool in Forensic anthropology and every document in the field of forensic medicine begins with identification (either a live person or remains – dead body or skeletal remains).¹⁰ The Mandible is extensively used in Forensic odontological studies as it forms a strong and nonperishable source of facial skeleton. In living individuals, remodeling of the mandible takes place throughout life and these changes are observed on dried mandible as well as on its radiographic image. The body of the mandible and the mental and the mandibular foramina have been used as points of reference in various morphometric analyses of the mandible, by virtue of their stable relation with the basal bone. The mandibular foramen (MF) is an important landmark on the medial surface of ramus of the mandible which transmits the inferior alveolar nerves and vessels.¹¹ ¹³ Vast amount of data is available of the studies on either the mandibular basal bone or the other morphologic landmarks on the mandible but very few studies include the Mandibular Foramen as a landmark for evaluating age and fewer still for correlating Gender. Hence this study was designed to include the change in position of mandibular foramen and its morphology as the individual grows older and whether it can contribute to establishing Gender.
Materials & Method

This observational study consisted of 200 randomized digital OPG’s from a database in the extra oral radiographic machine in the Department of Oral Medicine and Maxillofacial Radiology. Thus, patients were not unnecessarily exposed to radiation and their identities remained confidential. The radiographs were selected based on the following criteria.

Inclusion criteria

- Subjects within the age group of 20 - 60 Yrs belonging to Nagpur Division population.
- Good quality (with respect to contrast) OPG’s with complete lower border of mandible.
- Individuals with all the teeth normally present for that particular age in right & left lower jaw.
- Devoid of any jaw lesions and traumatic injuries in the mandible.
- Images without radiographic exposure or processing artifacts.

Exclusion criteria

- Poor quality radiograph.
- Presence of processing artifacts.
- Presence of jaw fracture in mandible.
- Presence of any pathology, bony abnormalities / bifid mandibular canal, any systemic diseases affecting bone remodeling in the mandible.

All radiographs were taken with a digital machine, Orthophos XG X-ray system version 2.53 SIRONA Germany with the following parameters:

- Kilo voltage of 62-73 kVp
- Tube current 8-15 mA
- Time for 15 s.

Position of Mandibular foramen was identified (Fig-1) and measured in three directions superoinferiorly (SI) posteroanteriorly (PA) and anteroposteriorly (AP). Superoinferiorly it was measured as a line drawn from the deepest point of the mandibular notch to the mandibular foramen and was marked. Posteroanteriorly measurements were made by drawing a line from the posterior border of the ramus at the level of mandibular foramen to the actual mandibular foramen. Similarly measurements were made anteroposteriorly by drawing a straight (perpendicular) line from mandibular foramen to the anterior border of ramus (AP). The distances from the MF to various landmarks were recorded as an average of two measurements which were measured independently by two different people. The mean and standard deviation for each distance were calculated separately for right and left sides. Statistical analysis was performed that included mean values in males and females on the right and the left sides, Discriminate Function Test, $t$-test and p-value were calculated.

Results

The mean distance of the MF from anterior border of the mandibular ramus on the right side was $14.88\pm1.91\text{mm}$ and left side was $14.60\pm1.83\text{mm}$. The MF was located $14.90\pm1.90\text{mm}$ (Right side) and $14.62\pm1.83\text{mm}$ (Left Side) from the posterior border of Mandible. The distance of the MF from the mandibular notch was $22.70\pm1.57\text{mm}$ and $22.02\pm1.57\text{mm}$ on the right and left sides respectively. Table 1 shows the mean and std deviation of the distance of Mandibular Foramen from the anterior, posterior and superior borders of the Mandible respectively. The values of the Antero-posterior measurements on right and left sides was $14.18\pm1.60\text{mm}$ & $14.59\pm2.07\text{mm}$ in females and $15.17\pm1.70\text{mm} & 15.02\pm1.95\text{mm}$ in males, Postero-anterior dimensions recorded were $14.20\pm1.60\text{mm}$ & $14.61\pm2.07\text{mm}$ in females and $15.04\pm1.95\text{mm}$ & $15.19\pm1.7\text{mm}$ in males and Supero-inferior dimensions were $22.20\pm1.60\text{mm}$ & $22.88\pm1.59\text{mm}$ in males as compared to females $21.84\pm1.52 \& 22.51\pm1.53\text{mm}$. Table 2 shows Gender wise comparison of parameters left and right side which is statistically significant for Anteroposterior and Postero-anterior dimensions. The Anteroposterior and
Postero-anterior dimensions are higher in males than in females and are greater on right side than on left side. Table 3 shows Discriminant Function Analysis, i.e., Tests of Equality of Group Means which demonstrates that the change in Anteroposterior and Posteroanterior dimensions to be statistically significant.

### Table I: Comparison of parameters left and right side Student’s Paired t test

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>t-value</th>
<th>p-value</th>
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<tr>
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<tr>
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<td>14.88</td>
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<td>2.10</td>
<td>0.036, S</td>
</tr>
<tr>
<td>Left Side</td>
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<td>0.12</td>
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<tr>
<td>Posteroanterior</td>
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<tr>
<td>Right Side</td>
<td>14.90</td>
<td>200</td>
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<tr>
<td>Left Side</td>
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<tr>
<td>Right Side</td>
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<td>296.43</td>
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### Table II: Gender wise comparison of parameters left and right side Student’s Paired t test

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<td>0.19</td>
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<tr>
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<td>100</td>
<td>15.19</td>
<td>1.70</td>
<td>0.17</td>
<td>2.16</td>
<td>0.031 S</td>
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<tr>
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<td>0.001,S</td>
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<td>0.16</td>
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<tr>
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### Table III: Discriminant Function Analysis

#### Tests of Equality of Group Means

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#### Variables Entered/Removed

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#### Eigenvalues

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</table>
Discussion

The mandibular foramen is located on the medial surface of the ramus approximately midway between the mandibular notch and the angle of the jaw and also midway between the internal oblique line and the posterior border of the ramus. Its relation to the occlusal plane varies by approximately 10.00 to 11.00 mm above the occlusal surface of the mandibular first molar and can vary depending upon the reference tooth that is used for measurement. The mental and mandibular foramina have been used as points of reference in various morphometric analyses of the mandible, by virtue of their stable relation with the basal bone.\(^\text{13, 9}\) It has been established that the mandibular foramen undergoes a shift in position from its location at birth into adulthood. This shift in its position is in a vertical plane and this study aimed to assess if there was a change in location with respect to gender of an individual and whether this information may have Forensic implication. In our study, the results showed that differences between gender for mandibular foramen at AP and PA was highly significant, which is similar to the study by Rashid et al 2011\(^\text{11}\), Samanta PP2013\(^\text{13}\) and Linganna CS2015 \(^\text{8}\). In our study the mandibular foramen shows a shift with age which is similar to the study by Lim MY et al\(^\text{7}\). The observation found in our study was a posterior shift because we studied adult population whereas in the study by Lim MY et al 2015\(^\text{7}\), the population studied were children. When compared between genders the mean values superoinferiorly were higher in males than females which is similar to the study conducted by Shendakar AT etal, 2010\(^\text{14}\). A similar finding was also reported by Direk F et al2018\(^\text{4}\) who used Multi detector computed tomography to assess mandible and suggested that the ramus dimensions are higher in males. The Posteroanterior and Anteroposterior dimensions were higher for males in our study; this can be attributed to the stronger masticatory muscles in males imparting greater stability to the ramus of the mandible. Sairam V et al2016\(^\text{12}\), Lasemi Eet al2019\(^\text{6}\), Bhardwaj D et al, 2014\(^\text{2}\) and Jalili MR, 2012\(^\text{5}\) studied mandibles through OPG and concluded that Superoinferior dimensions were higher in males. This difference was statistically significant between sexes, thus indicating a strong sexual dimorphism.

The difference in dimensions measured for the right and left sides showed values that were almost similar, with a non-significant difference and this applies for both the male and the female groups which is in accordance with study by C. Lavanya Varma et al\(^\text{2011}\)\(^\text{3}\) and Ashkenazi, 2011\(^\text{1}\). Thus the various parameters of the ramus of the mandible can be used for personal identification and also to identify gender, as they serve as stable landmarks and show gradual and steady modifications with age. These dimensional changes are greater in males than in females.

Funding: None

Conflict of Interest: No conflict of interest

Ethical Approval: Approved by IEC

References

6. Lasemi E, Motamedi MHK, Talaeipour AR, et


A Study to Assess the Effect of Food Consumption Pattern on Health Status among Nursing Students at Dinsha Patel College of Nursing, Nadiad

Dhara Vyas¹, Fiza Sherasiya², Ami Patel²

¹Associate Professor cum Head of Department Child Health Nursing, ²Clinical Instructor, Dinsha Patel College of Nursing, Nadiad, Gujarat, India

Abstract

College students are at risk for making poor dietary choices that can cause significant health problems. A descriptive study was conducted to assess the effect of food consumption pattern on health status among nursing students at Dinsha Patel College of Nursing, Nadiad. A sample of 101 students was selected by systematic random sampling technique. Five point Likert Scale was used to assess the food consumption pattern. The data was analyzed by using the descriptive and inferential statistics. The result of present study reveals 50(49%) students have more than 11 g/dl hemoglobin level, 56(55%) students are underweight, 73(72 %) students believes that they are taking balance diet. There is a statistically significant association with the perception regarding balanced diet and food consumption pattern and no significant association with other socio demographic variables such as age, sex, education, BMI, Hemoglobin level, religion, eating habits, residential status. The result shows 37(36.6%) students had unhealthy, 44(43.5%) students had average healthy, 19(18.8%) students had healthy diet pattern The study concluded that there is a need for health education programs that promote healthy food consumption pattern for nursing students.

Key Words: Food Consumption Pattern, Health Status, BMI, Hemoglobin level

Introduction

College students are at risk for making deprived dietary choices that can cause significant health problems. Majority of undergraduate students consume at college dining facilities with imperfect healthy food options. Moreover, if students do not accomplish sufficient nutrition daily, a decrease in educational or physical performance can result.¹⁻³

Despite the well-built emphasis on meeting dietary requirements every day to attain optimal health, many college students tend to care less about or neglect their nutritional requirements. Many factors come in to play as they transition to university life. Many of them leave the parental home, adapt to social and environmental changes, admit new financial responsibilities, build different social networks, and experience different time availability. Yet, meeting nutritional requirements leftovers important in achieving one’s health. It is very favorable for college students to formulize high-quality eating habits that lead them to obtaining health and finest function.⁶

Objectives of the study were

1. To describe the socio demographic profile of Nursing students at Dinsha Patel College of Nursing, Nadiad.

2. To assess the Food Consumption Pattern of Nursing students at Dinsha Patel College of Nursing, Nadiad.

3. To assess the health status of Nursing student in terms of BMI and Hemoglobin level

4. To find out the association between the Food Consumption Pattern and selected demographic variables of Nursing students at Dinsha Patel College of
Nursing, Nadiad.

**Research Methodology**

**Research Approach:** Descriptive survey approach

**Research Design:** Cross sectional study

**Sampling method:** Systematic random sampling technique

**Study population:** G.N.M., B.Sc (N), P.B.B.Sc (N), M.Sc Nursing students

**Study Setting:** Dinsha Patel College of Nursing, Nadiad.

**Sample Size:** 101

**DESCRIPTION OF TOOL:**

Tool consists of two parts.

**Part-I:** It includes socio demographic variable such as Age, Sex, Education, Height, Weight, BMI, Hemoglobin, taking balance diet, Religion, Family Income, Address, Residential Status, Permanent residence, Eating habits, Reason for skipping meals, Skipped meal substitute, Fasting

**Part-II:** Consists of Five point Likert Scale, it consists 30 items which food items. Total score is 150. The score more than 100 is considers as a healthy food consumption pattern and less than 90 is consider unhealthy food consumption pattern.

**Result**

**ASSOCIATION BETWEEN THE FOOD CONSUMPTION PATTERN AND SELECTED DEMOGRAPHIC VARIABLES**

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<th>Scores (Fr)</th>
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### ASSOCIATION BETWEEN THE FOOD CONSUMPTION PATTERN AND SELECTED DEMOGRAPHIC VARIABLES

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Table shows that there is a statistically association between food consumption pattern and selected demographic variables among nursing students. The data shows that the chi-square value computed between the perception regarding balanced diet and food consumption pattern among nursing students which was found to be statistically significant (p=0.002). In statistical data of Age group Degree of Freedom was (df= 1), Calculated Value Chi square ($x^2 = 9.56$), Tabulated value at 0.002 level of significance was 9.56. So, The calculated value is more than tabulated value which shows association between food consumption pattern among nursing students and perception regarding balanced diet and there is no significant association between food consumption pattern among nursing students with other socio demographic variables such as age, sex, education, religion, income, residential status, permanent residents, eating habits, reason for skipping meals, skipped meal substitute, fasting.

**Conclusion**

The health status of student indicated majority of students were undernourished and having hemoglobin level more than 11 g/dl. 37% students had unhealthy, 44% students had average, 19% students had healthy diet pattern. There is a statistically significant association with balanced diet and no significant association with other socio demographic variables such as age, sex, education, religion, income, residential status, permanent residents, eating habits, reason for skipping meals, skipped meal substitute, fasting.

**Ethical Clearance** - Ethical clearance by Institutional Ethical Committee

**Source of Funding** - Dinsha Patel College of Nursing, Nadiad

**Conflict of Interest**: Nil/NA

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5. Rathi N. Food consumption patterns of adolescents aged 14–16 years in Kolkata, India Nutrition Journal August 2017; Article number: 50
The Undernutrition Prevalence of Under-Two-Years Infant in Indonesia: Do breastfeeding Practices Ecologically Matter?

Fandaruzzahra Putri Perdani1, Agung Dwi Laksono2, Djazuly Chalidyanto2, Fandaruzzahra Putri Perdani3
1 Post Graduate Student of Department of Health Policy and Administration, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia, 2 Researcher, Ministry of Health of the Republic of Indonesia, Jakarta, Indonesia, 3 Lecturer of Department of Health Policy and Administration, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia

Abstract

Undernutrition due to inappropriate feeding practices for children increases morbidity. Breastfeeding is considered the most important in a baby’s growth and development. Breastfeeding started within one hour of birth and was maintained exclusively for the first six months until one year of age or more to obtain optimal benefits. The study aims to determine the ecological relationship between undernutrition in 2-year-old infant and breastfeeding practice in Indonesia. This study is based on environmental analysis using a report of the 2018 Indonesia Basic Health Survey. The dependent variable was the prevalence of undernutrition in 2-year-old infant. Besides, the independent variables were four optimum breastfeeding practices. The analysis used bivariate analysis with scatter plot in statistic application. The study results show . The higher the proportion of early initiation of breastfeeding in a province, the lower the levels of undernutrition in children aged two years in that province. The higher the ratio of starting to breastfeed less than one hour in an area, the lower the undernutrition levels in children aged two years in that area. On the other side, the higher the proportion of the mother giving all colostrum in a province, the lower the undernutrition levels in children aged two years in Indonesia in that province. The higher the proportion of still being breastfed 0-23 months in a region, the lower the undernutrition levels in children aged two years in that region. The study concluded that optimal breastfeeding practices negatively affect the levels of undernutrition in children aged two years.

Keywords: Undernutrition, breastfeeding, early initiation of breastfeeding, exclusive breastfeeding

Background

Malnutrition during the developmental period can increase the risk of morbidity and mortality. On the other side, the first 1000 days are critical for growth and development throughout the child’s life. Meanwhile, undernutrition due to inappropriate feeding practices for newborns and children can increase morbidity1.

Moreover, children with malnutrition are the trigger of the death of 10.5 million children each year worldwide. Malnutrition is associated with the end of 35% of children. The percentage of stunting is 32% of children less than five years old in developing countries, and 10% of them suffer from malnutrition. Suboptimal breastfeeding, especially non-exclusive breastfeeding in the first six months, causes 1.4 million deaths and causes a 10% disease burden in children aged <5 years2. Based on the Indonesian Demographic and Health Survey, the infant and under-five children’s mortality rate in Indonesia in Infant Mortality Rate is 35 babies per 1000 births; if the breakdown, 157,000 babies die per year 430 babies per day. Meanwhile, every year, the under-five children mortality rate is 46 out of 10003.

We considered breastfeeding to be one of the most critical factors for a baby’s growth and development. Breastfeeding offers tremendous health benefits for both the child and the mother. The American Academy of Pediatrics recommends exclusive six months of breastfeeding, followed by introducing supplementary...
foods for up to 1 year of age or more as long as the mother and baby want it\textsuperscript{4}. One of the programs for accelerating infant mortality reduction in the Early Initiation of Breastfeeding and exclusive breastfeeding\textsuperscript{5,6}. Breastfeeding must be started within one hour of the baby’s birth and must be maintained exclusively for the first six months of the baby’s life to obtain optimal benefits. Exclusive breastfeeding means that a newborn is mother fed the baby only breast milk, and no other fluids (even water) or solids are given, except for oral rehydration salts, vitamins, mineral supplements, or medicines\textsuperscript{2,7}. The World Health Organization (WHO) recommends that capable mothers practice and maintain exclusive breastfeeding for the first six months of their baby’s life\textsuperscript{8}.

Inadequate breastfeeding practices can interfere with the health and development of children and mothers\textsuperscript{9,10}. Breastfed babies have fewer cases of diarrhea, respiratory infections, ear infections, and other acute illnesses. While these benefits are more excellent in developing countries, they are also crucial in industrialized countries. Inadequate breastfeeding practice caused nearly half of diarrhea episodes and one-third of respiratory infections. During the first six months of life in America, breastfeeding saves almost 1,000 lives and the US $ 13 billion in excess health care costs associated with not breastfeeding\textsuperscript{4}. More prolonged breastfeeding was associated with a 13% reduction in the chance of being overweight and obesity prevalence and a 35% reduction in the incidence of type 2 diabetes. We can prevent an estimated 20,000 maternal deaths from breast cancer each year by increasing breastfeeding rates\textsuperscript{10}.

According to the recommendations, the fulfill optimal exclusive breastfeeding, including breastfeeding until the age of more than two years, start breastfeeding in the first 1 hour, the length of Early Initiation of Breastfeeding and the provision of colostrum are all factors for the success of breastfeeding. This study aims to determine the ecological relationship between undernutrition in two-year-old infant and breastfeeding behavior in Indonesia. This research, hopefully, can be implemented as a consideration for policymakers in promoting the importance of exclusive breastfeeding according to the recommendations.

**Methods**

The thesis was planned by the author using an ecological research approach. Ecological experiments compare populations rather than entities. The data studied is composite data at a given community or level, particularly the provincial level. Aggregate quantities, environmental measurements, or global measurements may all be used as ecological research variables\textsuperscript{11,12}.

The study used secondary data from the 2018 Indonesia Basic Health Survey national report. The Ministry of Health of the Republic of Indonesia published the survey report openly, and we can access it on the ministry’s website\textsuperscript{13}. There are data from 34 provinces throughout Indonesia. The survey carried out measurements on 33,376 children aged two years spread across 34 provinces from Sumatra to Papua. The dependent variable measured was “prevalence of malnutrition and deficiency status.” The nutritional status used is the number of malnutrition and deficiency status in children aged two years. The independent variables measured were the fulfillment of exclusive breastfeeding according to recommendations, namely breastfeeding until the age of more than two years, the time to start breastfeeding in the first 1 hour, the duration of Early Breastfeeding Initiation, and colostrum administration.

The study carries out bivariate analysis using a scatter plot. The review uses the linear fit line to determine the relationship between undernutrition in two-year-old infant and the independent variable. The entire analysis process utilizes SPSS 21 software.

**Result and Discussion**

The study found that the four factors of breastfeeding behavior negatively relationship with the undernutrition prevalence of two-year-old infants based on the scatter plot. The higher the proportion of early breastfeeding initiation in a province, the lower the undernutrition levels in children aged two years in that province.
The higher the proportion of starting to breastfeed less than one hour in an area, the lower the undernutrition levels in children aged two years in that area. Meanwhile, the higher the proportion of the mother giving all colostrum in a province, the lower the undernutrition levels in children aged two years in Indonesia in that province. Moreover, the higher the proportion of still being breastfed 0-23 months in a region, the lower the undernutrition levels in children aged two years.
These results are consistent with all measured factors for optimal breastfeeding behavior. Breastfeeding helps protect babies from acute and chronic illnesses. Babies who are not breastfed have 6-10 times the risk of dying than babies who are breastfed\textsuperscript{14}. Babies who are given foods other than breast milk are at risk of developing diarrhea and pneumonia\textsuperscript{5}. Diarrhea is common in infants given artificial food even with high hygiene levels, such as in Belarus and Scotland\textsuperscript{15}. Growth and development from birth to the first two years are critical for optimizing growth and development. Nutritional deficiencies at 0-2 years can lead to stunting, so this is a crucial period for implementing strategies to reduce the risk of undernutrition\textsuperscript{15}. 

Figure 3. The scatter plot diagram of the undernutrition prevalence of two-year-old infants and the behavior of the mother giving all colostrum in Indonesia in 2018

Figure 4. The scatter plot diagram of the undernutrition prevalence of two-year-old infants and the proportion still being breastfed 0-23 months in Indonesia in 2018
Based on data analysis, East Nusa Tenggara has the highest percentage of infants aged two years undernutrition of the total population (24.5%) but has breastfeeding behavior above the national average except for the early initiation of breastfeeding variable. Meanwhile, West Java is a province with the lowest percentage of two-year-old babies with malnutrition and malnutrition of the region's total population (10.6%) having breastfeeding behavior above the national average. Gorontalo, Maluku, and North Maluku are provinces with a higher prevalence of undernutrition infants than the national average (16.6%). As well as lower breastfeeding behavior than the national average, including early initiation of breastfeeding (54.8%), percentage breastfed <1 hour (29.0%), giving colostrum entirely (83.5%) and the proportion was still breastfed 0-23 months (76.50%). The highest percentage of early breastfeeding initiation was in Jakarta (74.1%), and the lowest was in West Papua (34.3%)

Based on the scatter plot diagram, the higher the early initiation of breastfeeding practice, the lower the number of undernutrition in a province. According to WHO, early initiation of breastfeeding (EIBF) defines as an initiation of breastfeeding in the first 1 hour after delivery. Early initiation of breastfeeding has the benefit of preventing infection, lowering the risk of diarrhea, and increasing survival rates in children\(^2\). In mothers who underwent EIBF, neonatal mortality decreased by 33%. Although WHO has recommended every mother to perform EIBF, the practice of EIBF is still low, especially in developing countries\(^{16}\). The study found that the average EIBF practice in 34 provinces was 54.8%. The results are consistent with research on EIBF practices in Ethiopia, where EIBF practices in the districts of Amibara, Dale Woreda, Gurage zone, and Debre Birhan are 39.6%, 83.7%, 47.3%, and 62.9%, respectively\(^{17}\).

Meanwhile, the lowest percentage of breastfed <1 hour was West Nusa Tenggara (38.5%) and the highest was the rate of breastfeeding in the first hour in North Sumatra (17.4%). The practice of breastfeeding in the first 1 hour is essential for the initiation and continuation of breastfeeding and to increase the emotional bond between mother and baby\(^2\). In initiating early breastfeeding, the mother’s skin-to-baby skin contact begins immediately after delivery or at least 1.5 hours and should last as long as possible or at least 1 hour without interruption\(^{18}\). Early connection is essential for mother and baby. Early contact stimulates the flow of oxytocin, helps release oxytocin, reduces the risk of bleeding, and facilitates emotional bonding between mother and baby. The neonatal mortality rate increased by 33% in infants whose EBF was longer than 1 hour. The Zimbabwean study showed a risk of sepsis in the first one week of infants who delayed breastfeeding\(^{17}\).

Giving colostrum entirely mostly done in Yogyakarta province (97.7%), while the lowest was in Papua province (68.8%). Colostrum is the first milk that is very important for newborns to prevent infection. Mother breast produced colostrum in small amounts, namely 40-50 ml in the first days, and all babies are needed. Colostrum is rich in white blood cells and antibodies, especially sIgA, and contains high amounts of protein, minerals, and water-soluble vitamins (A, E, and K) than breast milk\(^{18}\). Colostrum can prevent infectious diseases from bacteria, viruses, fungi, and protozoa. The study found that colostrum administration can reduce the risk of malnutrition and malnutrition in children aged two years. Based on other tasks, children who do not get colostrum can be at risk of infection, stunting, and undernutrition\(^{19}\).

For more than two years, the duration of breastfeeding was mainly in the province of Central Java (86.7%), while the lowest was in Gorontalo province (60.2%). Breastfeeding should be continued until two years of age or older, upon request, as often as possible for as long as the child requests. Breast milk meets half or more of the energy needs of children aged 6-12 months and one-third of energy and high-quality nutrition for children aged 12-24 months\(^{14}\). Breast milk provides a critical energy and nutrition source when a child is sick and reduces the risk of mortality in malnourished children. Breastfeeding also reduces acute and chronic illnesses\(^{20}\). In children who have received complimentary foods
where breastfeeding is reduced, breastfeeding is given actively to meet breast milk needs in children\textsuperscript{19}.

The nutrients can explain the nutrition and development of children in breast milk. Breast milk ensures optimal bone development, thereby preventing stunting. Oleic acid in breast milk can increase the absorption of fat and calcium. Meanwhile, breast milk consumption can improve the quality of nutrients and micronutrients in children, but not the energy needs of children\textsuperscript{21}. Apart from breastfeeding, several other factors influence the nutritional status of children. The success of breastfeeding optimal is affected by a pregnancy, namely the provision of good nutrition during pregnancy, preparation for breastfeeding, and the support of health workers and families to accompany during the optimal breastfeeding process\textsuperscript{16}.

**Conclusion**

The study concluded that optimal breastfeeding practices negatively relationship the undernutrition levels of children aged two years. The research shows the higher the optimal breastfeeding practices, the lower the undernutrition levels of children aged two-years-old.

**Source of Funding:** Self-funding

**Conflict of Interests:** The authors declared no potential conflicts of interest concerning the research, authorship, and publication of this article.

**Ethical Clearance:** The study was conducted by utilizing secondary data from published reports. For this reason, the study’s unrequired ethical clearance in the implementation of this study.

**Acknowledgments:** The authors are grateful to the Ministry of Health of the Republic of Indonesia for providing a report as material for analysis in this study.

**Reference**


Development of Surface Coating based Hybrid Acrylic Copolymers for Coating Application, Preparation, Characterization and Processing

Farah Safi Khliwi¹, Mohamed Ali Mutar², Ramaah Abdulhassan Sabit³, Entidhar Kadhim Sahib⁴
¹Asst. Lect., Department of Chemistry, College of Education, University of AL-Qadisiyah, Iraq, ²Prof., Department of Chemical Engineering, College of Engineering, University of AL-Qadisiyah, Iraq, ³Bachelor Student. College of Biotechnology University of AL-Qadisiyah, Iraq, ⁴Bachelor Student, Department of Chemistry, College of Education, University of AL-Qadisiyah, Iraq

Abstract

In this study, series of hybrid emulsion copolymers with different composition ratios of acrylic acid, methyl-acrylic acid with butyle-acrylate, methyl-metha-acrylate, polyvinyl alcohol were generated. The preparation was performed in a batch reactor on an industrial scale to obtain the resulting resin. While applying various processes of emulsion polymerization, batch polymerization was chosen to find optimum conditions by investigating the parameters of the monomer percentages, initiators, temperature and time. Dodecyl Benzene Sulphonic acid SDBA as emulsifier, as well as sodium dodecyl sulfonic acid SDBAS as co-emulsifier as redox couple initiator have been chosen as the best and most effective initiators to hybrid acrylic resin processing. Water-dispersed acrylic resin was developed as a hydrophilic monomer, utilizing acrylic acid. To avoid gelatin and agglomeration, the reaction temperature of the acrylic resin preparation was taken for 4 hours at 70°C. To understand the effect of silane coupling agent on the properties of hybrid emulsion copolymers, in the presence of trimetyl-chloro saline. FTIR spectroscopy, ¹H NMR spectra, thermal analysis as well as mechanical tests were widely studied confirmation of the hybrid acrylic resins. The various physic-chemical properties of hybrid emulsion acrylic copolymers including density, viscosity, chemical resistance and volatile matter were studied. The results show that hybrid emulsion acrylic copolymers are readily soluble in aprotic polar solvents such as (Toluene, Acetone, Benzene, xylene, DMF, DMSO, Methanol, and ethanol) without being in need for heating. The emulsion copolymers obtained had a great solid content as well as have been used as binder in emulsion paints. These polymers have very useful properties such as high anticorrosive and chemical resistance, the experimental results reveal.

Key Words: hybrid acrylic copolymers, Binder, Surface coating, Batch polymerization

Introduction

Drying Test: Ethanol was used to clean aluminium plates to ensure no pollutants are present to impact the test. A hand coating with a particularly defined thickness has been used to spray the resin uniformly on the test plate surface. Density Test

Viscosity Test: Brook field rotary Viscometer Ku-2 model RVDV-II+P8500 was used to measure the viscosity at 25°C, and using a variousspindle with velocities

Volatile Matter Test: In previously weighted watch glass as well as heated floor (2h) at (135-140) °C, three specimens were put in the oven within min after the
preparation of methacrylic emulsion copolymers. The non-volatile matter was determined from the original and final watch glass weights variation. The average value of three results has been stated as the non-volatile percentage matter.

**Corrosion Resistance Test:** A salt spray test (ASTMD1654) was conducted to study the corrosion resistance of acrylic emulsion copolymers over the coatings. A 5% NaCl salt water solution was used as a corrosive environment; a 144-hour test was conducted. Coated panels were regularly inspected for blistering or sign of corrosion after (24 hours = one day, 72 hours = 3 days, 144 hours = 6 days) (12).

**Abrasion Resistance Test:** The test has been conducted to assess a resistance for coating films for abrasion affected via an abrasive fall from a specified height through a guide tube onto a coated metal (ASTMD968). It has been used as an abrasive factor for silica sand. This was sprayed on the coated metal when some scratches were found. The volume of sand that changes to some degree the thickness of a film determines the resistance to abrasion. In terms of “l per micrometre,” abrasion is given.

**Results and Discussions**

**Synthesis and Characterization of (PFA1):**
These polymer were synthesized by the emulsion polymerization for MAA by Styrene in the presence of Dodecyl Benzene Sulphonic acid SDBA as an emulsifier, sodium dodecyl sulfonic acid SDBAS as co-emulsifier and trimethylchlorosilane (TMCS). At (70°C) for (4 hours).

**Characterization of (PFA1) FTIR Spectra:**

![Figure 1: FTIR for PFA1](image)

**Synthesis and Characterization of (PFA2):**
This polymer has been synthesized by a emulsion polymerization for MAA with Epoxy in the presence of Dodecyl Benzene Sulphonic acid SDBA as an emulsifier, sodium dodecyl sulfonic acid SDBAS as co-emulsifier and trimethylchlorosilane (TMCS). At (70°C) for (4 hours).

**Characterization of (PFA2) FTIR Spectra:**

![Figure 2: FTIR for PFA2](image)

**Synthesis and Characterization of (PFA3):**
This polymer has been synthesized by a emulsion polymerization of MAA with BA and P.V.A in the presence of Dodecyl Benzene Sulphonic acid SDBA as an emulsifier, sodium dodecyl sulfonic acid SDBAS as co-emulsifier and trimethyl-chlorosilane (TMCS). At (70°C) for (4 hours).

**Characterization of (PFA3) FTIR Spectra:**

![Figure 3: FTIR for PFA3](image)

**Synthesis and Characterization for (PFA4):**
This polymer has been synthesized by a emulsion polymerization for AA with BA and MMA, and styrene in the presence of Dodecyl Benzene Sulphonic acid SDBA as an emulsifier, sodium dodecyl sulfonic acid SDBAS as co-emulsifier and trimethyl chlorosilane
Characterization of (PFA4) FTIR Spectra: The FTIR spectra of (PFA3) indicated absorption bond for (OH carboxylic) in (3400 cm\(^{-1}\)), (C=O ester) in (1770 cm\(^{-1}\)), (CH\(_3\)) for (1350, 1420 cm\(^{-1}\)), (C=O carboxylic) in (1725 cm\(^{-1}\)), (C=O ester) in (1110 cm\(^{-1}\)), (C-H aromatic) in (3010 cm\(^{-1}\)), aromatic (C=C) in (1650 cm\(^{-1}\)), (C-H) aliphatic in (2900 cm\(^{-1}\)).

Synthesis and Characterization of (PFA5): This polymer has been synthesized by emulsion polymerization for AA with BA and MMA, and Epoxy in the presence of Dodecyl Benzene Sulphonic acid SDBA as an emulsifier, sodium dodecyl sulfonyl acid SDBAS as co-emulsifier and trimethyl chlorosilane (TMCS). At (70\(^{\circ}\)C) for (4 hours).

Characterization of (PFA5) FTIR Spectra: The FTIR spectra of (PFA6) indicated absorption bond for (OH carboxylic) in (3400 cm\(^{-1}\)), (C=O) carboxylic in (1650 cm\(^{-1}\)), (C=O) ester in (1730 cm\(^{-1}\)), (C-H) aliphatic in (1370, 1420 cm\(^{-1}\)), (C=O) ester in (1200 cm\(^{-1}\)), (C=O epoxy for 1200 cm\(^{-1}\)).

Density Test: A substance density and the volumetric mass density are its mass per unit volume. The results were observed for the emulsion copolymers prepared at the density of emulsion acrylic copolymers increase when the ratio of butyle-acrylic increases. The density of emulsion acrylic copolymers increases, when the no. of monomer increasing, and the density of emulsion acrylic copolymers with epoxy increases more than styrene.

Viscosity Test: A fluid’s viscosity is a measure of its resistance to slow but steady deformation caused by shear stress or tensile stress. For fluids, this equates for an indirect “thickness” concept. The viscosity of the solution is so vital to characterization polymers area measure of molecular weight of polymer as the viscosity of the solution is a measure of the size. The factors affecting viscosity: Pressure: Pressure viscosity little importance, but viscosity effect appears when the pressure increases (68 bar). Temperature: When temperatures decrease viscosity because at high temperature of liquid increase distances between molecules less friction and therefore less viscous. The ecology of different types of the formulated binders shows a reduction through viscosity as the shear rate increased. Appropriately, it has been found that all the designed binders are thinning polymers (a thixotropic material) that become more fluid with an increased period of application or increased shear average raised.

Drying Test: Denser layers (120\(\mu\)m) are in need of more time to dry in comparison for thinner layers (30\(\mu\)m). A thicker films mechanism for drying necessities a great deal to form cross-link between chains. The thicker films emulsion copolymers drying time can radically increase with the increasing of styrene (11).
**Table 1: Measurement of Drying**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample 2</th>
<th>Sample 1</th>
<th>Emulsion-co-polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:35</td>
<td>2:20</td>
<td>2:15</td>
<td>PFA1</td>
</tr>
<tr>
<td>2:50</td>
<td>2:45</td>
<td>-</td>
<td>PFA2</td>
</tr>
<tr>
<td>1:55</td>
<td></td>
<td></td>
<td>PFA3</td>
</tr>
<tr>
<td>1:45</td>
<td></td>
<td></td>
<td>PFA4</td>
</tr>
<tr>
<td>3:05</td>
<td></td>
<td></td>
<td>PFA5</td>
</tr>
<tr>
<td>3:20</td>
<td></td>
<td></td>
<td>PFA6</td>
</tr>
</tbody>
</table>

**Volatility Matter Test:** There is a difference in the value of the volatility between each emulsion copolymers prepared. The (PFA1) shows least value of volatility, because this monomer is hydrophilic, while (PFA2) shows the high value of volatility due to length chain of copolymer containing in its structure on hydrophobic monomers therefore the volatility increased.

**Testing Chemical Resistance:** In two media, distilled water, HCl, H2SO4 as well as NaOH solution, the resistance of emulsion acrylic copolymers was calculated, describing that after 18 hours of immersion in distilled water there was no impact on emulsion copolymers film, which was ample time to analyse the water resistance. While the emulsion copolymer film was submerged in the powerful alkaline solution as well as acid solution (3N NaOH) (3N HCl) as well as (3N H2SO4), the film was blanched for 8 hours after immersion, blistered for 16 hours after immersion as well as extracted for 24 hours after immersion.

**Table 2: Chemical Resistant for Emulsion Copolymers**

<table>
<thead>
<tr>
<th>PFA6</th>
<th>PFA5</th>
<th>PFA4</th>
<th>PFA3</th>
<th>PFA2</th>
<th>PFA1</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>H2O</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NaOH</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>HCl</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>H2SO4</td>
</tr>
</tbody>
</table>

(+) chemical resistant for polymer (no soluble) (-) chemical resistant for polymer (soluble)

Emulsion-methacrylic polymers demonstrated high solubility in various solvent forms. The sample solubility, determined by a solubility (0.01 g) of every sample formulated resin as well as melted by (2ml) of a solvent, including (DMF, acetone,) (Polaraprotic) non-polar solvents like (Benzene, CHCl3) as well as other solvents like (HCl, H2SO4). With the distance among the resin’s molecular series, solvent molecules raise. The gaps between long chains with a resin pendant side group are occupied with solvent molecules as they fill the space made accessible through the motion of a series. When moves put two series next to each other, hence, attractive forces for short-range are formed resulting in restricted chain movement and therefore the creation of the vicious system$^{(12,13)}$. 
Table 3: Solubility of Emulsions

<table>
<thead>
<tr>
<th>Solvent</th>
<th>CHCl3</th>
<th>THF</th>
<th>DMF</th>
<th>DMSO</th>
<th>CH2Cl2</th>
<th>Benzene</th>
<th>Acetone</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>PFA1</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>PFA2</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>PFA3</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>PFA4</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>PFA5</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>PFA6</td>
</tr>
</tbody>
</table>

**Corrosion Test:** Salt spray tests are perhaps the most common and most controversial tests pertaining to corrosion resistance. It is well known that salts like sodium chloride could cause rapid corrosion for ferrous substrates, so it is beneficial to have knowledge about the actions of a specific system in both intact as well as deteriorated coating films to protect those substrates from corrosion. Even so, they are well known, as well as in the absence of long-term corrosion data, despite the issue of reproducibility, they are very useful guides to perform. They are also unlikely to have been discarded. Some workers consider them impractical due to the degree of acceleration of the corrosion process they accomplish as well as the uncertainty in the extent of the damage sustained in some for the tests. It should be said that the incorporation for (tri chloro-methyl saline) in the backbone for acrylic polymers has helped develop a coating with improved resistance to moisture including salt spray. With the rise in monomers, the resistant characteristics in formulated polymers rise, showing that the coating consistency raises and is less corroded, so it provides the binder so much strength to withstand weather conditions like moisture and thus enhances the binder’s durability to protect the surface from corrosion damage to hybrid polymers.

**Thermo Gravimetric Analysis (TGA) Study:** The thermal properties of 2 specimens among these emulsion acrylic copolymers were gated in the vest by means of thermogravimetric analysis (TGA) in an Argon at a heating rate of 10°C / min. Outcomes like Ti, Top, Tf, T50 percent, percent residue at 700°C, as well as char yields at 600°C Treatment. Temperatures of 50 percent weight loss (PFA1, PFA2, PFA3, PFA4, PFA6) as a typical predictor for polymer thermal stability were all 700°C, char yields (PFA1), 55 percent (PFA2), 57 percent (PFA3), 52 percent (PFA4) 45 percent and (PFA6) 42 percent at 600°C in Argon atmosphere, where it means that they could meet the temperature-resistant specifications that can be applied to surface coating. The weight residue (PFA1) is 68% (PFA2), 56% (PFA3), 58% as well as 61% (PFA4) at 700°C.

**Differential Scanning Calorimeter Analysis (DSC) Study:** Is fully dissolved and afterward the sample’s rate of absorption to the sun, as well as the degree for crystallization (Tc) of the mix (250 °C), was set via the curve. The outcomes of (PFA2) polymer showed the glass transition (Tg) value of the mix (120 °C), suggesting a rise in temperature flow and then raising the specimen absorption rate to temperature until it reaches the melting point (Tm) at (490 °C). The crystallization rate (Tc) for the mix (350 °C) was calculated in the curve. The outcomes of (PFA3) polymer the glass transition (Tg) value for a mix (65 °C), indicating an increase in the temperature flow as well...
as then a raise in the absorption rate of a specimen at the melting point (Tm) at (480 °C) when completely melted and afterward the absorption rate of a specimen at the heat and by the curve was set the crystallization rate (TC) of a mix (20° C). The outcomes of (PFA4) polymer the glass transition (Tg) value of the mix (110 °C), suggesting a rise in the temperature flow and afterward the absorption rate of a specimen at the temperature until it reaches the melting point(Tm)at(460 ° C) and then the absorption rate of a specimen at the heat as well as the mix (370) forming a curve (Tc).

Conclusions

From the results, we can infer that all acrylic copolymers formulated for the emulsion had non-Newtonian pseudo-plastic form. High shear rate, high viscosity as well as high compliance were provided by the methacrylic acid. For the composition, the polymer having (MAA) monomer was of high thermal stability. Analysis of salt spray indicates enhanced corrosion resistance for hybrid binders with increased resin content. Due to the chemical reaction through the reactive groups incorporated for polymer as well as its main chain, the physical properties are subject to adjustment of thermal treatment temperature. The higher the temperature of thermal treatment, therefore better physical characteristics are reached for copolymers. A hybrid in acrylic through polymerization for emulsion, resulting in lower VOC compared to traditional systems. It shows that in a hybrid system, by adding two elements, we can boost the system’s properties as well as rise good properties as the quality of one element rises the other characteristics are not impacted. Thus these resins could be used in coatings at which good requires corrosion-resistant paints. Corrosion resistance, resistance to abrasion as well as resistance for different chemicals.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required

References

14. Laudone, G.M.; Matthews, G.P.; Gane, P.A.C. Effect of Latex Volumetric Concentration on Void Structure, Particle Packing, and Effective Particle Size Distribution in a Pigmented Paper Coating.


Evaluation of Primigravida Women’s Childbirth Self-efficacy at Al-Elwea Maternity Hospital in Baghdad City

Fatima Fadel Benyian¹, Rabea Mohsen Ali²

¹Assistant Instructor, ²Professor, University of Baghdad, College of Nursing, Maternal and Neonate Nursing Department, Baghdad, Iraq

Abstract

Pregnant women who believe themselves have capabilities to cope with childbirth, they feel able to control labor stress. On the other hand, if they believe themselves uncappable to cope with labor, they cannot control labor stress and may choose cesarean delivery.

Objectives: To evaluate women’s self-efficacy of childbirth and find out the association between women’s self-efficacy and study variables.

Methods: Descriptive study of non-probability (purposive sample) was used to collect the data from (100) women. A pilot-test was conducted to determine the reliability of the questionnaire. Data were analyzed through the use of SPSS.

Results: The higher percentage of women’s age was (20-29) years graduated from secondary school. About one-third of them in gestational age 32 weeks. More than two-thirds of them are preferred cesarean birth. They have a low childbirth self-efficacy. There are significant differences between women’s age, education, occupation, delivery preference, and childbirth self-efficacy.

Conclusion: This study finds that primigravida women have low childbirth self-efficacy, and there are significant differences between women’s self-efficacy with the demographic and reproductive variables.

Recommendations: Primigravida women need prenatal education and encouragement regarding labor to increase their believe of own capability to control and cope with labor stress.

Keywords: Primigravida, Childbirth, Self-efficacy, Women

Introduction

Self-efficacy is essential factor that required to successfully perform a certain behavior in a specific situation, also it is important for change behavior and self-control [1].

Self-efficacy believes to control labor stressors has a significant role in anxiety arousal.

Pregnant women who have high level of childbirth self-efficacy is associated with lower levels of labor pain, anxiety, require less obstetric intervention and analgesia compared to low levels of childbirth self-efficacy [2][3].

Self-efficacy has four sources, first mastery experiences (performance accomplishments); second vicarious experiences (modeling); third verbal (social) persuasion; and fourth physiological and affective states [4].

Corresponding Author:
Fatima Fadel Benyian,
Assistant Instructor, University of Baghdad, College of Nursing, Maternal and Neonate Nursing Department
Baghdad, Iraq.
Email: fatmaf@conursing.uobaghdad.edu.iq
Pregnant women’s self-efficacy can affect prenatal well-being such as to mood changes, anxiety, and childbirth fear. Fear of childbirth also associated with anxiety and depression\(^5\).

Vicarious experience is observing others perform a certain behavior in specific situation that an individual wants to perform. It does not always have a positive effect on self-efficacy, an individual must find a role-model to develops self-efficacy beliefs individuals, through observation and interpretation of others’ performance. Observation of successful performance may increase self-efficacy, while observation of failed performance may decrease self-efficacy\(^7\).

Pregnant women’s perception of pain during labor can vary according to their expectations and prenatal preparation; labor length; position of fetus; fear, anxiety; body image; self-efficacy; and support. Women who believe that they can control their situation (have self-efficacy) than are those who do not feel in control\(^8\).

**Materials and Methods**

Descriptive analytic study design was used to evaluate women’s childbirth Self-efficacy. The study was performed from October 2020 to February 2021. Non probability (purposive sample) used to collect the data from (100) women. Inclusion criteria (First pregnancy, Singleton pregnancy, Gestational age of (28 to 32) weeks, has no medical and obstetrical problem, Literacy, Accept participation in study). A pilot study conducted in order to determine the reliability of the questionnaire in a sample of (10) women (r1= 0.88). Content validity was determined through a panel of (21) experts their experience mean and SD 25.8 (11.1). The data was collected after obtaining the agreement from women to participate in this study. Data are analyzed through the use of SPSS version 26.

**Results**

**Table (1) Distribution of Study Sample According to Socio-demographic Characteristics**

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age / years</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-19</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>20-29</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>30-39</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>40-49</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Mean=20.67  SD=3.57</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Educational level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Read &amp;write</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Primary School</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Secondary School</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Institute graduate</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>College graduate</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Master and higher</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Occupational Status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employee</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Housewife</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
This table illustrates that the highest percentage (44%) of women’s age are (20-29) years, with mean (SD) 20.67 (3.57). Regarding educational level more than one-third of them (40%) are graduated from Secondary School. Regarding occupation the majority of them (92%) are housewives.

Table (2) Distribution of Study Sample According to Reproductive Characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planned pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Gestational Age/ weeks</td>
<td></td>
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</tr>
<tr>
<td>28</td>
<td>8</td>
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</tr>
<tr>
<td>29</td>
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<td>20</td>
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<td>32</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mean (SD) 30.4 (1.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration between marriage and pregnancy/ months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;6</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>7-12</td>
<td>13</td>
<td>13</td>
</tr>
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<td>13-18</td>
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<td>8</td>
</tr>
<tr>
<td>19-24</td>
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<tr>
<td>&gt;24</td>
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<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mean (SD) 9.37 (12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conception assistant methods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>No</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Delivery Preference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>NVD</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
This table show that the majority of women (94%) have planned pregnancy. About one-third of them (31%) their gestational age 32 weeks, with mean (SD) 30.4 (1.3). Regarding Duration between marriage and pregnancy more than two-thirds of them (65%) are less than six weeks, with mean (SD) 9.37 (12.5). The higher percentage of them (63%) didn’t use conception assistant methods. More than two-thirds of them (64%) are preferred cesarean birth.

![Pie chart showing childbirth self-efficacy](image1)

*Figure (1) Women’s Childbirth Self-efficacy*

![Bar chart showing self-efficacy subdomains](image2)

*Figure (2) Women’s Childbirth Self-Efficacy Subdomain*
Table (3) Association between Women’s Childbirth fear and Demographic Characteristics

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>Chi square statistics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value</td>
<td>df</td>
</tr>
<tr>
<td>Age / years</td>
<td>21.1</td>
<td>6</td>
</tr>
<tr>
<td>Educational level</td>
<td>63.23</td>
<td>10</td>
</tr>
<tr>
<td>Occupational Status</td>
<td>40.88</td>
<td>2</td>
</tr>
</tbody>
</table>

(df) degree of freedom, (Sig) significant Probability value (P < 0.05), (NS) Non-Significant. (S) significant, (HS) High Significant

Table (3) results presents that there are significant differences between women’s age and childbirth Self-efficacy. Also, there high significant differences between women’s educational level, occupational status and childbirth self-efficacy.

Table (4) Association between Women’s Childbirth fear and Reproductive Characteristics

<table>
<thead>
<tr>
<th>Reproductive Characteristics</th>
<th>Chi square statistics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value</td>
<td>df</td>
</tr>
<tr>
<td>Planned pregnancy</td>
<td>1.4</td>
<td>2</td>
</tr>
<tr>
<td>Gestational Age/ weeks</td>
<td>10.3</td>
<td>8</td>
</tr>
<tr>
<td>Duration between marriage and pregnancy/ months</td>
<td>19.2</td>
<td>8</td>
</tr>
<tr>
<td>Delivery Preference</td>
<td>9.53</td>
<td>2</td>
</tr>
</tbody>
</table>

(df) degree of freedom, (Sig) significant Probability value (P < 0.05), (NS) Non-Significant. (S) significant, (HS) High Significant

Table (5) results shows that there are no significant differences between women’s pregnancy planning, gestational age and childbirth Self-efficacy. There are significant differences between duration of marriage before pregnancy, delivery preference and childbirth Self-efficacy.

**Discussion**

The findings of the present study are consistent with Matinnia et al., who found that the mean age (25) years in the study sample which ranged from (18-34) years, most of them had planned pregnancies and more than two-thirds (62.6%) requested cesarean birth without any medical indication [8].

Kabukçu et al., found the majority of nulliparous women (94%) had a planned or a desired pregnancy, and more than half (56%) of nulliparous women preferred cesarean delivery [9].

The results of the study are inconsistent with Serçekuş et al., who mentioned the mean age and SD (27.7 ± 4.5) years of pregnant women which ranged from (25-63) years, and their mean gestational age and SD (26.9th week ± 0.8). The highest percentage of them
(86.8%) were university graduates and the majority of them (81.2%) were employed [10].

Zhang et al., who found that the mean age and SD (29.33 ± 3.09) years in the study sample (n=1211) which ranged from (21-36) years, and their mean gestational age and SD (23.35 \text{th} week ± 3.22) ranged from (13.6-27.5) weeks. The majority of them (80%) were had completed Diploma level or postgraduate education and more than two third (67%) employed [11].

The differences in the above studies could be due to this study conducted among different sample in the different setting.

The current study show that the highest percentage of study sample have low childbirth self-efficacy, with mean score (1.49) and relative sufficiency (29.8%).

Toohill et al. reported a significant higher percentage of women in the intervention group scoring higher on the CBSEI (76.3%) [12]. While Gao et al., (2015) found that average total score of childbirth self-efficacy among the pregnant women was 219.69 (SD¼50.56, range51-320). They found women have high level of outcome expectancy Mean (SD) \( \frac{1}{4}112.60 \) (\( \frac{1}{4}16.95 \)) range (20–160) and efficacy expectancy Mean (SD) \( \frac{1}{4}107.00 \) (\( \frac{1}{4}27.02 \)) range16–160 [13].

The present study found there are significant differences between women’s age, education, occupation, delivery preference and childbirth self-efficacy.

Lower efficacy expectancy was associated with higher FOC while preference for a caesarean section was not. Improvement of self-efficacy could be a part of care for women with FOC during pregnancy; however, it would not be enough for fearful women who wish to have a caesarean section [14].

The results of the study are inconsistent with Schwartz et al., (2015) who found no relationship between age, education, or of having a history of abortion and self-efficacy for first stage of labor in nulliparous or multiparous women [15].

Fadhil, stated that women’s perception of childbirth pain may be influenced by several factors such as culture, childbirth knowledge, fear of childbirth. Utilization of childbirth emotional support, holding hands can make a difference to childbirth coping [16].

**Conclusion**

This study concluded that the majority of study sample have low childbirth self-efficacy. more than two-thirds of them were preferred cesarean delivery. There are significant statistically differences between women’s age, education, occupation, gestational age, delivery preference with childbirth fear.

**Recommendation**

Physicians and nurse-midwives should provide education and support to pregnant women, especially for primigravida women during prenatal and antenatal period to increase their childbirth self-efficacy. Study factors that affecting childbirth self-efficacy.

**Funding:** this research was funded by Author. Moreover,

**Conflict of Interest:** None declared.

**Ethical Approval:** was taken from committee in College of Nursing/ University of Baghdad.

**Acknowledgments:** we would like to thank all the pregnant women who participated in this study.

**References**


Neoplasia of the Nictitating Membrane in A Domestic Short-Haired Cat based on Cytology of Fine Needle Aspiration

Fatimah Alaydrus¹, Lita Rakhma Yustinasari², Juliano Mwenda Ntoruru³

¹Veterinary Practitioner, Maha Pet Care Clinic, Jln. Phh Mustofa Surapati Core F-20, Bandung, Indonesia,
²Lecturer, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia,
³Research Assistant, Meru University of Science and Technology, Kenya

Abstract

A 3-year-old male domestic short-haired cat was presented with the complaint of a dark red mass of the nictitating membrane of the right eye covered most of the cornea, mucopurulent eye secret, lost appetite, medium dehydration, and cough. Physical examination was performed and showed high temperature (38.6°C). Ultrasonographic examination was not performed due to tool limitations. Cytology of fine-needle aspiration masses suggested an ophthalmic neoplasm with the characteristic of neutrophils infiltration, multinucleated giant cells, and cells having granular cytoplasm. Enucleation bulbi was performed subsequently to exenteration the eye and the contents of the orbit.

Keywords: cat, cytology, neoplasia, nictitating membrane

Introduction

Tumors are the frequent cause of death in cats (32%); which 0.34% of those affect the ophthalmic area.¹ Most cases of the feline ophthalmic disease were neoplastic. The neoplasms were organized according to location (orbit, conjunctiva, eyelids, nictitating membrane, globe). Neoplasia of the nictitating membrane (NM) is relatively uncommon in veterinary species, especially cats.³ However, the following neoplasms have been reported such as adenocarcinomas, adenomas, carcinomas, ependymomas, fibrosarcomas, histiocytic sarcoma, mast cell tumors, melanocytomas, melanomas, osteosarcomas, papillomas, and squamous cell carcinomas.⁴ ⁵ When considering cases with neoplasia, an evaluation of how the health and longevity of the eye and patient is essential to determine biologic behavior. Inflammation and secondary glaucoma may arise from neoplasms, which are considered vision-threatening and may cause loss of the eye.⁶ The most common cause of enucleation in cats is the ophthalmic neoplasm.²

Case History

A 3-year-old (3.6 kg) male, domestic short-haired cat was referred to the Maha Pet Care Clinic, Bandung, Indonesia with the complaint of irregular roughed masses affecting the bulbar and nictitating membrane of the right eye. The cat’s eye was injured in a fight, but the cat disappeared and returned two weeks later in a very bad condition. The nictitating membrane was severely hyperemic and covered most of the cornea (Figure 1) with inflammation on the eyelid, so we could not evaluate the internal ocular structures. The cat appeared to be in pain. On physical examination findings were normal. The cat had no history of other medical problems, and its vaccination status was current.

Ultrasonographic examination was not performed due to tool limitations. Fine-needle aspiration of the nictitating membrane’s masses suggested an ophthalmic neoplasm. Cytological evaluation was of an unencapsulated, well-demarcated, multilobular neoplastic mass composed of basal cells seated within a moderately dense fibrovascular stroma (Figure 2). Based on the cellular morphology, the neoplasm was diagnosed as a presumptive basal cell tumor. Due to the lack of natural borders and the size of the tissue fragments, it was difficult to determine whether the neoplasm represented
a benign process or a malignant process. There was no evidence of sebaceous or squamous differentiation in the examined sections. Considering the extensive nature of the lesion, the cat’s pain, and the owner’s financial constraints, we recommended enucleation bulbi to remove the eye and the contents of the orbit.

**Treatment**

Pre-anesthetic drugs included atropine sulfate (0.08 ml/kg) and acepromazine (0.02 ml/kg) given intramuscularly. Anesthesia was induced with intramuscular ketamine (0.05 ml/kg).

The exenteration was performed by enucleation bulbi approach. Scalpel blade No. 22 was used to incise the skin from the eyelid edge in an elliptical fashion to allow for smooth skin apposition at closure. The orbital contents were removed which were kept as close to the bones of the orbit as possible. The medial and lateral orbital ligaments were transected, and the nictitating membrane was completely removed. The dissection was continued around the eye and mass until the optic nerve was encountered. The nerve was then transected, and the tissue was completely removed. The subcutaneous tissue was closed with vicril 2.0 in a simple continuous suture, and the skin was closed in a simple interrupted suture(Figure 2).

The surgery was successful and the cat recovered from anesthesia. It was released to the owner following day. For pain management, the cat was given tramadol (0.1 ml/kg intramuscularly) for three days. A two-week postoperative reevaluation was scheduled and the cat was in a good condition.

**Discussion**

Following 2 weeks history of eye enlargement and ocular discharge from the right eye, an ophthalmic examination was performed on both eyes. Severe mucoid discharge was detected on right eye, inflammation of the nictitating membrane was covered almost entire the right eye. Pupillary light reflexes both direct and indirect were present. Incisional biopsy sections were of an infiltration of neutrophils, multinucleated giant cell, granular cytoplasm, different cells sized, irregular outlines of nucleus, and no mitotic cells was found. Based on the cellular morphology, the neoplasm was diagnosed. Due to the lack of natural borders and the size of the tissue fragments, it was difficult to determine whether the neoplasm represented a benign process or a malignant process. The nictitating membrane is composed of numerous tissue types including conjunctival epithelium, vascular substantia propria, lymphoid tissue, supporting hyaline cartilage, and associated glandular tissue which contributes to lacrimation. Once nictitating membra removed, the eye become dry and possibility of corneal ulcers. In this case, neoplasm is located in the nictitating membrane, metastasis/recurrence to the eyeball will sooner or later occur. Based on the case, we did not have any choice except surgical enucleation bulbi and the cat will loss of the right eye. The cause of the neoplasm remains unknown.

**Conflict of Interest:** There is no conflict of interest among the authors.

**Source of Funding:** This study was self-funded.

**Ethical Clearance:** Compliance with ethical standards.

**References**


Nursing Intervention Program for Enhancing Coping Patterns among Nursing Students

Fatma Adel Ahmed Ahmed¹, Sorayia Ramadan Abd El-fattah², Galila Shawky El-Ganzory², Hanaa Ezz El-din Prince³

¹Research Scholar, ²Professor, ³Lecture, Psychiatric-Mental Health Nursing, Faculty of Nursing, Ain-Shams University

Abstract

Background: Nursing students face many sources of stress during undergraduate nursing education and training as, fear of the unknown, lack of professional nursing skills. Aim: to enhance coping patterns among nursing students. Design: a quasi-experimental design was utilized to conduct this study. Setting: The study was carried out at a faculty of nursing Ain-shams University. Subjects: A convenient sample was selected for this study was performed on 80 nursing student. Tools: 1) Self-administered questionnaire sheet. 2) student clinical stressors Scale. 3)Coping patterns Scale. Results: The present study revealed that there were highly statistically significant differences were found between pre-post program implementation after five months regarding student clinical stressors and coping strategies among nursing students. Also, there were statistically significant correlations between total clinical stressors and coping patterns at a pre - post-program after five months. Conclusion: nursing students had high levels of clinical stressors, which decreased after the implementation of the management program with a highly statistically significant difference (P≤ 0.001). Recommendations: Designing systematically continuous Special remedial programs need to be constructed for students with high clinical and academic stressors and low coping patterns to provide proper information and psychological support that help them improve their academic performance which in turn increase their coping patterns and reduce the sense of stress.

Keywords: Clinical Stressors, Coping Patterns, Nursing Students, Management program.

Introduction

A nursing student is a student in a post-secondary educational program that leads to certification and licensing to practice nursing. The title ‘nursing student’ usually applies to students enrolled in a registered nurse or practical nurse program. A nursing student can be enrolled in a program that leads to a diploma, an associate degree or a Bachelor of Science in nursing (1).

Nursing is regarded as a demanding and stressful profession. Nursing students and nursing educators need to understand the concept of coping, the application of the coping concept in pre-registration nursing may provide fresh insights into nursing recruitment, retention and ultimately patient/client outcomes (1)(2). Coping has been highlighted as an important theoretical and practical construct. It has the potential to enable individuals to cope better and experience less stress thus contributing to a healthy and stable workforce (2).

There are many ways of coping with stress. Their effectiveness depends on the type of stressor, the particular individual, and the circumstances, for example, if we consider the way students deal with stresses such as exams, we will see a range of different coping responses (3). Higher education students cope in different ways with varying levels of success (4)(5).

The main sources of stress are academic and time concerns, fear of failure, classroom interactions, and...
economic issues. Apart from this, the parental system also affects a lot of these students. The parents have unlimited expectation from their children and therefore they impose their desires on them. The impact of these influences results in many students reporting emotional problems, anxiety, stress, and other neurotic problems😃😕.

Considerable stress is involved in nursing education. It is found that higher levels of stress and higher levels of physiological and psychological symptoms were found among nursing students than students in other health-related disciplines. Nursing Students experience increased tension before their clinical rotation and written examination especially their final examination📮.

An understanding of the stress experienced by students enables nurse educators to assist students in their stress and to provide a supportive learning environment for them, and there should be a collaborative effort among academic staff members to reduce the unnecessary stressors, this may create a positive attitude among nursing students and enrich emotions📝.

A nursing intervention program for enhancing coping patterns among nursing students include health education, stress management, decreasing maladaptive responses to the identified stressor, enhancing adaptation toward stressors, improving psychological wellbeing, communication skills, psychosocial skills, assertive techniques, relaxation techniques, problem-solving, crisis intervention, environmental change, social support, stigma reduction, role modelling, counselling, motivation, providing emotional support, lifestyle changes and the teaching of coping skills. managing stress effectively and using healthy coping strategies is an important skill required for students who enrol in nursing, for optimum performance in their future life📝.

Significance of the study:

Nursing students often work with vulnerable patients who. are in need of strong support to advocate and assist them to manage their health, and these students must be prepared and ready to assume their role with confidence and competence to free any stress.

Stress is a condition faced by students when they are unable to bear the burdens involved in higher nursing education. Students who learn and use effective coping patterns to deal with stress will most likely develop a positive self-concept. Students who become overwhelmed with stress may feel hopeless and powerless, leading to a feeling of low self-confidence and self-esteem. Thus, the present study will be made for enhancing coping patterns among nursing students.

Aim of the Study

This study aims to enhance coping patterns among nursing students.

The aim will be achieved through:

1-Assessing the practical stressors and coping patterns among nursing students.

2-Designing nursing intervention program for nursing students based on assessment.

3-Implementing nursing intervention program for enhancing coping patterns among nursing students.

4-Evaluating the effect of a nursing intervention program for enhancing coping patterns among nursing students.

Hypothesis:

Improving nursing students coping patterns will decrease practical stressors after the implementation of a nursing intervention program.

Subjects and Methods

- Research design: A quasi-experimental research design was used for the current study.

- Sampling: The sample size included all available nursing students in the faculty of nursing from fourth grade were around 80 students.
Tools of data collection

1- Self-administered questionnaire sheet (Appendix I):

It was developed by the researcher based on a comprehensive reviewing of the literature. It was reviewed and accepted by supervisions to collect data concerning nursing students perception and expectations about the nursing profession. The questionnaire was in the form of multiple-choice questions (MCQ). The nursing students were requested to circle the statement of choice in each question. The questionnaire consisted of two parts:

- Demographic data of the nursing students such as age, sex, academic year, place of residence and monthly income.

- The nursing students’ perception and expectations about the nursing profession such as what are the reasons for joining the college, what do you think of the nature of the college, what is the community’s view of the profession in your opinion, is nursing in Egypt similar to that of developed countries and the role of media for nursing.

2- Scale to assess student clinical stressors (SCSS):

This scale was developed by (El-Sebaie, 1991). It is intended to measure the stress experienced by undergraduate nursing students during clinical practice. This scale includes 34 statements on a 4-point scale: Unstressful, mild stressful, moderate stressful and severely stressful. the scale assesses three subscales as follows:

- Clinical setting.
- Work with different types of patients.
- Evaluation process.

3- Coping strategies scale:

This scale was developed by Ibrahim (1994). It consists of 69 statements measuring coping strategies on a 3-point scale: never, sometimes, and always. The sheet is divided into two main categories:

- **Problem-focused coping:** this part deals with 24 problem-oriented coping behaviours. These behaviours are primarily aimed at using cognitive skills in handling stressful situations through problem-solving techniques. They are classified into four subscales which refer to:
  - Seeking out information (3 statements)
  - Positive reinterpretation (6 statements)
  - Exert of restraint (5 statements)
  - Active coping (10 statements)

- **Emotional-focused coping:** this part deals with 45 emotional-focused coping behaviours. These behaviours are primarily adopted to manage the emotions accompanying a stressful situation. They are classified into seven subscales which refer to:
  - Helplessness (10 statements)
  - Mental disengagement (7 statements)
  - Wishful thinking (8 statements)
  - Turning to religion (3 statements)
  - Emotional discharge (13 statements)
  - Acceptance (3 statements)
  - Denial (1 statement).

**Results**

Table (1) shows that more than two-thirds of nursing students (67.5%) were in the age group 18->20, with Mean±SD: 1.49±0.50. Two-third of nursing students (60.0%) were Sufficient monthly income. More than half of the nursing students (55.0%) were males. More than two-thirds of nursing students (68.8%) were lived in the urban area and more than two-thirds of them (71.3%) were lived with their family.
Table (1): Frequency distribution of Socio-demographic characteristics of nursing students (n = 80)

<table>
<thead>
<tr>
<th>Items</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>· Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>§ 19-&gt;21</td>
<td>54</td>
<td>67.5</td>
</tr>
<tr>
<td>§ 21-&gt;24</td>
<td>26</td>
<td>32.5</td>
</tr>
<tr>
<td>Mean±SD: 1.49±0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>§ Male</td>
<td>44</td>
<td>55.0</td>
</tr>
<tr>
<td>§ Female</td>
<td>36</td>
<td>45.0</td>
</tr>
<tr>
<td>· Accommodation during study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>§ With family</td>
<td>57</td>
<td>71.3</td>
</tr>
<tr>
<td>§ With relatives</td>
<td>4</td>
<td>5.0</td>
</tr>
<tr>
<td>§ In the university city</td>
<td>19</td>
<td>23.8</td>
</tr>
<tr>
<td>· Place of residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>§ Rural</td>
<td>25</td>
<td>31.3</td>
</tr>
<tr>
<td>§ Urban</td>
<td>55</td>
<td>68.8</td>
</tr>
<tr>
<td>· Monthly income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>§ Insufficient</td>
<td>28</td>
<td>35.0</td>
</tr>
<tr>
<td>§ Sufficient</td>
<td>48</td>
<td>60.0</td>
</tr>
<tr>
<td>§ Saving</td>
<td>4</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Table (2): clarifies that, there are highly statistically significant differences between the pre-and post-program of nursing student total clinical stressors, “P-value <0.001”.

Table (2): Distribution of nursing student total clinical stressors pre and post-program (N= 80)

<table>
<thead>
<tr>
<th>Total</th>
<th>Pre Program</th>
<th>Post Program</th>
<th>Chi-Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Low</td>
<td>49</td>
<td>61.3 %</td>
<td>70</td>
</tr>
<tr>
<td>High</td>
<td>31</td>
<td>38.8 %</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>100.0%</td>
<td>80</td>
</tr>
</tbody>
</table>

Table (3): clarifies that, there are highly statistically significant differences between the pre-and post-program nursing student total coping patterns, “P-value <0.001”.
Table (3): Distribution of nursing student total coping patterns pre and post-program (N= 80)

<table>
<thead>
<tr>
<th>Total</th>
<th>Pre-program</th>
<th>Post-program</th>
<th>Chi-Square</th>
<th>X2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Ineffective</td>
<td>46</td>
<td>57.5 %</td>
<td>35</td>
<td>43.8 %</td>
<td>45.99</td>
</tr>
<tr>
<td>Effective</td>
<td>34</td>
<td>42.5 %</td>
<td>45</td>
<td>56.3 %</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>100.0%</td>
<td>80</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>

Table (4): Correlation between Pre-post total score of clinical Stressors and total score of coping patterns among nursing students (n=80).

<table>
<thead>
<tr>
<th>Items</th>
<th>The total score of coping patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-program</td>
</tr>
<tr>
<td>The total score of the clinical stressors Scale</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td></td>
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</tbody>
</table>

Discussion

The current study was conducted on eighty students of the College of Nursing (N= 80), which revealed that most students were in age 19-21 years. This may be due to admission requirements, this result consistent with the study conducted by (13), who assessed socio-demographic and academic profile of nursing students of a public university, stated that the possibility of students aged 19 years feeling insecure regarding the requirements of professional education and of the profession they have chosen.

Concerning sex, the current study revealed that more than half of the students were male. This may be because males are the prominent gender in the nursing practice because of the phenomenon of the general trend of students towards the field of nursing as a work for him a good future. This finding is inconsistent with (3). Who assessed stress levels and coping patterns of nursing students in an international program practicum, showed that the majority of respondents were female.

About residence, the current study revealed that the majority of students were living in urban areas. This may be due to increased urban people aware of the nursing profession, in particular women in urban areas are independent but seek employment to make money but in rural areas rural cultures have been prevented from working as nurses because of rural culture, this justification consistent with the study conducted by (4), who assessed recruit nursing students and stated that students from rural backgrounds are more challenged in adapting to the large urban academic environment, often far from family and community supports.

Concerning monthly income, more than half of the students studied have sufficient monthly income to meet all the requirements of the study, this due to the financial burden and associated additional costs, such as study
costs and the continuing increase in the prices of books. Besides, The students who try to solve the financial burden of the study fees through the working night and have their academic performance affected by the lack of time available for study. This result is also congruent with (5) who discussed The key sources of stress emanated from clinical, academic and financial issues.

About the reasons for joining the college, we find that the most selected answer was a work with a good future. This explains the turnout of male nursing colleges more than females and in recent years it is consistent with (6) who described the many benefits of the nursing profession.

In relation to the effect of the nursing intervention program on total clinical stressors score, the results indicated that about a third of the study were under high clinical stress due to the stresses they were exposed to during practical training. This finding may be due to the most studied students’ fear of the new, unknown clinical environment, the conflict between ideal and real clinical practice, lack of knowledge of clinical history, lack of professional nursing skills, diagnosis and treatment of unknown patients, and the physical. Psychosocial care for patients, fear of making mistakes, giving medicines to children, and the death of the patient. Other sources of reported stress include negative interaction with coaches, monitoring and delaying teachers, bad relationships with medical staff and even talking to doctors.

A significant decrease in the percentage of stress among students was observed after applying the nursing intervention program. These results may be due to the students’ awareness created through the nursing intervention program to enhance coping patterns among nursing students, and it may also be because the nursing intervention program sessions provide the nursing students with specific information about clinical stressors such as the sources of clinical stressors, the effect and methods of prevention, and thus their knowledge increases after undergoing the program, and this means increasing students’ awareness through the Nursing Intervention Program to enhance the coping patterns among nursing students.

These results matched with (14) in the study entitled “Effects of an Online Mindfulness Program on Depression, Anxiety, Stress, and Coping among Undergraduate Second-Year Nursing Students” which revealed that there was highly statistical significant improvement between pre-post program about clinical stressors.

Furthermore, this results matched with (10), in the study entitled “Decreasing stress and supporting emotional well-being among senior nursing students: A pilot test of an evidence-based intervention” who stated that Overall feedback was positive, with participants describing how skill modules helped them establish relationships and manage stress in clinical, academic, and personal settings. Significant reductions in stress and improvements in well-being were also reported.

Also these results agreed with (15), in the study entitled” The effects of group mindfulness-based cognitive therapy in nursing students” who mentioned that stress scores decreased significantly in the experimental group.

On the other hand, these results were contraindicated with (16), in the study entitled” The effect of a peer mentoring strategy on student nurse stress reduction in clinical practice “ who found that knowledge of students in the preprogram equal the post and not changed after implementation of the program these may be due to awareness of students about hazards of substance abuse before program implementation. These findings may be due to, the difference of cultures and the range of education from country to country effects on student responsiveness to programs.

In relation to the effect of the nursing intervention program on total coping patterns score, the findings of the present study revealed that, there were highly statistical significant improvement between pre-post intervention program. This results could have been due to, the program sessions contribute to the improvement of coping related to clinical stressors by provides nursing
students with special information about coping patterns such as identify the concept of coping, the resources for effective coping and altering the mistaken beliefs and replacing them with right beliefs.

This finding were consistent with (17) which conducted study about “Effect of Nursing Intervention on Students’ Stressors related to their Training in Pediatric Critical Care Settings”. The study concluded that, the application of nursing intervention reduce the students’ stressors related to their training in pediatric critical care settings. The study recommended orientation program, identifying students’ needs in the pediatric critical care settings and developing effective nursing interventions to reduce their stress.

In the same line, this results came in agreement with the study of(18), in the study entitled; The effect of a stress and anxiety coping program on objective structured clinical examination performance among nursing students in shiraz, Iran, that found that there were The anxiety coping program improved the examination results of nursing students in the final exam compared to the midterm results.

Furthermore, these results were in harmony with the study conducted by(19), who noted that, there were highly statistical significance improvement pre –post intervention in the study entitled “The effects of group mindfulness-based cognitive therapy in nursing students: A quasi-experimental study”.

On the other hand, these result was contradicted with study conducted by (8) in the study entitled as “Stress and anxiety among nursing students: A review of intervention strategies in literature between 2009 and 2015” they clarified that, there were no statistical difference improvement pre-post intervention. The review concluded further methodologically sound, adequately powered studies, especially randomized controlled trials, are needed to determine which interventions are effective to address the issue of excessive stress and anxiety among undergraduate nursing students.

In correlation to the effect of the nursing intervention program on total clinical stressors score and total coping patterns score. The current study revealed that there was a highly statistical correlation between coping patterns and clinical stressors, these results may be due to, the more information about different coping patterns can cause the better attitudes and beliefs toward clinical stressors, also could attribute these findings to, the booklet which had an excellent source of information and references to the students which helped to increase their knowledge and improve their coping and.

This could be due to using different methods of educational guidelines program as face to face interaction, laptop, discussion and demonstration supported by using real objects, posters, models and handouts which are effective approaches for conveying information.

Additionally, the educational program can increase students level of information through the use of basic principles which are acceptance, understanding, empathy and communication which are helping students to make decisions for themselves according to their information and problems.

These results matched (20) in the study entitled “Stressors and coping of nursing students in clinical placement: A qualitative study contextualizing their resilience and burnout” who mentioned that there was a highly statistical correlation between coping and stressors.

**Conclusion**

Based on the results of the current study; it can be concluded that:

nursing students had high levels of clinical stressors, which decreased after implementation of the nursing intervention program with a highly statistically significant difference (P≤ 0.001). Also, there were statistically significant positive correlations between the total score of student clinical stressors and total score of coping patterns during the pre-post implementation of the nursing intervention program after five months (P≤ 0.001).
Recommendation

In the light of these findings, it was recommended that:

- Replication of the current study on a larger sample is recommended to achieve generalization of the results and wider utilization of the designed program.

- Designing systematically continuous Special remedial programs need to be constructed for students with high clinical and academic stressors and low coping patterns to provide proper information and psychological support that help them improve their academic performance which in turn increase their coping patterns and reduce the sense of stress.

- Implement psychosocial counselling sessions for social workers, instructors who work in the faulty of nursing to teaching them the physiological and psychological changes of students to help their students how to face their problems and provide a chance for students to express themselves and develop their ability to coping effectively.

- Workshops organization at the beginning of each semester for university students, especially those in the first year. In these workshops, they will learn how to deal with those clinical and academic stress, how to manage it and learn some effective time management.

- Training courses and workshops should be organized to improve self-esteem for nursing students and increase their awareness and appreciation of their profession, thus relieving stress towards their perception of careers.

- Communication channels to express students’ reactions should be more positive to increase the assessment of nursing students for themselves and their profession, thus relieving stress within them.

- Further researches about provide attention to clinical and academic stressors and coping patterns among students and identify the factors that affect them and work to address them. Also their instructors, nurses and parents should be offered methodological guidance to work on this throughout the educational process.

Ethical Clearance: The study was approved from ethical and research committee faculty of nursing Ain Shams University, Egypt.

Source of Funding: Self-funding

Conflict of Interest: Nil

Reference


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Molecular Docking Human Plasma Kallikrein to Prevent Acute Respiratory Distress Syndrome (ARDS) in COVID-19 Patient

Feriawan Tan 1, Cindy Aprilia Eka Prasany 1, Anna Surgean Veterini 2, Yuani Setiawati 3, Rizki Awaluddin 4, Fadilah Fadilah 5, Siti Khaerunnisa 6

1 Student, Faculty of Medicine, Airlangga University, Surabaya, East Java 60132, Indonesia, 2 Lecturer, Department of Anesthesiology and Reanimation, General Hospital Dr. Soetomo-Faculty of Medicine, Airlangga University, Surabaya, East Java 60132, Indonesia, 3 Lecturer, Department of Anatomy Histology and Pharmacology, Faculty of Medicine, Airlangga University, Surabaya, East Java 60132, Indonesia, 4 Lecturer, Department of Pharmacy, Faculty of Health Science, University of Darussalam Gontor, Ponorogo, East Java 63471, Indonesia, 5 Lecturer, Department of Medicinal Chemistry, Faculty of Medicine, University of Indonesia, Central Jakarta, Jakarta 10430, Indonesia, 6 Lecturer, Department of Physiology and Medical Biochemistry, Faculty of Medicine, Airlangga University, Surabaya, East Java 60132, Indonesia

Abstract

SARS CoV-2 infection causes various clinical manifestations ranging from mild to severe. Acute Respiratory Distress Syndrome (ARDS) is a severe complication of COVID-19 caused by activation of the kallikrein-kinin system which produces bradykinin which is a potent proinflammatory mediator. This research is an in silico study which aims to determine the potential of active medicinal plant compounds in inhibiting the kallikrein-kinin system. Molecular docking in this study using Autodock 4.2 with Lamarckian GA criteria. Human plasma kallikrein (PDB ID: 5TJX) was docked with 70 compounds and one native ligand and analyzed using Autodock 4.2. The smallest binding energy obtained from docking 5TJX with several compounds in sequence, namely, xanthohumol, nafamostat, demethoxycurcumin, epicatechingallate, beta mangostin, alpha mangostin (-9.52, -9.35, -9.33, -9.28, -9.19, -9.06 kcal/mol). Therefore, the compound shows the best potential as a plasma kallikrein inhibitor. However, further research is still needed to determine the potential of drugs and medicinal plant active compounds for medical treatment.

Keyword: COVID-19, ARDS, Kallikrein, Medicinal Plant, Docking, Health Risk

Introduction

SARS Cov-2 infection causes various clinical manifestations, from asymptomatic, mild, or moderate symptoms, to severe cases requiring intensive care 1. In severe cases, one of the serious complications that often occurs is Acute Respiratory Distress Syndrome (ARDS). Several studies from Wuhan, China reported the incidence of ARDS in COVID-19 patients of 14-29%, and among critically ill patients around 67%. ARDS is caused by extensive endothelial-barrier damage and an uncontrolled ‘cytokine storm’. Increased levels of cytokines in SARS Cov-2 infection result in endothelial dysfunction, deregulation of coagulation, and increased microvascular permeability, leading to tissue edema and shock that can develop into acute lung injury (ALI) and ARDS 2.

The Kallikrein-kinin system mechanism can also contribute to lung injury and angioedema which can lead to ARDS. Kallikrein is a serine-protease enzyme that converts kininogens into kinin plasma protein.
Dysregulation of the Kallikrein-kinin system in COVID-19 patients potentially affected by decreased ACE2 expression due to SARS Cov-2 infection. SARS Cov-2 also expresses cysteine proteases which can activate the kinin pathway by interacting with kininogens. Activation of the Kallikrein-kinin system causes the release of bradykinin. Bradykinin-receptor B2 (BKB2R) contributes to the pro-inflammatory pathway by activating nitric oxide (NO) and prostaglandins (PGs) thereby increasing vascular permeability. Bradykinin can contribute to lung injury and inflammation in patients with COVID-19.

The development of natural compounds that may have similar therapeutic potential with smaller adverse effects is considered to be used as therapeutic alternatives or as complementary therapies to current ALI therapies. Natural compounds can also form the basis of new drugs for the treatment of diseases. In recent decades, various natural compounds extracted from plants have been reported to have anti-inflammatory activity. Several polyphenolic compounds have shown important in vivo and in vitro anti-inflammatory activity, such as flavonoids, lignans, phloroglucinols, quinones, stilbenes, phenylpropanoids, and diarylheptanoids. Also, terpenoid compounds have shown anti-inflammatory effects.

Kallikrein-kinin system inhibitor is one of the ways to minimize widespread inflammation due to SARS Cov-2 infection, so it is supposed to reduce the incidence of ALI and ARDS in critically ill COVID-19 patients. Currently, there has not been much research on natural compounds that have the potential to inhibit the Kallikrein-kinin system. In this study, the authors conducted a molecular docking study using human plasma kallikrein (PDB ID: 5TJX) as a protein target and several natural compounds which aimed to determine the potential of these active compounds in inhibiting the formation of bradykinin.

**Material and Methods**

**Protein Receptors Preparation**

The 3D structure of human plasma kallikrein (PDB ID: 5TJX) was downloaded from the Protein Data Bank (https://www.rcsb.org/) in .pdb format. The active site of the protein was determined using Discovery studio 2016. Protein optimization by removing water, adding polar hydrogen, and adding charge using Autodock 4.2. The structure of the protein was saved in .pdb format for further analysis.

**Ligand Preparation and Drug Likeness Activity**

The 3D structure of each natural compound was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in .sdf format and being optimized using Avogadro 1.2 and converted format into .pdbqt by using Open Babel 2.4. The natural compound with anti-inflammatory effect was obtained from Dr. Duke’s Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search).

The drug-likeness analysis was conducted to know the molecules with good permeation and oral absorption have molecular weight <500 Da, C logP<5, H-bond donor <5, H-acceptor <10, violation <2, this was calculated using the SWISSADME (http://www.swissadme.ch/).

**Molecular Docking**

The device used in this study is a laptop with an Intel Core i5-7200U CPU @ 2.50-2.71GHz, 8GB RAM. Operating System using Windows 10 pro ver 1903, 64-bit OS. Firstly molecular docking was performed with the native ligand of the protein to validate the reliability of the molecular docking process with root mean square deviation (RMSD) value <2 Å. The grid coordinate (X,Y,Z) -11.377, -2,117, -16,39 with dimensions of the Grid Box 36x36x36. Throughout the docking process, the macromolecule was kept rigid and the ligand was flexible. The molecular docking was performed using Autodock 4.2 by adjusting the Genetic algorithm (GA) parameter, using 10 runs of the Lamarckian GA criteria. Human plasma kallikrein (PDB ID: 5TJX) was docked with 70 compounds and one native ligand and analyzed using Autodock 4.2.
Result and Discussion

In the 5TJX protein, there is only one native ligand, namely GBT with active sites LEU418, HIS434, HIS472, GLN473, SER478, GLU479, GLY480, TYR555, ARG560, MET561, ASP572, ALA573, CYS574, LYS575, GLY597, SER578, THY599, CYS602. Several drug candidate compounds have been selected according to the Lipinski Rule of Five (Table 1) then sorted according to their binding energy. The smallest binding energy obtained from docking 5TJX with 70 compounds in sequence, namely, xanthohumol, nafamostat, demethoxycurcumin, epicatechingallate, beta mangostin, alpha mangostin, avoralstat, camostat (-9.52, -9.35, -9.33, -9.28, -9.19, -9.06, -8.41, -8.23 kcal/mol).

Table 1. Lipinski Rule of Five Properties of Human Plasma Kallikrein Potential Inhibitors

<table>
<thead>
<tr>
<th>No</th>
<th>Compound (Molecular Formula)</th>
<th>Lipinski’s rule of five</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Molecular weight (&lt;500 Da)</td>
<td>LogP (&lt;5)</td>
</tr>
<tr>
<td>1</td>
<td>Xanthohumol (C21H22O5)</td>
<td>354.40</td>
</tr>
<tr>
<td>2</td>
<td>Nafamostat (C19H17N5O2)</td>
<td>347.37</td>
</tr>
<tr>
<td>3</td>
<td>Avoralstat (C28H27N5O5C30H26F4N6O)</td>
<td>513.54</td>
</tr>
<tr>
<td>4</td>
<td>Camostat (C21H26N4O8S)</td>
<td>494.52</td>
</tr>
<tr>
<td>5</td>
<td>Demethoxycurcumin (C20H18O5)</td>
<td>338.35</td>
</tr>
<tr>
<td>6</td>
<td>Epicatechin gallate (C22H18O10)</td>
<td>442.37</td>
</tr>
<tr>
<td>7</td>
<td>Beta mangostin (C25H28O6)</td>
<td>424.49</td>
</tr>
<tr>
<td>8</td>
<td>Alpha mangostin (C24H26O6)</td>
<td>410.46</td>
</tr>
</tbody>
</table>

Kallikrein is a serine protease enzyme that converts kininogens into kinin plasma protein. Activation of kallikrein causes the release of bradykinin which contributes to the pro-inflammatory pathway. The kallikrein-kinin system can be a factor that plays a role in the formation of lung injury and angioedema in COVID-19 patients. This study used human plasma kallikrein (PDB ID 5TJX) as a potential protein target for the prevention of ARDS in critically ill COVID-19 patients. The ligands that will be tested are berotralstat, avoralstat, nafamostatmesylate, camostatmesylate, and several natural compounds that have anti-inflammatory effects to assess potency as a plasma kallikrein inhibitor.

In this molecular docking study, we used berotralstat, avoralstat, nafamostat, and camostat as standard drugs for comparison. Berotralstat is a specific plasma kallikrein inhibitor therapy given orally once-
daily dose. Berotralstat has completed its phase III study as long-term prophylaxis for Hereditary Angioedema due to deficiency of C1-inhibitor (C1-INH-HAE). Berotralstat was reported to reduce attacks of C1-INH-HAE angioedema in a phase III trial. Avoralstat is a potent plasma kallikrein inhibitor. Oral avoralstat therapy has been tested in phase III trials to shorten angioedema episodes and improve the quality of life of patients with C1-INH-HAE compared to placebo therapy.

Nafamostat mesylate and camostat mesylate are synthetic serine protease inhibitors. Nafamostat inhibits several enzyme systems, namely the coagulation and fibrinolytic systems (thrombin, Xa, and XIIa), the kallikrein-kinin system, the complement system, pancreatic proteases, and protease-activated receptor (PARs) activation. In COVID-19, Nafamostat may prevent disease progression by controlling the immune system such as the complement cascade, preventing disseminated intravascular coagulation (DIC), and preventing viral invasion by inhibiting viral fusion in cell membranes. Camostat inhibits cholecystokinin, proinflammatory cytokines, and serine proteases. Camostat is used for the treatment of chronic pancreatitis, drug-induced lung injury, and has the potential as COVID-19 therapy by preventing the entry of the virus into lung cells.

The results of docking between human plasma kallikrein (PDB ID: 5TJX) and ligands using Autodock 4.2 showed binding energy ranges of -9.35 and -3.59. The natural compound that has the smallest binding energy is xanthohumol with -9.52. Nafamostat, Avoralstat, Camostat, and Berotralstat have binding energies of -9.35, -8.41, -8.23, and -3.59 respectively. Xanthohumol is a prenylflavonoid found in the hop plant (Humulus lupulus L.) which is used in brewing and medicine. In the previous study, Xanthohumol could downregulate inflammatory mediators by inhibiting transactivation of NF-κB in LPS-activated macrophages and inhibiting STAT-1α and IRF-1 activation in IFN-activated macrophages. Xanthohumol could effectively inhibit LPS-induced oxidative stress and inflammatory damage of lungs, which may be associated with upregulation of the Nrf2 pathway depending on AMPK activation and GSK3β inhibition.

Demethoxycurcumin has a binding energy of -9.33. Demethoxycurcumin can be found in turmeric (Curcuma longa) and ginger (Curcuma xanthorriza). This compound has anti-inflammatory, antioxidant, and antiproliferative effects. Demethoxycurcumin significantly reduces inflammation due to increased expression of IL-1β, IL-6, NF-κB, TNF-α, iNOS, and COX-2. Epicatechingallate exhibits binding energy of -9.28. Epicatechingallate is mostly found in the tea plant (Camellia sinensis). Epicatechingallate has been studied to have anti-inflammatory effects, which reduce the expression of TNF-α, IL-1β thereby attenuating the expression of iNOS and COX-2. Beta mangostin has a binding energy of -9.19. Beta mangostin can be isolated from hexane and chloroform extracts of mangosteen stem bark (Garcinia mangostana). Beta mangostin has anti-inflammatory effects by inhibiting the production of nitric oxide (NO). Based on the binding energy from the docking result, xanthohumol, demethoxycurcumin, epicatechingallate, beta mangostin, and alpha mangostin, are natural compounds that are recommended as potential inhibitors of plasma kallikrein for further in vitro and in vivo studies.

Conclusion

COVID-19 has become a global health problem due to the high spread and until now there has been no adequate therapy for ARDS complications which are triggered by activation of the kallikrein-kinin system which produces potent pro-inflammatory mediators. Therefore we recommend xanthohumol, nafamostat, demethoxycurcumin, epicatechin gallate, beta mangostin, and alpha mangostin which show the best potency as plasma kallikrein inhibitors. However, further research is still needed to determine the potential of drugs and medicinal plant active compounds for medical treatment.

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Conflict of Interest: The authors declare that there is no conflict of interest.

Funding: The authors declare that there is no funding source.

Ethical Approval: There is no ethical clearance because this study didn’t use animal or human samples.

References


Effectiveness of Training Program Regarding Gullian Baree Syndrome Maneuver on Pediatric Nurses’ Knowledge and Practices

Amina Mohamed Thabet¹, Mahmud, Huda Shawky², Eman Hassan³, Zahra Ahmed Sayed⁴, Nora Abd- Elhamid Zaki⁵, Naglaa Gamal Eldien Abdelhafez Hariedy⁶

¹Assistant Professor of Pediatric Nursing, Faculty of Nursing, Sohage University, ²Lecturer in Pediatric Nursing Department, Faculty of Nursing, Helwan University, ³Assistant professor of Pediatric Nursing, Faculty of Nursing, Helwan University, ⁴Lecturer of Critical Care Nursing, Faculty of Nursing, Aswan University, ⁵Assistant Professor of Pediatric Nursing, Faculty of Nursing, Assiut University, ⁶Assistant Professor of Critical Care and Emergency Nursing, Sohage University, Egypt

Background: Guillain-Barre syndrome is an acquired inflammatory polynuropathy characterized by rapidly progressive symmetrical flaccid limb weakness and a reflexia.

Design. A quasi-experimental design was used to guide this study. The study included a sample of 30 nurses who work at pediatric intensive care and neurology unit in Assuit University Children Hospital.

Data were collected using a questionnaire and an observation checklist, used in pre-post testing to measure the effectiveness of the training program among nurses.

Results: The study revealed deficiency in pre-intervention nurses’ knowledge and practices. Statistically significant improvements on knowledge and practices were demonstrated at the post-intervention time for the studied nurses. Statistically significant relations were shown between knowledge and practices among studied nurses.

Conclusion: Implementation of the training program significantly improved nurses’ knowledge and practice. More experienced and aged nurses had better knowledge.

Keywords: Guillain Barre Syndrome, Nurses’ Knowledge and Practices

Introduction

Guillain Barre Syndrome (GBS) is classically defined as an acute acquired sensitive-motor polyradiculo neuropathy post infectious, immunologically mediated, usually of demyelinating nature. It is the leading cause of acute flaccid paralysis in developed countries, in which polio has been eradicated (¹).

Guillain Barre Syndrome is a relatively rare disorder, with an incidence ranging between 0.5 to 1.5 cases per 100,000 individuals in the population of 0-17 months. It is a well-recognized disease worldwide and it is the most frequent cause of acute flaccid paralysis in countries where polio vaccination has allowed polio eradication. GBS affects patients of all ages. The incidence of GBS in children is 0.8 cases per 100,000. It affects both sexes in a ratio M/F 1.5-1 (¹).

Nursing care is essential in all phases of disease because children with GBS are immobilized and have respiratory depression. The emphasis care is on close observation to assess the extent of paralysis and

Corresponding author:
Huda Shawky,
Pediatric Nursing Department, Faculty of Nursing, Helwan University, Egypt,
Email: dr-hudashawky@yahoo.com
on prevention of complication including autonomic dysfunction as hypertension, orthostatic intolerance, respiratory dysfunction, pain management and life-threatening dysrhythmias (2).

Increasing nurses’ awareness of Guillain Barre syndrome and the related childcare management strategies for all phases of the illness; to enhance care provided to child, provide a more informed and higher level of care for children and their families impacted by this unique and challenging illness (3). This study aims to evaluate the effectiveness of training program regarding Guillain Barre Syndrome maneuver on nurses’ knowledge and practices through.

**Significance of the Study**

The GBS is a significant cause of long-term disability for at least 1,000 children per year in the United Stat and is considered a medical emergency. It is affecting approximately 100,000 children per year. The incidence of non-polio acute flaccid paralysis in Egypt from 2014 to 2015 was 1534 children and the incidence of using plasma pharesis as a first line for treatment was 60 % of total number of previous cases. So, the expected cases for Egypt are 2/100,000 among children < 15 years of age (4).

Children who are suffering from GBS require special care from nurses to ensure maximum performance. so, during the researchers’ work at neurology and pediatric intensive care unit in Assiut University Children Hospital, it was observed that nurses were lacking the necessary basic knowledge and practices related to caring of children with GBS. So, this study was designed in a trial to improve nurses’ knowledge and practices concerning the care offered to children with GBS.

**Materials and Methods**

**Research design**

A quasi-experimental research design was used to guide this study.

**Setting**

The study was conducted at neurology department and pediatric intensive care unit in Assiut University children Hospital.

**Sampling:**

A convenience sample of 30 nurses (3 baccalaureate and 27 practical nurse) working at the pervious selected setting in Assiut University Children Hospital.

The first tools utilized to collect data is a structured questionnaire interview sheet which includes the personal and sociodemographic characteristics (age, qualification, job position, duration of nursing total and current job experience, and previous attendance of training courses). The nurses’ knowledge form about GBS includes 28 items about definitions of GBS, types, incidence, causes, complications, management and prevention of GBS. These items were scored as 2 grades for each complete answer, (1) for incomplete, and (0) for wrong one. The total score of knowledge is 115. The cut-off-point of knowledge is satisfactory for > 60% and unsatisfactory for < 60%.

The second tool is observation checklist which was developed by Kumar (5) that evaluates nurses’ practices given to child with GBS. It includes 55 items about practices related to vital signs, 8 items related to blood sample, 6 items related assist during lumber puncture, 10 items related to intravenous fluid, and 21 items related to suctioning. These items were scored as (1) for those be done correctly and (0) for these not done. The practice was considered competent if the percent score was 60% or more and incompetent if less than 60%.

**The training program**

Handout about the training program content was developed by the researchers based on the knowledge and practices needs in a form of printed (Arabic booklet). It was also supplemented with information based on review of relevant literature (nursing textbook, journals, internet resources, etc.) about care provided to children in with GBS. Then the program was reviewed by a panel of experts before its implementation.
The program included two parts:

**Theoretical part:** It includes two lectures, one session for each. The first lecture included definition of GBS, types of meningitis, dangers type, causes and incidence. The second lecture included sign and symptoms of GBS, complications of each one, treatment and prevention.

Practical part: This part covered the nursing procedures offered for children with GBS such as vital signs, intravenous fluid, blood sample, lumber puncture and suctioning. One session of each procedure and each group has 1-2 nurses. The time required for the program implementation was 7 months with approximately 280 hours divided in 80 hours theoretical and 200 hours practical.

**Data collection**

An official letter was obtained from the Dean of the Faculty of Nursing to the Heads of the Neurology Unit, as well as the Head of Pediatric Intensive Care, soliciting the necessary approvals to conduct the research.

An Arabic translation of all study tools was done and preparation of the program lectures in form of Arabic module.

**Validity**

The validity of the study tool was assessed to check its content coverage, and clarity of the questions by a jury panel of professors from five expert pediatric field and its result was 96%.

**Reliability**

Reliability was estimated by Cronbach’s Alpha test for study \( r = 0. 68 \).

**Ethical consideration:**

The written consent was taken from all nurses participate in the study.

**Results**

**Table 1. Nurses’ sociodemographic characteristics (N = 30)**

<table>
<thead>
<tr>
<th>Item</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 24</td>
<td>8</td>
<td>26.7</td>
</tr>
<tr>
<td>24 -</td>
<td>14</td>
<td>46.7</td>
</tr>
<tr>
<td>29 -</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>≥ 34 y</td>
<td>3</td>
<td>10.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>27.19±8.77</td>
<td></td>
</tr>
<tr>
<td>Years of experience</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5 years</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>5 – 10 years</td>
<td>14</td>
<td>46.7</td>
</tr>
<tr>
<td>&gt; 10 years</td>
<td>11</td>
<td>36.7</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>11.24±4.90</td>
<td></td>
</tr>
<tr>
<td>Years of experience in current place</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5 years</td>
<td>9</td>
<td>30.0</td>
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<tr>
<td>5 – 10 years</td>
<td>12</td>
<td>40.0</td>
</tr>
<tr>
<td>&gt; 10 years</td>
<td>9</td>
<td>30.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>9.88±3.74</td>
<td></td>
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<tr>
<td>Attending training courses:</td>
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<td>Yes</td>
<td>18</td>
<td>60.0</td>
</tr>
<tr>
<td>No</td>
<td>12</td>
<td>40.0</td>
</tr>
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</table>
Table 2. Comparison between mean score of the nurses’ practice of GBS care over time

<table>
<thead>
<tr>
<th>Score</th>
<th>Pre</th>
<th>Post</th>
<th>Follow-up</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Vital signs</td>
<td>156</td>
<td>24.67 ± 19.19</td>
<td>134.37 ± 16.82</td>
<td>97.20 ± 23.61</td>
</tr>
<tr>
<td>Intravenous fluid</td>
<td>8</td>
<td>1.97 ± 2.20</td>
<td>7.60 ± 1.54</td>
<td>6.80 ± 1.65</td>
</tr>
<tr>
<td>Lumber puncture</td>
<td>42</td>
<td>14.80 ± 7.50</td>
<td>35.03 ± 7.05</td>
<td>28.37 ± 7.34</td>
</tr>
<tr>
<td>Blood sample</td>
<td>26</td>
<td>1.47 ± 3.41</td>
<td>19.10 ± 1.90</td>
<td>17.20 ± 3.49</td>
</tr>
<tr>
<td>Suctioning</td>
<td>22</td>
<td>2.60 ± 3.11</td>
<td>20.57 ± 1.85</td>
<td>15.33 ± 4.16</td>
</tr>
</tbody>
</table>

P-value < 5

Table 3. Correlation between nurses’ knowledge and practices over time

<table>
<thead>
<tr>
<th>Group</th>
<th>Knowledge Mean ± SD</th>
<th>Practices Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>10.43 ± 2.65</td>
<td>45.50 ± 26.69</td>
</tr>
<tr>
<td>Post</td>
<td>96.87 ± 12.10</td>
<td>216.67 ± 23.20</td>
</tr>
<tr>
<td>Follow-up</td>
<td>58.90 ± 13.58</td>
<td>164.90 ± 35.10</td>
</tr>
<tr>
<td>P-value</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

P-value < 5

Level of knowledge in pre, post and follow-up tests

Figure 1. Level of nurses’ knowledge over time
Table (1) shows that less than a half (46.7%) age 24-28-years with mean age (27.19±8.77). The majority (70%) had 5-10-years of experience mean duration of 9.88 and 60% of them had Supportive Strategies courses related to emergency need.

None of nurses had complete answers before program implementation about GBS is, among children. GBS is caused by many factors such as children at risk of GBS are and the most dangerous type of GBS. This percentage increased to 100% at post I and decreased to (60%, 70%, 50%, 43.4%, 20.0% & 36.7%) respectively at post II.

It was found that none of the nurses knew the sign and symptoms and treatment before the program implementation. On the other hand, all nurses mentioned that sign and symptoms and treatment, with statistically difference significant post-intervention (p <0.000*).

Moreover, when asked about the Supporting family of children with GBS, it was found that none of them had complete answers before program implementation, while 73.3% of nurses mention them completely at post I and 57.6% at post II (follow-up).

Table (3) indicates increases in the practice scores in all areas at the post-intervention. However, this increase reached statistical significance for nurses’ basic practice about vital sign, lumber puncture, intravenous fluid, blood sample and suctioning (p< 0.00*). However, the practice scores slightly declined after three months at follow-up. Nevertheless, the scores were still higher than the baseline scores.

Table (4) indicates statistically significant improvement in total knowledge over time (I) (96.8 and 216.67, respectively) compared to preprogram (10.43 and 45.58, respectively) (p=0.000).

Figure 1 showed in this figure the majority of nurses (90%) were poor knowledge before program implementation, while after program their knowledge was satisfactory and only 10% were poor.
Figure 2 displayed that only 10% of nurses had adequate level of practice before program implementation, while after program the majority (96.7%) had adequate level of practice.

**Discussion**

Concerning the nurses’ knowledge of GBS before the program implementation, it was found that none of them know the common types, causes, the sign and symptoms, and its treatment. On the other hand, all nurses at the post answered correctly the questions related to the sign and symptoms and treatment of GBS with statistically significant difference. While the percentage of the correct answered was increased to 100% at post I and slightly decreased at post II. These findings agree with that of Kudhaer and Mua’ala (7) who revealed that the majority of nurses had poor knowledge in relation to general information (only answer 5 items correctly out of 20 items) and nursing care (only answer 3 items correctly out of 12 items) of children with Guillain-Barre Syndrome.

The present study showed a comparison between mean score of nurses’ performance of GBS care throughout intervention program phases (pre, post and follow-up). The indicated increases in the practice scores in all practice areas at the post-program phase. However, this increase reached statistical significance for nurses’ basic practice about vital sign, lumber puncture, intravenous fluid, blood sample and suctioning. However, the practice scores have demonstrated some declines after three months at follow up testing. Nevertheless, the scores were still higher than the pre-program baseline scores.

As regards the nurses’ knowledge, the majority have poor knowledge before program implementation, while after program the majority were satisfactory level of knowledge. This result agrees with that of Kudhaer and Mua’ala who stated that, in general, nurses’ knowledge was poor before program (7).

Regarding nurses’ practices, they have improved over time. This study finding contradicts another study finding by Mohamed which revealed that more than three quarters of them (76.0%) had competent practices (8).

The current study indicated statistically significant improvement in total knowledge and practice over time. This finding was inconsistency with another study finding conducted by Abdelmonem et al. (9) who stated that nurses had unsatisfactory knowledge pre teaching program compared to post teaching program. The majority of nurses’ practice have been inadequate pre teaching program compared with post teaching program.

**Conclusions**

It was concluded that the implementation of the training program significantly improved nurses’ knowledge and practices. Program attendance was the only independent predictor of nurses’ knowledge and practices scores.

**Recommendations**

Continuous evaluation for nurses caring of children with GBS is crucial, as they may lose knowledge and skills in the course of time.

Providing educational guidelines, posters, pamphlets and manuals about GBS should be available at each nursing station in wards and encourage nurses to get use of them.

Encourage the nurses to participate in training courses and congresses held by specialists in GBS to update their knowledge.

**Conflict of Interest**: The researchers confirm that there is no any conflict of interest.

**Source of Funding**: This study is self-funded.

**Ethical Clearance**: The researchers obtained the ethical approval from the Bilad Alrafiadain University College

**References**

2. Modupalli C, Sriramulu C, Gopineni D, Yalavarthi


Molecular Detection of Helicobacter Pylori in Sheep at AL-Muthana Province of Iraq

Ftima Atiya Kareem¹, Nabeel M.H. Al-Maaly²
¹Scholar Researcher, ²Assistant Professor Internal and Preventive Medicine, College of Veterinary Medicine, Baghdad University, Iraq

Abstract

Helicobacter pylori (H. Pylori) was discovered in 1982. Over 50% of the world population is infected by this bacterium. H. Pylori is the main cause of gastritis, peptic ulcers, and gastric cancer in human. Foods with animal origins play a substantial role in the transmission of H. Pylori. The present investigation was carried out to study the molecular detection of H.pylori in stomach gastric tissue of the sheep. Collecting of the samples occur randomly from various area of Al-Muthana province slaughter house(AL-Muthana, AL-Khudur, AL-Rumethah) from October 2019 to February 2020. One hundred and fifty (n 75 male, n 75 female) abomasums samples were collected and dissection in aseptic manner then stored in deep freeze (Liquid nitrogen -196) until processing and analyzed for the presence of 16s RNA and VACA genotypes. This study showed that the Prevalence of H. Pylori in the gastric samples of sheep at slaughterhouses (Al-Muthana, Al-Khur, Al-Rumethah) were (9 positives) 18%, and (7) 14%, (15) 30% respectively. The most commonly detected genotypes in the gastric samples of slaughterhouses were 16s RNA (20.67%) in ewes(25.33%). AL-Rumetha was the most commonly detected H. Pylori (30%). Results showed that gastric tissue of sheep can act as a reservoir to H. Pylori and disseminate the pathogen in feces and milk then transmitted to human during uses of unpasteurized milk, or meat. samples could be the potential sources of virulent strains of H. Pylori. Application of sanitary measures in the storage, transportation and sale of meat is essential for reducing the levels of H. Pylori cross contamination.

Keywords: Molecular detection, helicobacter pylori, H. Pylori, sheep

Introduction

H. Pylori is a bacterium that infects the mucus lining of the stomach and Duodenum. Helicobacter bacteria are the only known microorganisms that can thrive in the highly acidic environment of the stomach with a diameter 2 to 4 μm in length and 0.5 to 1 μm in width. H pylori is gram negative, S-shaped or curved, microaerophilic, none sporulating, spiral coccoid flagellated bacterium. It consist from two to six flagella that give it the motility to withstand rhythmic gastric contractions and penetrate the gastric mucosa. Hp-pathogenesis is not yet clear. Hp is genetically heterogeneous and all strains may not play the same role in the development of malignancy. Specific allele polymorphism exists in domains of this gene: signal region (s1a, s1b, s1c and s2), the intermediate (i1, i2 and i3) and mid region (m1a, m1b and m2). Waist named due to its ability to cause “vacuole”-like membrane vesicles in the cytoplasmatic gastric cells, but the mechanisms in H. Pylori pathogenesis remain unclear. In addition to the induction of vacuolation, Vac A exert severity of other effects on target cells, including disruption of mitochondrial functions, stimulation of apoptosis and blocked of T-cell proliferation. Vac A is also important for colonization of H. Pylori in gastric mucosa, another virulence factor is cytotoxin-associated gene A (cagA), which is a gene of pathogenicity island of H. Pylori and can be a predictive marker for the bacterium pathogenicity. H. Pylori strains can be divided into two groups: cag positive and cag negative.
negative. According to previous studies, *cag* positive strains are more prevalent in patients with more severe clinical symptoms\(^\text{(4)}\).

Different modes of transmission have been suggested that gastric tissue of several animals, taking part in the human food chain, such as sheep 16 % and this suggest them as reservoirs\(^\text{(13)}\). Sheep may are natural host for *H. Pylori*\(^\text{(3)}\), *H. pylori has ability* to survive in complex foodstuffs such as milk, butter, cheese 30%\(^\text{(10)}\), feces of animals (sheep, cow, buffalo) play main shedding site, which agrees with the findings that *H. Pylori* colonizes the abomasum of animals and crosses the intestine. In accordance with previous studies, *H. Pylori* in milk might be attributed to contamination from feces or from the surrounding environment\(^\text{(5)}\).

**Materials and Method**

**Gastric Sample Collection**

Collection of samples from sheep gastric tissue of stomachs, including the antrum area from abomasum.

**Experimental animals**

Samples were collected from 150 sheep (75 male & 75 female) with different ages at a slaughterhouse. Dissection of samples occur in laboratory of Microbiology College of veterinary medicine and done under aseptic conditions. Tissue collection took place in the AL- Muthana province, Iraq. All animals prepared for consumption in this slaughterhouse are from AL-Muthana, the collection take place from different area of provence (rural, and city center) and many slaughter houses of (AL-Muthana, AL- Khudur, AL- Rumetha). After dissection the samples stored in deep freeze (-20) until processed.

**DNA Extraction and Genomic Characterization Of Helicobacter by Polymerase Chain Reaction**

Extraction of genomic DNA was from sheep stomachs antrum area of abomasums that preserved in deep freeze (-20), and using the G-DEX™ Ilc Genomic DNA Extraction Kit for Cell/Tissue (korea), according to the manufacturer’s instructions. To determine the presence of *Helicobacter pylori* 16S rRNA two 22-base oligonucleotide primers (Hp1 and Hp2) that amplified 1200 bp were used as described elsewhere. In addition, a set of primers specific for the *H. Pylori vacA* gene

(VAG-F: 59-CAA TCT GTC CAA TCA AGC GAG-39; and

VAG-R: 59 GCG TCA AAA TTC CAA GG-39) with a reported sensitivity of 98.4%\(^\text{(3)}\) was used to amplify a 570-645 bp product for *m1 & m2* from the middle region of the *vacA* gene. Furthermore, primers specific for *Helicobacter* genus–specific 16S rRNA described by Fox *et al.* were also used for polymerase chainreaction (PCR) amplification of DNA extracted from tissue samples. The primer pairs (C97, 59 GCT ATGACG GGT ATC C-39 (276–291 forward); ; and C05, 58-ACT TCA CCC CAG TCG CTG-38 (1478–1495 reverse)\(^\text{(6)}\).

After amplification, the reaction products were separated by electrophoresis through 1% agarose gels containing ethidium bromide ((5μl of a 10mg/ml stock solution per 100 ml of agarose gel) and the PCR products were visualized by ultraviolet fluorescence. All reactions were performed in duplicate.

**Sequence analysis**

The sequence of the nucleotide of Helicobacter pylori 16S Rrna gene was known in 6 samples, as 25 microliters of each sample of the PCR product with the Primers of the nucleotide of Helicobacter pylori 16S Rrna gene were sent to Macrogen in the Korea and after obtaining the results , all the results were compared directly with the nucleotide of the nucleotide of Helicobacter pylori 16S Rrna gene Available in the internet (http: NCBI Reference Sequence) by computer program (BioEdit Pro. version: 7.0.0)

**Statistical Analysis**

program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage in this study(19).

Results

Genomic Characterization of Helicobacter Organisms

The result show 31 of the 150 gastric tissue samples positive for the 16S rRNA also amplified specific vacA that 25 of 150 gastric tissue is positive. Statistically significant differences (p<0.01) were observed in the prevalence of H. Pylori in sheep gastric samples collected from different provinces (data shown in table 1), while table 2 observed that female had more tendency (25.33%) to harbor H. Pylori in it gastric mucosa than male (16%) also in this study the result shown this bacteria compare between rural and city of AL-Muthana provinces that AL-Rumetha with percentage 15%, AL-Khudur 7%, AL-Muthana slaughter house 9% data shown in table 3.

Sequence analysis

The results were registered in NCBI under accession numbers (LC589440, LC589441, LC589442). Which is available on the website (https://www.ncbi.nlm.nih.gov/nuccore/LC589441).

H- pylori 16S Rrna gene. The results of the analysis of the H- pylori 16S Rrna gene sequence were summarized in Table (4) and showed that there were 14 mutation in 6 samples with the H- pylori 16S Rrna gene. It was found that there is one mutation in some samples and more than one mutation in other samples, this shows that the type and location of the mutations that have been found may lead to a different effect of the mutation. Some of these mutations lead to changes in the genetic codes (Figure 1, 2).

<table>
<thead>
<tr>
<th>Table 1: Results of 16sRNA gene in sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>16sRNA gene</strong></td>
</tr>
<tr>
<td>Positives (+)</td>
</tr>
<tr>
<td>Negative (-)</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Chi-Square ((\chi^2))</td>
</tr>
<tr>
<td>P-value</td>
</tr>
<tr>
<td><strong>VAC gene in sheep</strong></td>
</tr>
<tr>
<td>Positives (+)</td>
</tr>
<tr>
<td>Negative (-)</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Chi-Square ((\chi^2))</td>
</tr>
<tr>
<td>P-value</td>
</tr>
</tbody>
</table>

** (P≤0.01)-Highly significant.
### Table 2: Distribution of 16sRNA gene according to Sex of sheep

<table>
<thead>
<tr>
<th>16sRNA gene</th>
<th>Male No. (%)</th>
<th>Female No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positives (+)</td>
<td>12 (16.00%)</td>
<td>19 (25.33%)</td>
</tr>
<tr>
<td>Negative (-)</td>
<td>63 (84.00%)</td>
<td>56 (74.67%)</td>
</tr>
<tr>
<td>Total (150)</td>
<td>75</td>
<td>75</td>
</tr>
</tbody>
</table>

Chi-Square ($\chi^2$) = 13.561 **  

P-value = 0.0001

** (P≤0.01)-Highly significant.

### Table 3: Distribution of 16sRNA gene according to Location of sheep

<table>
<thead>
<tr>
<th>16sRNA gene</th>
<th>AL- Muthana center No. (%)</th>
<th>AL- Khudur No. (%)</th>
<th>AL- Rumethah No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positives (+)</td>
<td>9 (18.00%)</td>
<td>7 (14.00%)</td>
<td>15 (30.00%)</td>
</tr>
<tr>
<td>Negative (-)</td>
<td>41 (82.00%)</td>
<td>43 (86.00%)</td>
<td>35 (70.00%)</td>
</tr>
<tr>
<td>Total (150)</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Chi-Square ($\chi^2$) = 13.319 **  

P-value = 0.0001

** (P≤0.01)-Highly significant.

### Table (4): The most common types of mutations in the Helicobacter pylori 16S Rrnagene, sequence in this study.

<table>
<thead>
<tr>
<th>No. of sample F</th>
<th>Wild type</th>
<th>Mutant type</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>A</td>
<td>T</td>
<td>201</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>C</td>
<td>388</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>C</td>
<td>199</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>C</td>
<td>199</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>C</td>
<td>224</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>T</td>
<td>339</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>T</td>
<td>394</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>T</td>
<td>339</td>
</tr>
</tbody>
</table>
Figure (1) :- Gel electrophoresis for PCR product of (C97&C05 primer) show 1200bp. (11,6,1,2,16,28,24,33,40,12) represented positive results of bacterial DNA isolates, Lanes (23) represented Negative results lane L represent Ladder.

Figure (2) Gel electrophoresis for PCR product of (VAC A gene primer) show 645bp. Primer TM at (55C). Lane L : DNA ladder (100-1500bp), Lanes(40,12,33,28,16,2,1,6,50c,17c,43c,38c,27c,46c,20c,31c,7c,1c) represented positive results of bacterial DNA isolates, Lanes (42) represented Negative results.
Discussion

Results of the present study showed that *H. Pylori* had a considerable prevalence in meat samples. Total prevalence of *H. Pylori* was 20.67%. This level of prevalence of *H. Pylori* in meat samples was higher than that of Talimkhani and Zohreh (23) (Iran, 7.33% in meat samples), Talaei et al (22) (13.79% sheep milk samples), Gilani et al (14) (Iran, 5% in meat samples) and Momtaz et al (11) (16% in gastric biopsy specimens), while was lower than that of Dore et al (3) (30% sheep gastric tissue samples), Saeidi and Sheikhshahrokh (18) (Iran, 21.90% in milk and 26.25% in meat samples), Chehelgerdi and Ranjbar (16) (70% gastric tissue of sheep), our result disagree with an investigation of study Tabatabaei Mohammad (21) that sheep cannot play a role in transmission of *H. Pylori* microbe. High prevalence of *H. Pylori* strains in different types of foods with animal origins showed that animals and especially ruminants may play an important role in the maintenance and transmission of infection to humans (16). It may be due to the high ability of sheep stomach to harbor *H. Pylori* and its transmission into the environment (23). Our data revealed that *h pylori* set in abomasums tissue of adult sheep than in lamb due to it need a period to settle in gastric tissue (long incubation period) (9,15), and occurring in people with bad socio-economic habitat or rural area (7), (that may be a reason for that sheep in area with bad sanitation and rural our (Al-Rumetha) have percentage of *h pylori* more than in another part of province 15% may relate to high infection of *h pylori* in people of this part.

Conclusions

In conclusion sheep in Iraq harbor *H. Pylori* in their gastric suggest that sheep may are the natural reservoir of the bacteria and can transmit *H. Pylori* to human community. Meat, harbor *H. pylori* strains with considerable distribution of *vacA* genotypes. Considerable incidence of *H. Pylori* proposes similarity in the genotyping pattern of *H. Pylori* strains of various samples represents their similar sources of infection. Simultaneous presence of these genotypes together in some of our strains showed their high pathogenicity. Regarding the high prevalence rate of pathogenic *H. Pylori* beside the high consumption rate of meat, among Iraqi people represent an important public health issue, which should be addressed before the vast spread of the *H. Pylori* infection

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

References


Level of Knowledge and Perception Public of Forensic Odontology

Fuad Husain Akbar¹, Muliaty Yunus², Nadya Shefira Salsabillah³

¹Department of Dental Public Health, ²Department of Radiology, ³Dental Hospital, Faculty of Dentistry, Hasanuddin University, Indonesia

Abstract

Background: Awareness of the importance of forensic odontology and the need for national forensic odontology data collection is still lacking attention both in the community and at the government level. Aim: to determine the level of knowledge and public perceptions about forensic odontology. Method: This type of observational analytic study with cross sectional design with the population of Makassar City using snowball sampling technique. This research was conducted September 2020. This study has obtained ethics from the Health Research Ethics Committee of the Hasanuddin University Dental Hospital. This research tool was an online questionnaire. The knowledge domain consists of 10 questions and the perception domain consists of 10 questions. To assess knowledge, each indicator of knowledge is assessed using the Guttman scale. Total research sample is 213 respondent with the test equipment used chi-square and value p 0.05. Results: The average level of public knowledge was 6.15 ± 2.30 (moderate category), the perception level was 6.25 ± 2.13 (moderate category). Conclusion: The level of knowledge and perceptions about forensic odontology in Makassar City people aged ≥ 18 years is still around 60% who answered questions correctly and responded positively.

Keywords: Forensic odontology, Public knowledge, Public perception, National forensic odontology registry.

Introduction

In Indonesia, regulations regarding post mortem have been regulated in Law No. 36 of 2009 on health. Article 118 paragraph 1 of the Law states that every unidentified corpse must be identified.¹ In European and American countries, conducting national forensic odontology data collection is an obligation as a citizen.² In Indonesia, the awareness of the importance of forensic odontology and the need for national forensic odontology data collection is still lacking attention both at the community and at the government level.³

Forensic odontology is a specialist field in dentistry that helps resolve a number of criminal cases.⁴ Humans have their own unique characteristics regarding tooth structure (enamel / dentin), lip pattern, and palatal rugae.⁵ Age, sex, race, occupation, socioeconomic status, and dental history can be determined by a forensic odontologist.⁶ Therefore, during criminal investigations, representatives of the court frequently consult with odontologists as part of the prosecution process, to protect living victims, and/or to identify corpses.⁷

Although the benefits of establishing a national forensic odontology data collection are significant, their feasibility depends on the level of people’s willingness to contribute to their dental profile, because the criminal investigation system does not involve public participation in this kind of national data collection.⁸

Before attempting to undertake a national forensic odontology data collection, there is a need to evaluate public awareness of forensic odontology. Therefore, this study was conducted to examine public knowledge regarding the aspects of forensic odontology and public perceptions regarding forensic odontology. Given the fact that the promotion of forensic odontology and
forensic odontology data collection nationally has not yet been carried out.

**Material and Method**

This research was an observational analytic study with a cross sectional study design. This research was conducted on 15-20 October 2020 in the city of Makassar. This study has obtained ethics from the Health Research Ethics Committee of the Hasanuddin University Dental Hospital with Ethical Approval number No.0091 / PL.09 / KLPK FKG-RSGM UNHAS / 2020. The population of this research is the people of Makassar city. Determination of the sample using snowball sampling. The inclusion criteria in this study were Makassar city people aged ≥ 18 years, having a smartphone and social media (whatsapp application). Meanwhile, the exclusion criteria were people who did not complete the questionnaire completely. In this study, 213 samples were obtained.

The instrument used in this study was a questionnaire adapted from the research of Salam M, et al.\(^8\) Saudi Dental Journal which consisted of two domains. The knowledge domain consists of 10 questions and the perception domain consists of 10 questions.

To assess knowledge, each indicator of knowledge is assessed using the Guttman scale. Having 2 indicators for the answer “yes” is given a score of 1 while the answer “no” or “don’t know” is given a score of 0. To assess perception, each indicator of perception is assessed using the Guttman scale. Has 2 indicators of answers “agree” and “strongly agree” classified as positive responses and answers “strongly disagree”, “disagree”, and “neutral” are classified as negative responses. The total new scores were categorized into 3 categories, poor: 1 - 4.3, moderate 4.4 - 7.7, good 7.8 – 10.

**Results**

**Table 1 Distribution of respondent characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>71 (33.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>142 (66.7%)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
</tr>
<tr>
<td>18-25</td>
<td>87 (40.8%)</td>
</tr>
<tr>
<td>26-35</td>
<td>47 (22.1%)</td>
</tr>
<tr>
<td>&gt;36</td>
<td>79 (37.1%)</td>
</tr>
<tr>
<td><strong>Marital Status</strong></td>
<td></td>
</tr>
<tr>
<td>Unmarried/Separated</td>
<td>109 (51.2%)</td>
</tr>
<tr>
<td>Married</td>
<td>104 (48.8%)</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
</tr>
<tr>
<td>School</td>
<td>53 (24.9%)</td>
</tr>
<tr>
<td>University</td>
<td>160 (75.1%)</td>
</tr>
<tr>
<td><strong>Job Status</strong></td>
<td></td>
</tr>
<tr>
<td>Work</td>
<td>135 (63.4%)</td>
</tr>
<tr>
<td>Not working</td>
<td>78 (36.6%)</td>
</tr>
<tr>
<td><strong>Have Health Insurance</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>180 (84.5%)</td>
</tr>
<tr>
<td>No</td>
<td>33 (15.5%)</td>
</tr>
<tr>
<td><strong>Number of Clinical visits year</strong></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>61 (28.6%)</td>
</tr>
<tr>
<td>1–2 times</td>
<td>118 (55.4%)</td>
</tr>
<tr>
<td>More than 2 times</td>
<td>34 (16.0%)</td>
</tr>
</tbody>
</table>
Table 2 Responses to knowledge questions

<table>
<thead>
<tr>
<th>Response</th>
<th>Yes n(%)</th>
<th>No n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1. Everyone has a different palate</td>
<td>151 (70.9%)</td>
<td>62 (29.1%)</td>
</tr>
<tr>
<td>A2. Each person has different dental characteristics</td>
<td>207 (97.2%)</td>
<td>6 (2.8%)</td>
</tr>
<tr>
<td>A3. Everyone has a different lip line</td>
<td>200 (93.9%)</td>
<td>13 (6.1%)</td>
</tr>
<tr>
<td>A4. Forensic dentistry can determine the sex of a person</td>
<td>77 (36.2%)</td>
<td>136 (63.8%)</td>
</tr>
<tr>
<td>A5. Forensic dentistry helps in estimating a person’s age</td>
<td>121 (56.8%)</td>
<td>92 (43.2%)</td>
</tr>
<tr>
<td>A6. Forensic dentistry assists in medico-legal cases</td>
<td>143 (67.1%)</td>
<td>70 (32.9%)</td>
</tr>
<tr>
<td>A7. Forensic dentistry assists in sexual harassment investigations</td>
<td>53 (24.9%)</td>
<td>160 (75.1%)</td>
</tr>
<tr>
<td>A8. Forensic dentistry assists in the investigation of accident victims</td>
<td>125 (58.7%)</td>
<td>88 (41.3%)</td>
</tr>
<tr>
<td>A9. Forensic dentistry assists in the investigation of bite marks</td>
<td>156 (73.2%)</td>
<td>57 (26.8%)</td>
</tr>
<tr>
<td>A10. Forensic dentistry assists in investigations of sexual violence</td>
<td>78 (36.6%)</td>
<td>135 (63.4%)</td>
</tr>
</tbody>
</table>

Table 3 Responses to perceptual statements

<table>
<thead>
<tr>
<th>Perceptual statement</th>
<th>Positive response (%)</th>
<th>Negative response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1. I think refusing to give out my dental information is for privacy</td>
<td>55 (25.8%)</td>
<td>158 (74.2%)</td>
</tr>
<tr>
<td>B2. I have the right to refuse the national dental registration.</td>
<td>59 (27.7%)</td>
<td>154 (72.3%)</td>
</tr>
<tr>
<td>B3. I have the right to collect my dental information from the national dental</td>
<td>162 (76.1%)</td>
<td>51 (23.9%)</td>
</tr>
<tr>
<td>B4. I believe forensic dentistry assisted in the identification of criminal</td>
<td>158 (74.2%)</td>
<td>55 (25.8%)</td>
</tr>
<tr>
<td>B5. I believe the national dental data collection can help in legal prosecution</td>
<td>130 (61.1%)</td>
<td>83 (38.9%)</td>
</tr>
<tr>
<td>B6. I believe forensic dentistry helps identify disaster victims</td>
<td>139 (65.2%)</td>
<td>74 (34.8%)</td>
</tr>
<tr>
<td>B7. I believe the dentist is competent in making my dental data</td>
<td>181 (85.0%)</td>
<td>32 (15.0%)</td>
</tr>
<tr>
<td>B8. I believe dentists are able to keep my dental information</td>
<td>187 (87.8%)</td>
<td>26 (12.2%)</td>
</tr>
<tr>
<td>B9. Dental information should be maintained and controlled by the government</td>
<td>129 (60.6%)</td>
<td>84 (39.4%)</td>
</tr>
<tr>
<td>B10. I want to provide dental information in the national dental data collection in the future</td>
<td>130 (61.0%)</td>
<td>83 (39.0%)</td>
</tr>
</tbody>
</table>
Table 4 Average responses to questions of knowledge and perceptions

<table>
<thead>
<tr>
<th>Category</th>
<th>Mean</th>
<th>SD</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge</td>
<td>6.15</td>
<td>2.30</td>
<td>Moderate</td>
</tr>
<tr>
<td>Perception</td>
<td>6.25</td>
<td>2.13</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Category: Poor 1 - 4.3, moderate: 4.4 - 7.7, good 7.8 – 10

Table 5 Chi-square test related to the relationship between knowledge level and respondent characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Knowledge n (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poor</td>
<td>Moderate</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19 (26.8%)</td>
<td>27 (38.0%)</td>
</tr>
<tr>
<td>Female</td>
<td>35 (24.6%)</td>
<td>63 (44.4%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-25</td>
<td>19 (21.8%)</td>
<td>39 (44.8%)</td>
</tr>
<tr>
<td>26-35</td>
<td>17 (36.2%)</td>
<td>15 (31.9%)</td>
</tr>
<tr>
<td>&gt;36</td>
<td>18 (22.8%)</td>
<td>36 (45.6%)</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unmarried/ separated</td>
<td>28 (25.7%)</td>
<td>47 (43.1%)</td>
</tr>
<tr>
<td>Married</td>
<td>26 (25.0%)</td>
<td>43 (41.3%)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>School</td>
<td>22 (41.5%)</td>
<td>22 (41.5%)</td>
</tr>
<tr>
<td>University</td>
<td>32 (20.0%)</td>
<td>68 (42.5%)</td>
</tr>
<tr>
<td>Job Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Work</td>
<td>29 (21.5%)</td>
<td>56 (41.5%)</td>
</tr>
<tr>
<td>Not working</td>
<td>25 (32.1%)</td>
<td>34 (43.6%)</td>
</tr>
<tr>
<td>Have Health Insurance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>42 (23.3%)</td>
<td>77 (42.8%)</td>
</tr>
<tr>
<td>No</td>
<td>12 (36.4%)</td>
<td>13 (39.4%)</td>
</tr>
<tr>
<td>Number of clinical visits year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>19 (31.1%)</td>
<td>21 (34.4%)</td>
</tr>
<tr>
<td>1-2 times</td>
<td>28 (23.7%)</td>
<td>57 (48.3%)</td>
</tr>
<tr>
<td>More than 2 times</td>
<td>7 (20.6%)</td>
<td>12 (35.3%)</td>
</tr>
</tbody>
</table>

Category: poor 1 - 4.3, moderate: 4.4 - 7.7, good 7.8 - 10, p* value: significant at <0.05.
Table 6 Chi-square test related to the relationship between the level of perception and the characteristics of respondents

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Perception n (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poor</td>
<td>Moderate</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (16.9%)</td>
<td>32 (45.1%)</td>
</tr>
<tr>
<td>Female</td>
<td>35 (24.6%)</td>
<td>62 (43.7%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-25</td>
<td>20 (23.0%)</td>
<td>40 (46.0%)</td>
</tr>
<tr>
<td>26-35</td>
<td>13 (27.7%)</td>
<td>17 (36.2%)</td>
</tr>
<tr>
<td>&gt;36</td>
<td>14 (17.7%)</td>
<td>37 (46.8%)</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unmarried/ separated</td>
<td>24 (22.0%)</td>
<td>52 (47.7%)</td>
</tr>
<tr>
<td>Married</td>
<td>23 (22.1%)</td>
<td>42 (40.4%)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>School</td>
<td>18 (34.0%)</td>
<td>26 (49.1%)</td>
</tr>
<tr>
<td>University</td>
<td>29 (18.1%)</td>
<td>68 (42.5%)</td>
</tr>
<tr>
<td>Job status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Work</td>
<td>23 (17.0%)</td>
<td>61 (45.2%)</td>
</tr>
<tr>
<td>Not Working</td>
<td>24 (30.8%)</td>
<td>33 (42.3%)</td>
</tr>
<tr>
<td>Have Health Insurance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>37 (20.6%)</td>
<td>79 (43.9%)</td>
</tr>
<tr>
<td>No</td>
<td>10 (30.3%)</td>
<td>15 (45.5%)</td>
</tr>
<tr>
<td>Number of clinical visits year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>15 (24.6%)</td>
<td>25 (41.0%)</td>
</tr>
<tr>
<td>1-2 times</td>
<td>30 (25.4%)</td>
<td>51 (43.2%)</td>
</tr>
<tr>
<td>More than 2 times</td>
<td>2 (5.9%)</td>
<td>18 (52.9%)</td>
</tr>
</tbody>
</table>

Category: poor 1 - 4.3, moderate: 4.4 - 7.7, good 7.8 - 10, p * value: significant at <0.05 .

**Discussion**

The Federation Dentaire Internationale (FDI) defines forensic odontology as the branch of dentistry that deals with the proper handling and examination of dental evidence and with the proper evaluation and presentation of dental findings. Implementing national medical or dental data collection requires great effort at the government level. National forensic odontology data collection requires the establishment of a security infrastructure for data services, training of dentists nationwide, suitable tools for obtaining prints, and a
competent data analysis team.\textsuperscript{10}

Table 2 shows the responses to the knowledge questions. In this survey, the majority of respondents knew that the palatine rugae pattern, teeth and lip lines were different for each individual. This contrary to research conducted in Saudi Arabia, the majority of respondents did not know that the palatine rugae and tooth characteristics were different for each individual.\textsuperscript{8}

In the survey it was found that the majority of respondents knew that forensic odontology helped in medico-legal cases, identifying accident victims and investigating bite marks. This survey is in line with the empirical role of forensic odontologists in disaster management and victim identification by maintaining well-structured, comprehensive and accurate dental records for teaching and research purposes, as well as for legal matters.\textsuperscript{11}

Another study highly recommends mandatory-quality dental records that are kept efficiently and easily accessible.\textsuperscript{12}

Knowledge related to maintaining dental records for forensic and medicolegal purposes may be insufficient indicating the need for proper education and training and the need for further training.\textsuperscript{13} This contradicts research carried out in Saudi Arabia where the majority of people in Saudi Arabia do not know that forensic odontology helps in medico-legal cases.\textsuperscript{8} In this survey, the majority of respondents did not know that forensic odontology can help in cases of sexual harassment and violence. A systematic review paper revealed that a large proportion of the public has insufficient knowledge of the ability of forensic odontologists to handle cases of abuse.\textsuperscript{14}

Table 3 shows the responses to the perception statement. The willingness and rejection of respondents is important for successful data collection. Most of the respondents in this survey stated that they at least have the right to refuse to be recorded and the right to collect data from the national forensic odontology data collection. Applicants’ participation for research purposes is entirely voluntary, however the criteria for participating in databases government-maintained for personal identification are usually different.\textsuperscript{15} In some countries, it is mandatory for citizens to register their personal identities.\textsuperscript{16}

When asked about their perceptions of the role of dentists in national forensic odontology data collection, the majority of respondents would trust them in their ability to maintain confidentiality, and in terms of expertise and knowledge. This is very important, because the patient-doctor relationship depends on trust, communication and respect.\textsuperscript{17} In general, most people consider the use of their personal information in data collection, to overcome this problem requires appropriate cybersecurity measures, which are more appropriate when dealing with government levels.\textsuperscript{18}

In this survey, the majority of respondents wanted to provide their dental information in the future national forensic odontology data collection, this is contrary to research conducted in Saudi Arabia where the majority of Saudi Arabians refuse to register themselves in the database national forensic odontology.\textsuperscript{8}

In table 4, the average level of public knowledge and perception regarding forensic odontology is still categorized as moderate. This is in line with previous research which assessed the insufficient knowledge and awareness of forensic odontology in the community and research conducted in Saudi Arabia said the knowledge and awareness of the people in Saudi Arabia was still very low.\textsuperscript{19,8}

Although crime films and documentaries are available on television, not all societies will be exposed to this. The most important factor identified for data collection is the advertiser media related to the benefits of data collection.\textsuperscript{20} This is the reason why researchers believe that a vital component in the success of increasing people’s knowledge, changing their awareness, and increasing their attitudes towards forensic odontology is its marketing, which involves disseminating knowledge regarding forensic dentistry through social media, television and health care facilities.

Table 5 shows the relationship between the level of knowledge and the characteristics of the respondents. Based on the education level, the respondents who
attended school and university had different levels of knowledge. This is in line with research conducted in Saudi Arabia which states that there are differences in the level of knowledge between respondents who attend school and university level. Education affects the learning process, the higher a person’s education, the easier that person will receive information.8,21

Table 6 shows the relationship between the level of perception and the characteristics of the respondents. Based on the level of education, respondents who attended school and university had different levels of perception. There are also differences in the level of perception between respondents who work and do not have a job. A person’s perception can be influenced by individual attitudes and characteristics, family background, information obtained by knowledge and the needs of the surroundings.22,23

People’s perceptions are a reflection of what they feel and what they fear and / or want more. Increasing participants’ knowledge is an important factor where misunderstandings can be corrected and expectations improved.8 Researchers believe that every perception statement used in this survey represents an opportunity to increase knowledge. However, there are limitations in this research. Some of the anatomical terms of the oral cavity may be unclear to some respondents, even when translated into local dialects.

**Conclusion**

The level of knowledge and perceptions about forensic odontology in Makassar City people aged ≥ 18 years is still around 60% who answered questions correctly and responded positively. It is hoped that the improvement of public knowledge regarding the importance of forensic dentistry is expected to increase public awareness and encourage participation in data collection. Therefore, socialization is needed through social media, television and health care facilities. The public’s desire to carry out dental data collection is very helpful in accelerating the legal process and also in identifying individuals.

**Conflict of Interest:** Nil

**Source of Funding:** Not Applicable

**Ethical Clearance:** Given by Institutional Health Research Ethics Committee of the Hasanuddin University Dental Hospital with Ethical Approval number No.009I / PL.09 / KLPK FKG-RSGM UNHAS / 2020.

**References**


Serum Uric Acid Level in Oral Cancer Patients - Original Study

G. Florence Sangeetha¹, K.M.K. Mastan², N. Aravindha Babu³, S. Leena Sankari³, Jayasri Krupa⁴, T. Gopala Krishnan⁵

¹Post Graduate Student, ²Professor and Head, ³Professor, ⁴Reader, Department of Oral Pathology and Microbiology, Sree Balaji Dental College And Hospital, Chennai, ⁵Reader, Department of Oral Pathology And Microbiology, Sri Venkateswara Dental College And Hospital, Thalambur, Chennai

Abstract

Background Uric acid is a result of the metabolic breakdown of purine digestion. Serum uric acid ensures against carcinogenesis by means of its cell reinforcement properties and forestalls the development of oxygen extremists. High blood groupings of uric acid can prompt gout and are related with diabetes and development of kidney stones. Serum uric acid fixation mirror the harmony between uric acid combination and discharge. Studies have indicated that high uric acid is a danger factor for hypertension, diabetes and Cardio Vascular illness. Squamous cell carcinoma is characterized as threatening epithelial neoplasm showing squamous separation as described by the development of keratin or the bury cell spans. It also includes the initial presence of a precancerous lesion. Moreover, specific medical conditions can contribute to the oral potentially malignant disorders prevalence. Oral squamous cell carcinoma is the most well-known neoplasm speaking to over 80% of all oral malignancy cases. Serum uric acid has been proposed to be related with the danger of malignant growth and it was seen that raised degrees of serum uric acid was related with an expanded disease frequency contrasted with typical qualities.

Aim and Objectives: The aim of this study is to evaluate the serum uric acid level in oral cancer patients, to find out the association between low serum uric acid level and risk of squamous cell carcinoma and to compare the serum uric acid levels in oral cancer patients with normal individuals.

Materials and Methods: This is a hospital based study to find out the uric acid level in oral cancer patients. The participants were selected from the out patients, Department of Oral Pathology and Microbiology. The study included a total number of 28 persons. Out of the 28 persons 15 oral cancer patients were selected for the experimental group. The remaining 13 subjects were in the normal group.

Results: In this study, mean uric acid level in this stroke population with CAD is 6.37 mgs/dl and in those without CAD is 5.00 mgs/dl and thus establishes a statistically significant relationship (‘p’ 0.0004). When males and females are considered, males have a significant association with a ‘p’ value of 0.0003. The buccal mucosa of the oral cavity was the most common involved site followed by the vestibule.

Conclusion: The studies showed that serum uric acid level was significantly lower in oral cancer patients compared to the control group. Uric acid may prevent the formation of oxygen radicals and there by protect against carcinogenesis. In human’s uric acid is the most abundant antioxidant and is an important intra cellular free radical scavenger. Early detection of oral cancer and Habit should be controlled to reduce mortality rates and help to provide successful cancer treatment.

Key Words: Serum uric acid, High uric acid, Oral potentially malignant disorders, Squamous cell carcinoma, Oral cancer.
Introduction

Uric acid is a result of the metabolic breakdown of purine digestion. Serum uric acid ensures against carcinogenesis by means of its cell reinforcement properties and forestalls the development of oxygen extremists. Cell reinforcements, may have a defensive impact against disease.

Uric acid is a heterocyclic compound of Carbon, Nitrogen, Oxygen and Hydrogen with the equation $C_5H_4N_4O_3$ It structures particles and salts known as urates and acid urates.

High blood groupings of uric acid can prompt gout and are related with diabetes and development of kidney stones.

Serum uric acid fixation mirror the harmony between uric acid combination and discharge. Hyperuricemia follows utilization of diets wealthy in Purines, intense liquor utilization, ongoing fructose utilization and high effect exhausting activities. Debilitated kidney work likewise causes hyperuricemia because of aggregation of sodium urate.

Squamous cell carcinoma is characterized as threatening epithelial neoplasm showing squamous separation as described by the development of keratin or the bury cell spans. It also includes the initial presence of a precancerous lesion. Oral leukoplasia, and oral submucous fibrosis are the most common oral potentially malignant disorders, with reported malignant transformation rates ranging from 0.13% to 34% and from 1.9% to 9.13%, individually.

Studies have indicated that high uric acid is a danger factor for hypertension, diabetes and Cardio Vascular illness.

1) SMOKE LESS TOBACCO TYPES: - free leaf biting tobacco, clammy snuff and dry snuff are the three sorts of Smokeless tobacco generally utilized in the oral depression.

2) HUMAN PAPILLOMA VIRUS: - Human papilloma infection may assume an etiological part in cancer-causing agents in the oral hole regular in old people with propensities in lower financial gathering.

Oral tumors are harmful neoplasms that influence the mouth it might emerge from the mouth, metastasize from an inaccessible site or an expansion from connecting site. 90% of oral tumors are squamous cell carcinomas. The mean time of event of oral malignancy is the fifth and 6th decade of life.

The ordinary estimation of uric acid in human blood is 1.5mg to 6mg/dl in ladies and 2.7mg/7.0 mg in men.

Serum uric acid has been proposed to be related with the danger of malignant growth and it was seen that raised degrees of serum uric acid was related with that target organs including the oral cavity, pharynx, larynx and throat. Smokeless tobacco has been related with oral disease for a long time.

It is important to note that oxygen radicals may cause cell damage that ultimately donates to oral carcinogenesis through numerous molecular mechanisms including DNA damage, protein damage, oxidation of important enzymes and activation of specific cytokines. Uric acid acts as antioxidant and is affected by abundant factors including alcohol consumption and dietary intake.

Specifically, alcohol was lightly consumed by the subjects recruited in our study, but tobacco habits were chiefly prevalent. These findings are consistent with those reported in earlier studies from the Indian subcontinent.
an expanded disease frequency contrasted with typical qualities.

1) The aim of this study is to evaluate the serum uric acid level in oral cancer patients, to find out the association between low serum uric acid level and risk of squamous cell carcinoma and to compare the serum uric acid levels in oral cancer patients with normal individuals.

Materials and Methods

This is a hospital based study to find out the uric acid level in oral cancer patients. This study was conducted in the Department of oral pathology and microbiology of Sree Balaji Dental College and Hospital, Pallikaranai, Chennai.

The participants were selected from the out patients, Department of Oral Pathology and Microbiology. The study included a total number of 28 persons. Out of the 28 persons 15 oral cancer patients were selected for the experimental group. The remaining 13 subjects were in the normal group.

Inclusion Criteria

Group 1 – 15 Oral Cancer patients were selected in the study.

Group 2 – The control group consisted of 13 subjects. They were selected on the basis of no oral cancer and any other disease that affect serum uric acid.

Exclusion Criteria

1. The individuals suffering from diseases such as gout, renal disease, cardiovascular disease and diabetes.

2. Patients who are taking regular medical treatment. The medicines may affect serum uric acid levels.

3. Patients with other malignancies except oral cancer.

4. Patients undergoing treatment for oral cancer.

Two millilitres of infravenous blood was taken from all participants after an overnight fast. The blood was centrifuged at 3000 rpm for 5 minutes and separated serum was filled into tubes and analysed for uric acid using the reagent kit and BTS 350. Semi – auto analyser (Biosystems S.A. Costa Brava Barcelona (Spain).

The data was recorded using Microsoft Excel, statistical analysis was done using SPSS version 23. Descriptive statistics is given by frequency, graphs, mean and sd. Chi-square test is used to find the association between two categorical variable. Kruskal Wallis test is used to find the mean difference between two groups. P-value<0.05 is considered to be statistically significant throughout the study.

Results

In this study, mean uric acid level in this stroke population with CAD is 6.37 mgs/dl and in those without CAD is 5.00 mgs/dl and thus establishes a statistically significant relationship (‘p’0.0004). When males and females are considered, males have a significant association with a ‘p’ value of 0.0003. The buccal mucosa of the oral cavity was the most common involved site followed by the vestibule.

Uric acid levels and their association with risk factors:

The distribution of uric acid levels in the study population are as under:

Ø Less than 5mg / dl – 48% (25 males and 23 females)

Ø Between 5 – 6.9 mg / dl – 27% (12 males and 15 females)

Ø Above and equal to 7mg / dl – 25% (12 males and 13 females)

Age wise distribution of uric acid is found statistically significant. As age advances the uric acid level also rises with the ‘P’ value of 0.0001.

This
The mean uric acid value for the 40-49 yrs group is 4.34 mg/dl while the elderly age group of above 70 yrs has the mean value 6.65 mg/dl.

There is no statistically significant association found in this study between sex and uric acid. The mean uric acid level among male population is 5.39 mg/dl and among female population it is 5.51 mg/dl.

### Age and Uric Acid Levels

<table>
<thead>
<tr>
<th>Age group</th>
<th>Uric Acid mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>41-50</td>
<td>4.342</td>
</tr>
<tr>
<td>51-60</td>
<td>5.076</td>
</tr>
<tr>
<td>61-70</td>
<td>5.930</td>
</tr>
<tr>
<td>70 &amp; above</td>
<td>6.656</td>
</tr>
</tbody>
</table>

| 'p'         | 0.0001 (Significant) |

Mean uric acid level in males is 5.39 mg/dl and in females it is 5.51 mg/dl.
Age wise distribution of uric acid is found statistically significant. As age advances the uric acid level also rises with the ‘P’ value of 0.0001. This significance is maintained even when male and female populations are considered separately. (‘P’ of 0.0056 for males and 0.0077 for females).

Habit:

Out of 15 study group, 10 (35.71%) patients had only tobacco intake (in different from) as habit and 4 (14.28%) patients consumed tobacco and alcohol. Among 13 control subjects, only 3 (10.7%) had tobacco (in any form) as habit and 2 (7.14%) subjects had tobacco and alcohol. The results showed a very high significance with a habit of tobacco ($c^2 = 29.68$, $p<0.001$) subjects with a habit of consuming both tobacco and alcohol showed a significant result. ($c^2 = 4.86$, $p<0.05$) (Graph – 3).

Serum uric acid levels:

In the study group, out of 15 patients 3(20%) patients had low serum uric acid levels (<3mg/dl) 10(66.6%) patients has normal serum uric acid levels (3.6mg/dl) and 2(13.3%) patients had high serum uric acid levels (>6mg/dl) among 13 subjects in control group. 1(7.6%) subjects had low serum uric acid levels (<3mg/dl). 8(61.5%) subjects had normal serum uric acid levels (3.6 mg/dl) and 4(30.7%) subjects had high serum uric acid levels (<6mg/dl). The distribution of serum uric acid levels among the study group and the control group showed statistically very high significance ($c^2 =19.86$, $p<0.001$) (table 3).

### Smoking And Uric Acid

<table>
<thead>
<tr>
<th>Tobacco</th>
<th>Uric Acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Present</td>
<td>5.16</td>
</tr>
<tr>
<td>Absent</td>
<td>5.60</td>
</tr>
<tr>
<td>‘p’</td>
<td></td>
</tr>
</tbody>
</table>

Mean uric acid level in tobacco user is 5.16mg / dl and among non- tobacco user is 5.60 mgs /dl. Thus in this study there is statistically significant relationship between tobacco user and uric acid.
The mean age among study group was 53.2 ± 16.8 and that of the control group was 52.02 ± 14.63. The comparison of this shows no statistical significance \((t = 0.25, P > 0.05)\).

Subjects with a habit of consuming both tobacco and alcohol showed a significant result \(\chi^2 = 4.95, P << 0.05\).

The distribution of serum uric acid levels among the study group and the control group showed statistically very high significance \(\chi^2 = 18.86, P < 0.001\).
Discussion

From this study we come to know that uric acid is “lower” in oral cancer patients than the normal people. Only few studies have tested the association between uric acid and carcinogenesis with inconsistent results. Voruganti VS et al., found that serum uric acid could protect against cancer. This was corroborated by Khalili J, who reported a significantly lower serum uric acid in lung cancer patients compared to healthy controls. Since the high oxygen environment of the tissue could be more susceptible to the carcinogenic activity.

Our study consists of 28 samples. Out of the 28 samples 15 Squamous Cell Carcinoma patients were selected. The remaining 13 individuals have no signs of carcinoma and no known systemic disease that affects serum uric acid levels.

Age:

In this study 15 patients were in the age group of 30-80 Years with a mean age of 53.2 Years. This is comparable to the mean age of 49.73 specified by Shenoi et.al.

In our cases 6 (21.4%) were in the age of 41 to 50 Years. Most of the patients were in the fourth and fifth decades of life. This is in the agreement with study conducted by Sankar Narayan et.al where they have found that the peak age frequency of occurrence in India is at least a decade earlier than that described in the western literature.

Sex:

This has revealed the dominance with the male to female ratio of 1.4:1. Similar male prevailing nature was reported by Shenoi et.al in most cases.

Site of Oral Cancer:

Regarding the site of distribution, the buccal mucosa was the most commonly involved site with 7 (25%), vestibule was the second most commonly affected site with 6 (21.42%) patients and in retromolar area with 4 (14.28%), Tongue in 3 (10.74%) patients, alveolus in 2 (7.14%) patients. Floor of the mouth 2 (7.14%) and lips was 3.57%.

Habits:

In this study out of the 15 oral cancer patients, 10 (35.71%) patients had consumption of tobacco (in any form) and 4 (12.28%) patients consumed tobacco and alcohol. Among 13 in control group only 3 members (10.71%) had a habit of consuming tobacco (in any form) as habit and 2 (7.14%) subjects had a habit of tobacco and alcohol consumption.

Comparison of mean serum uric acid as risk factor for oral cancer

In this study, the Study group had 15 Participants and Control group has 13 participants. Out of them 3 (20.0%) patients and 1 (7.6%) subject with low serum uric acid. This is of statistically high significance.

Out of 15 study group 10 (35.71%) patients had only tobacco intake (in various forms) as habit and 4 (14.28%) patients consumed tobacco and alcohol. Among 13 control group only 3 members (10.71%) had consumed tobacco (in any form) as habit and 2 (7.14%) subjects had tobacco and alcohol. (The result showed a very high significance with subject taking only tobacco). The participants with the habit of consuming both tobacco and alcohol showed statistically significant results.

Dhanuthai K et.al. in his study has explained that reduced serum urate in smokers compared with non-smokers in the patient group may reveal unexpected harmful results for the smokers. The same inference is accepted by Bassam E. Hanna et.al. Williams HK, Zygogianni AG et al, Markopoulos and A.O. Lawal et al. They all have showed that low SERUM URIC ACID in regular smokers and reduction of antioxidants including uric acid in smokers indicating that oxidative stress increases every time a cigarette smoked.

The damage of DNA leads to the development of cancer. In fact, it has been found that Reactive Oxygen Species and Reactive Nitrogen Species are involved in
the initiation and promotion of carcinogenesis. Both are initiated by antioxidants. When the equilibrium is broken either by a reduction in levels of antioxidants or by enhancement of Reactive Oxygen Species and Reactive Nitrogen species level, DNA is oxidized and Cancer occurs.

Toxicity by oxygen radicals has been suggested as a major cause of cancer. Uric acid has been found to be an antioxidant. It acts as a scavenger of oxygen radicals in humans. It is one of the main radical trapping antioxidants in plasma and reported to protect the erythrocyte membrane against lipid peroxidation.

This study has proved that serum uric acid was lower in oral cancer patients when compared with normal group and low serum uric acid was associated with increased risk of oral cancer.

**Conclusion**

Oral cancer is the sixth most common cancer in the world. The aim of this study is to determine the serum uric acid level in oral cancer patients and compare them with those of normal patients in order to determine the role of uric acid in the etiology of oral cancer. Oral squamous cell carcinoma is the most common neoplasm representing more than 80% of all oral cancer patients.

Squamous Cell Carcinoma is defined as a malignant epithelial neoplasm exhibiting squamous differentiation as characterized by the formation of keratin /or the presence of intercellular bridges. The most common cause is smoking and alcohol intake. Also, exposure to carcinogens, infectious with papilloma viruses and the nutritional status are cancer risk factors. Poor oral hygiene is accompanied by tooth loss is another suspected risk factor.

The studies showed that serum uric acid level was significantly lower in oral cancer patients compared to the control group. Toxicity by oxygen radicals has been suggested as an important cause of cancer, Heart disease and aging. Uric acid is the product of purine metabolism in humans. Uric acid may prevent cancer by mopping up free radicals that may cause cellular and genetic injury. Uric acid may prevent the formation of oxygen radicals and there by protect against carcinogenesis. In human’s uric acid is the most abundant antioxidant and is an important intra cellular free radical scavenger.

Serum uric acid levels are influenced both by endogenous production and by ingestion. Serum uric acid may be determined by diet and foods such as organs meats, sausages, mussels and sardines have high number of purines that lead to the formation of uric acid.

Low serum uric acid levels may due to nutritional compromise of patients due to [Tumor Necrosis Factor] and Interleukin 6 produced in cancer patients which cause loss of appetite. The development of cancer is multifactorial depending on the extent of DNA damage that is proportional to the magnitude of oxidative and nitrative stress. It has been found that both Reactive Oxygen Species and Reactive Nitrogen Species have been inhibited by antioxidants which have ability to initiate carcinogenesis.

Early detection of oral cancer and Habit should be controlled to reduce mortality rates and help to provide successful cancer treatment. The delay in diagnosis may be due to patient’s delay, professional delay or both. Surgery has been the main mode of treatment for oral cancer.

Poverty, illiteracy and home remedies lead to delay by patients. Such conditions should be avoided and early diagnosis of cancer and proper treatment facilities should be prov.

**Conflict of Interest :** Conflict of interest declared none.

**Ethical Clearance-** Taken from ethical committee of BHARATH INSTITUTE OF H

**Source of Funding-** Self

**References**


To Study the Effectiveness of Rotational Vestibular Stimulation on Improving Balance and Gravitational Insecurity in Children with Down Syndrome - A Research Protocol

Gauravi Desale¹, Ketaki Naik², Dipti Shinde¹, Rinkle Malani²

¹Final Year BPT, MGM School of Physiotherapy Aurangabad Maharashtra, ²Professor and Principal, MGM School of Physiotherapy

Abstract

Background: Children with Down Syndrome have delayed motor development and coordination due to which they have poor balance. Balance is the condition in which all the forces acting on body are balanced such that the center of mass (COM) is within the stability limits, the boundaries of base of support (BOS). Several scales are available for assessing balance. However, standardized and reliable scales have been chosen to prevent the error while testing. Many studies have been conducted showing that sensory integration therapy improves balance. However, there is paucity of study on Rotational Vestibular Stimulation in improving balance.

Objectives: To study whether the rotational vestibular stimulation will improve balance and gravitational insecurity in children with down syndrome. To study the differences in pre-treatment and post-treatment parameters after rotational vestibular stimulation using outcome measures.

Methods: Balance will be assessed prior to treatment and after the treatment. The measures used for assessing will be Pediatric Balance Scale, Bruininks-Oseretsky Test, Movement Assessment Battery for Children.

Results: Once the study is completed, the parameters of outcome measures will be statistically analyzed.

Conclusion: Based on previous researches, it may be predicted that there will be significant improvement in balance in children with Down syndrome.

Keywords: Down syndrome, rotational vestibular stimulation

Introduction

Down syndrome is a genetic disorder of chromosome number 21 present in triplicate, the origin of extra chromosome 21 being either maternal or paternal. The estimated incidence of down syndrome is between 1 in 1000 to 1 in 1,100 live births worldwide. Each year approximately 3000 to 5000 children are born with this chromosomal disorder. There are many neuromuscular and musculoskeletal characteristics in Down Syndrome that can result into developmental delays. Children with down syndrome show motor dysfunctions which are poor balance, motor incoordination and hypotonia with oculomotor dysfunction and vestibular deficits. A smaller cerebellum and brain stem is seen in down syndrome causing loss of neurons in many parts of brain due to generalized hypopcellularity of brain with decreased myelination of brain hemispheres, basal ganglia, cerebellum, brain stem in first year of life. These factors may cause motor and postural delays.
The main function of vestibular system is development of motor skills, integration of postural reflexes, coordination of eye movements and visual attention skills. Developmental disorders such as motor incoordination and learning disabilities are observed in vestibular dysfunction. (5) Vestibular end organs produce signals on stimulation which are transmitted via eight cranial nerves to the vestibular nuclei in the brain stem to vestibular portion of cerebellum then to motor cortex which is responsible for coordination of motor responses, head position, posture and eye movements. It was found that rotational vestibular stimulation of children with Down syndrome showed improvement in motor performance of these children. (6) Vestibular dysfunction result in abnormal reflex and lead to sensation that respond to abnormal information about motion from vestibular receptors. Acceleration or Rotation of head movement result in asymmetric stimulation of neuroreceptors in the labyrinth and produce vestibulo-ocular reflex (VOR), resulting instimulation of the sensory canal integrating the sensed angular head acceleration with output head velocity information to brain. (4)

Balance or Stability defined as status of keeping balance between mutual forces. It is also called as “Postural Control”. Postural control is the ability to maintain balance in gravity field by staying on or returning to body’s center of gravity within the base of support. In children with Down syndrome with balance issues increase their base of support while sitting, standing and walking in order to achieve to stability. (7) Due to coordination of many brain structures movement control is achieved which was organized both hierarchically and in parallel. In hierarchically, a signal is processed within the ascending levels of central nervous system (CNS). In parallel distributed processing it shows that same signals may be processed simultaneously among many different brain structures. In perception, action and cognitive systems of movement control hierarchical and parallel processing occur in alliance. (8) An object when translated or rotated simultaneously a challenge arises leading to how much locally recognized translational motion signal is generated by object and how much is generated by rotation of object. (9)

Daryoush Didehdar, Ameneh Kharazinejad, 2019, in their study showed that there is improvement in static and dynamic balances with sensory motor integration activity in children. In this children have participated in their favorite games with their peers. They compared effectiveness of sensory integration treatment and combination of sensory integration therapy with vestibular stimulation and treatment of nerve growth. It was seen that there was significant improvement with subtest pertaining to sensory integration and subtle motor skills in therapy group. (7)

Annabelle Nommensen, Frikkie Maas, 1993, Their study revealed that vestibular stimulation was unclear. The sensory integration therapy approach studied on children with Down syndrome had been inconsistent and dominated by methodological flaws. The failure to the study was due to inadequate sample size and lack of control for heterogeneity evident in that population, also there was failure to employ standardized measures of gross motor function, fine motor function and vestibular function and they did not apply statistical analysis to outcome results. (10)

Mine Uyanik, Gonca Bumin and Hulya Kayihan, 2003, in their study concluded that there was significant improvement in all three groups. In first group sensory integrative therapy was given, they found there was improvement in sensory integration subtest and fine motor skills. In second group sensory integrative therapy and vestibular stimulation showed improvement in sensory integrative subtest, vestibular system, fine hand skills, reflex development and gravitational insecurities. In third group where NDT showed significant difference in all capabilities (5).

Sarah Sunderman, 2016, Concluded that group 1 included 10 participants with Down Syndrome and group 2 consisted of 8 participants with intellectual and developmental disabilities. Test were done based on Bruininks-osetetsky test of motor proficiency which was shown to be effective. During this analysis it was observed that there was lack of understanding of
participants about the equipment’s used. Limitation of the study was small sample size and lack of control group and limited participants from the particular program (4).

Objectives:

i. To study whether the rotational vestibular stimulation will improve balance and gravitational insecurity in children with down syndrome.

ii. To study the differences in pre-treatment and post-treatment parameters after rotational vestibular stimulation using outcome measures.

Methods:

• Study design: Randomized Controlled Trial
• Type of study: It will be an experimental study.
• Setting: MGM School of Physiotherapy, Aurangabad.
• Sample size: 30 calculated based on prevalence of ADHD in school going children using G power

Materials:

• Bosu ball
• Swiss ball
• T swing
• Square swing
• Tilt board
• Platform swing

Participants:

Inclusion Criteria:

• Age between 6-10 years.
• Down syndrome children with balance affection.

Exclusion Criteria:

• Children with Downs syndrome with cardiac illness like Atrial Septal Defect, congenital heart defect, Tetralogy of Fallot, etc.
• Children with Downs syndrome with Atlantoaxial instability.
• Children with Down syndrome with any orthopedic deformity, nystagmus, aversive response to movement.
• Children with Downs syndrome with severe mental retardation, IQ=20-35.

Variables:

Outcome Measures:

Name, Age, Gender, Gestational age, Prenatal history, Perinatal history, post-natal history and Drug Therapy will be recorded prior to the intervention with the use of following scale.

1. Movement Assessment Battery for Children (M-ABC)-

In this test general motor ability of children was measured. This test is designed to identify and describe the impairment in motor performance of children of 4-12 years of age. According to the Movement-ABC manual the test has acceptable Validity and reliability. The test reliability ranges from 0.70-0.89. The component of this scale include Balance which was further subdivided into 3 categories in which only the balance performance of the children was being focused. The total score of the scale ranges from 0-40. As the highest score indicates poor balance in the children (11).

2. Bruininks - Oseretksy Test of motor proficiency (BOT)-

Scale was used to assess gross motor skills with age group of 4-11 years. It includes components such as Balance with score of 32 points, running speed and agility with 15 points, bilateral co-ordination with 20 points and strength with 20 points. Reliability of scale range from 0.60-0.90(4).
3. **Pediatric Balance Scale (PBS)** -

Pediatric Balance Scale is used to measure the balance in such a way that minimal assistance is required with specialized equipment. The scale is performed in the children with the age group of 4-12 years. Pediatric Balance Scale is a reliable measure of functional balance in the children with mild to moderate motor impairment. The test reliability ranges from 0.89-0.99. The component of the scale included were 14 which were the task performed routinely by the children in day-to-day activity. Pediatric Balance Scale incorporates 0-4 grading scale to assess the performance. The total score ranges from 0-56. The highest score indicates no affection in balance and least score has affection of balance in children\(^{(12)}\).

**Study Size:**

**Expected Results:**

Once the data will be collected and statistical analysis will be done.

Significant Improvement may be seen in improving balance using sensory integration therapy. However, in previous researches on rotational vestibular stimulation was no statistical improvement in balance due to small treatment duration. In this study, the duration of treatment will be increased and large sample size will be used as compared to previous study on rotational vestibular stimulation. Hence, this study will help in investigating whether there will be improvement in balance using Rotational Vestibular Stimulation or not.

**Discussion**

The vestibular system is important for achievement of normal motor development and coordination. Vestibular dysfunction leads to motor discoordination. The vestibular system is one of the wide sensory system. Improving balance is important as it has several beneficial roles in an individual’s life. Improvement in balance leads to reduction in fear of fall which increases confidence amongst the children and increases their participation in various functional activities. Many studies have been done on balance in past years. Such studies revealed improvement in balance in children with down syndrome. These studies were conducted using techniques such as Neurodevelopmental Technique, Sensory Integration Therapy, Treadmill training, etc. But these studies faced some limitations such as inadequate sample size, inadequate treatment time, environmental disturbances during treatment, etc. However, in this study we will be eliminating these barriers by increasing the duration of session per day as 1.5 hours a day, frequency will be increased as 3 days per week, total duration of the treatment will be extended for 3 months. Moreover, this study will employ widely used methods of assessment which have appropriate reliability and validity as well as accepted by researchers, found in literature and research papers.

**Conflict of Interest**_ there is no conflict of interest

**Source of Funding**_ Self

**Ethical Clearance** taken from Institute

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Assess the Effectiveness of Planned Teaching Program on Knowledge Myths, Belief and Misconception Related to Complementary Feeding Practices

Geeta¹, Sunil Kumar Dular², Anu Grover³, Akoijam Mamata Devi⁴

¹PG Student, Obstetrics & Gynecological Nursing, Faculty of Nursing, SGT University, ²Associate Professor, Community Health Nursing, Faculty of Nursing, SGT University, ³Assistant Professor cum SLTO, Obstetrics & Gynecological Nursing, NRSC, SGT University, ⁴Professor, Obstetrics & Gynecological Nursing, Faculty of Nursing, SGT University

Abstract

Background: Complimentary feeding practices are a gradual process of introducing supplementary foods, starting around the age of 4-5 months. It should be supplemented by suitable foods rich in protein and other nutrients which is called as supplementary foods. The limited knowledge of rural women regarding complimentary feeding practices and myths and misconceptions about complementary feeding practices are evident in rural areas.

Methods: Quantitative research approach and Pre experimental. It is one group pretest – posttest design to assess the effectiveness of planned teaching program on knowledge myths, belief and misconception related to complementary feeding practices among women in selected rural areas of Gurugram. The data was collected from 60 rural women using Structured knowledge questionnaire and myths and misconception checklist by purposive sampling technique.

Result: The study results showed that in Pre test 51(80%) were having average knowledge, 7(11.66%) were having poor knowledge and 2 (3.33 %) were having good knowledge and in post test 59(98.33%) were having good knowledge, 1(1.66%) were having average knowledge on complimentary feeding practices. About the myths and misconceptions 34(56.66%) samples had the myths and misconceptions that complimentary feeding should be delayed as it causes vomiting and 19(31.66%) samples had the misconceptions that complimentary feeding to be begin when they start chew their fists. Significant increase in knowledge was found as the mean percentage of knowledge score was improved from 58% in pre test to 80.93% in the post test shows that structure teaching program is an effective tool to improve the knowledge of Primi-Para mothers regarding complimentary feeding practices. There is no significant association between post test knowledge score and any of the demographic variables.

Conclusion: The study concluded that rural women have less knowledge regarding complimentary feeding practices. There is evident of myths, misconceptions and barriers related to complimentary feeding practices. The health education programme is imperative to impart awareness and education to rural women to eradicate those barriers to complimentary feeding practices.

Keywords: Planned Teaching programme, Complimentary feeding practices, Structured knowledge questionnaire, misconceptions, myths.

Introduction

Health of the developing child is always a matter of great apprehension to the parents. Physical and mental health is necessary, as it is associated with physical,
mental and social, spiritual development of the children. India is a traditional society in the context of myths and beliefs. Starting from the period of infancy, breast feeding has been the only way of feeding the newborn child. In Kashyap Samhita, suggested offering fruits by 6 months and semisolid cereal preparation at tenth month. All these have contributed to the current beliefs which have been adapted with variation in the country due to socio-cultural, religious beliefs, literacy status, family size and availability of health services. 2-4

Complimentary feeding practices are a gradual process of introducing supplementary foods, starting around the age of 4-5 months which should proceed gradually and be based on the infant’s rate of growth and developmental skills. Child may suffer from diarrhea, obesity, under-weight, allergy, refusal to take food, choking, regurgitation, vomiting constipation and abdominal colic. These issues will obstruct the growth and development of children. Most of these issues occur during the period of weaning due to insufficient knowledge and also due to defective feeding practices.4 Complimentary feeding practices education to the mothers is very essential to provide knowledge regarding weaning and to promote healthy weaning practices.5

Material and Methods

A Pre-experimental design was adopted for the study. The study was carried out in the village Farukhnagar of Gurugram Haryana. Total 60 Breast Feeding mothers were enrolled in study using Purposive sampling technique. Structured knowledge questionnaire was used to assess the knowledge regarding the complimentary feeding and checklist regarding the myths and misconceptions regarding the complimentary feeding practices. Tools were developed after extensive review of literature and were validated by experts in field. Ethical permission for the study was taken from institutional ethical committee of SGT University, Gurugram and Sarpanch of Farukhnagar village of Gurugram.

Results:

Majority of the study population 17 (28.34%) belong to above 37 years and 9 (15%) belong to 23-32 years. Majority 21(35%) of the study participants have primary education and 9(15%) have no formal education. Majority 20 (33.34) of the study participants were housewives and 4(6.66 %) were health professionals. Majority 25 (41.66%) of the participants had a family income of less than 5000 and 3 (5%) had the income of 15000 to 20000. Majority 21 (35 %) of the samples belongs to joint family and 8(13.66%) belongs to extended family. Majority 19(31.66%) of the feed every 2 hours and 7 (11.66) samples feed once or twice a day. Majority 28(46.66) of the samples started complimentary feeding at 4 months and 9(15%) started between 6 and 8 months. Majority 13(21.68%) had information from magazine and from other sources and 10 (16.66%) had information from health professionals.

Data presented in the table showed that in pre test majority 51(80%) were having average knowledge, 7(11.66%) were having poor knowledge and 2 (3.33 %) were having good knowledge. In post test 59(98.33%) were having good knowledge, 1(1.66%) were having average knowledge on complimentary feeding practices
### MYTHS AND MISCONCEPTIONS ABOUT COMPLIMENTARY FEEDING

<table>
<thead>
<tr>
<th>Myth</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat/daliya/moti suji is heavy for kids</td>
<td>21</td>
</tr>
<tr>
<td>Vegetables may choke in the food pipe of the baby</td>
<td>28</td>
</tr>
<tr>
<td>Saur food cannot be given as it causes cold</td>
<td>28</td>
</tr>
<tr>
<td>Food is just for fun until they are one year old</td>
<td>25</td>
</tr>
<tr>
<td>Child is ready for weaning when they chew their fists</td>
<td>19</td>
</tr>
<tr>
<td>Child cannot eat lump of food without teeth</td>
<td>21</td>
</tr>
<tr>
<td>Solid and thick consistency food should not be given hence it causes constipation</td>
<td>28</td>
</tr>
<tr>
<td>Rice, rice water, dhal only good for the baby</td>
<td>28</td>
</tr>
<tr>
<td>Ghee is very heavy to digest and causes cough for the baby</td>
<td>28</td>
</tr>
<tr>
<td>Weaning foods are indigestible for the baby hence it should be delayed</td>
<td>33</td>
</tr>
<tr>
<td>Jaggery spicy food, papaya, eggs, mangoes are hot for the child</td>
<td>32</td>
</tr>
<tr>
<td>Banana, curd, buttermilk, ice-cream and fruits like guava causes cold for the child</td>
<td>25</td>
</tr>
<tr>
<td>Dhal or Pulses causes gas in the child's stomach</td>
<td>27</td>
</tr>
<tr>
<td>Undiluted milk is harmful as a weaning food</td>
<td>21</td>
</tr>
<tr>
<td>Complimentary feeding should be delayed since it causes vomiting for the baby</td>
<td>34</td>
</tr>
</tbody>
</table>

Data shows in the above table that 34(56.66%) samples had the myths and misconceptions that complimentary feeding should be delayed as it causes vomiting and 19(31.66%) samples had the misconceptions that complimentary feeding to be begin when they start chew their fists.
Data shows in the table that there is a significant difference between mean scores on knowledge as the ‘t’ value obtained 27.34 is higher than the tabulated value 1.67 at 0.05 level of significance.

There is no significant association between post test knowledge score with any of the selected demographic variables.

Discussion

To assess the myths and misconceptions about complimentary feeding practices among women with the help of myths and misconceptions about complimentary feeding practice checklist. It was found that 34(56.66%) samples had the myths and misconceptions that complimentary feeding should be delayed as it causes vomiting and 19(31.66%) samples had the misconceptions that complimentary feeding to be begin when they start chew their fists. Uzma Eram(2017) conducted a study on Myths, Beliefs and Malpractices Relating to Breastfeeding and Complementary Feeding
Practices. Myths, Beliefs and Malpractices Relating to Breastfeeding and Complementary Feeding Practices. It is concluded that most of the women are aware about exclusive breastfeeding although they do not practice 100%. This is because of the customs and traditions prevailing in our society.

To evaluate the effectiveness of planned teaching program on complementary feeding practices. A well-structured planned teaching program on Complimentary feeding was given to the participants with proper guidelines and explanations. After 7 days a post test was conducted by using same tool intervention, paired t-test was used to find out effectiveness.

In pre test maximum numbers of samples i.e. Majority 51(80%) were having average knowledge, 7(11.66%) were having poor knowledge and 2 (3.33 %) were having good knowledge in the pre test .In post test 59(98.33%) were having good knowledge, 1(1.66%) were having average knowledge on complimentary feeding practices. The calculated value was more than table value & mean pre interventional and post international score of women were checked, it was found that there was significant difference in pre and post interventional score of the subject i.e.post interventional score with mean score with 21.9 with SD1.91 from the pre interventional score of 14.51 score of with SD of 1.45. Nirmal Raj E.V. (2015) conducted a study on Effectiveness of STP on knowledge regarding complimentary feeding practices among primi-para mothers. The findings of the study with regard to the pre test knowledge assessment, revealed that the mean of the primi para mother’s knowledge was 58. In post test, very significant increase in knowledge was found as the mean percentage of knowledge score was improved from 58%in pre test to 80.93% in the post test. This study concluded that structure teaching program is an effective tool to improve the knowledge of Primi-Para mothers regarding complimentary feeding practices.

To find the association between knowledge and demographic variable chi square was used and result showed that the obtained P value is more than 0.05 and none of the variables has association with the post test knowledge score. Hence there is no significant association between post test knowledge score and any of the demographic variables.

**Conclusion**

The present study assessed the effectiveness of planned teaching program on knowledge myths, belief and misconception related to complementary feeding practices among women in selected rural areas of Gurugram, NCR. After the assessment it was found that myths and misconceptions about complimentary feeding is prevalent among women in rural areas. After the assessment it was found that there is optimal knowledge of women of complimentary feeding practices. There has been sharp increase in the knowledge of women on complimentary feeding practices after planned teaching programme. The study concluded that planned teaching programme could be significant in improving the knowledge of women regarding complimentary feeding practices and it would be beneficial in the rural settings.

**Ethical Clearance**: Taken from institutional ethical committee of SGT University, Gurugram and Sarpanch of Farukhnagar village of Gurugram.

**Source of Funding**: Self

**Conflict of Interest**: Nil

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Effect of an Educational Program for Patients Post Coronary Artery Bypass Surgery on the Compliance with Symptoms Management Strategies

Gehan Karawan Sayed Sallam¹, Kamelia Fouad Abdalla², Sara Fathi Mahmoud³
¹PhD Candidate, ²Professor, ³Assistant Professor, Medical Surgical Nursing, Faculty of Nursing-Ain Shams University

Abstract

Background: Patients undergoing CABG often experience a varied series of discomfort symptoms post hospital discharge include; shortness of breath, chest pain or angina pain, incisional pain, atrial fibrillation, fatigue, leg swelling, sleep quality, loss of appetite, anxiety and depression. Specific educational program regarding symptoms management strategies (SMS) are used to improve patient outcome, therefore patients’ compliance with this program is very essential to relief, manage it.

Aim: to evaluate the effect of an educational program for patients post CABS on the compliance with (SMS).

Design: a quasi-experimental design.

Setting: conducted at outpatient clinics in Cardio Thoracic Academy at Ain Shams Hospital.

Subject: a purposive sample of (150) patients post CABG were recruited.

Tools: demographic, medical data, Cardiac Symptoms Survey (CSS) and compliance scale are used.

Results: The subjects were complaint towards (SMS) pre-program (12.7%) changed to (83.3%) post program. As evidence, 53.3% of the subjects who had severe level of perception of symptoms pre, changed to 5.3% post. Moreover, (62.7%, 59.4% respectively) of the subjects who had severe level of frequency, severity of symptoms pre changed to (4 %, 2.7% respectively) post. In addition, (57.3% respectively) of the subjects who had severe level of interference with physical activity, enjoyment of life pre, changed to (4%,4% respectively) post.

Conclusion: Educational program showed positive effect on the subjects experienced discomfort symptoms post CABG and their compliance towards the management strategies.

Key words: Experienced discomfort symptoms post CABG, Symptoms management strategies, compliance, educational program

Introduction

Coronary heart disease (CHD) is a main cause of morbidity and mortality throughout the world. World Health Organization (WHO) expects 11.1 million deaths will occur from CHD in 2020. CABG has been the mainstay of treatment for revascularization in CHD patients, since 1960, in providing symptomatic relief and increasing life expectancy (14).

The main goal of (CBG) is releasing of angina, improved life expectancy. Although modern advances have enhanced the success rate of CABG they are not adequate to eliminate altogether the physical, psychological and social problems that patients tackled in the period following discharge. Patients post CABG conveyed that they experienced postoperative discomfort symptoms such as sleep disturbances, fatigue, swelling, shortness of breath, fatigue, loss of appetite, chest pain, gastrointestinal disturbance, weight loss, anxiety related to the treatment and their capability to adhere to the recommended physical activity, swelling of leg incisions, weakness, dizziness and depression (4).

The most important component of nursing care following CABS is concentrated on teaching patients to distinguish, manage postoperative symptoms. Nurses involved in postoperative teaching in acute care, home settings should deliver symptoms management strategies.
to patients and their families. Patients often learn through vicarious experience or modeling from others with similar experiences. SMS that other post-CABS patients have used successfully may improve a patient’s preparedness to try a strategy. SMS teaching could be enhanced with more explicit knowledge of strategies essentially used by patients (12).

Compliance is the process whereby the patient follows the prearranged, dispensed regimen as reinforced by the prescriber and dispenser. Compliance with treatment is a sign of a positive behaviour in which the patient is motivated appropriately to adhere to the prescribed treatment as a result of a perceived self-benefit and constructive consequence. Many studies suggested that patients who adhere to their treatments have better outcomes; they get long life, enjoy a higher quality of life (9).

The occurrence rate of CAD is growing all over the world including Egypt. According to ministry of health in Egypt, 2014 stated that CAD deaths reached 107,232 (23.14%) of all deaths. Age adjusted death rate is 186.36/100,000 population; this ranks Egypt #23 in the world. CAD deaths were 78,897 (21.73%) of all deaths, which make CAD the first killer in Egypt (7).

**Method of Study**

A quasi-experimental design was followed. The study was conducted among 150 post CABG patients for first time, discharged within 7 days after surgery and coming to follow up in outpatient clinics in Cardio Thoracic Academy at Ain Shams Hospital. Tools & data collection it was developed by the researcher based on the recent and related literatures it was written in Arabic to suit the level of education for subjectsthe data were collected through the following tools. **Tool I**, constructed multiple-choice questions to assess patient’s demographic characteristics which had two parts. Part A, concerned with socio-demographic variables. Part B included patients’ medical data. **Tool II**, It was used to assess patients’ level of the discomforts symptoms experienced by patients post CABS. It was adopted from (10). It consisted of 10 symptoms which included the following; (angina, shortness of breath, incision pain, a fluttering or racing heartbeat, fatigue, leg swelling sleep disturbance, loss of appetite, anxiety, and depression). Each of these ten symptoms was measured in regards to three an individual’s dimensions which included (perception evaluation and response to symptoms). Then each one of 10 symptoms was each rated on four different response scales (frequency, severity, interference with physical activity, and with enjoyment of life). **Tool III** It was used to assess extent to which patients’ behaviors comply with symptoms management strategies based on (11) and modified by researcher. Patient’s response were assessed through three numerical rating scale ranged from (1 = none or rarely, 2= sometimes and 3 = always). It was consisted of (10) common symptoms and management strategies items for each one of the symptoms it included 74 items.

The content validity was obtained from 7 experts in medical surgical nursing from different academic categories at the faculty of nursing, Ain Shams University. The expertise opinion’s elicited regarding the tools for clarity, consistency, accuracy relevance, comprehensiveness, simplicity and applicability and minor modification was done. **Testing reliability**, the test for this tools was carried out by Cronbach alpha test.

**Pilot Study**

A pilot study carried out for 10% of the subjects who the criteria. Based on the finding of pilot study the essential modification was done to the tool.

**D. Field Work:**

**Assessment Phase**

The aim of study explained to medical team of outpatient clinic. Interviewed subject who meet the inclusion. The required data were collected over 12 month. Six month spent for data collection and other six month spent for follow up the baseline patients’ data were assessed from the studied subjects that include demographic, medical data, cardiac survey and compliance of the first month of follow up visit in the
outpatient clinic.

Planning Phase

The educational program prepared and designed for the studied patients according to patient assessment needs and their level of understanding. The researcher assessed the compliance with SMS for the studied patients.

Educational booklet contain four main parts as following:

Part 1: consisted of anatomy & physiology of the heart, definition, indications, preoperative preparation prior to CABG and emergency instruction post CABG.

Part 2: included patient compliance with symptoms management strategies, experienced discomfort symptoms, importance of patients compliance.

Part 3: contained instruction regarding symptoms management strategies and how to deal with it post CABG for the common experienced symptoms e.g SMS for shortness of breath, chest pain /angina pain, incisional pain, a fluttering or racing heartbeat, fatigue, sleeping disturbance, leg swelling, poor appetite, anxiety, and depression.

Part 4: instructions for medication, movement, return to work, activity, follow up schedule, healthy diet. Each participant obtained a hard copy an education booklet.

Implementation Phase

The educational program covered the 4 parts of the booklet content by holding 3 main sessions each session aimed to teach two strategies to be taught to the patients. It was held on patient waiting area or any other empty room according to the situation of the clinic, it was around 60 minutes, the educational session conducted either during waiting time or after the patients appointment with physician the session were conducted on the morning time, afternoon. The assigned number of patients to educational session coordinated by researcher, clinic nurses and head nurse of outpatient clinic, maximum capacity per session was 10 patients, the educational program repeated from the researcher 8 times for 8 groups of patients to cover the total number of assigned patients in the study group.

Total hours for sessions for studied patients 3 hours one hour/session in each appointment and completed in the next appointment this phase took almost 3 months, the educational program as follows:

- **First session**: covered the first two parts of the booklet the researcher introduce the educational program to the patient and their families and discussed, (anatomy, physiology of heart, definition of CABG, compliance, discomfort symptoms)

- **Second session**: covered the third part of the booklet the patients trained about symptoms management strategies (chest pain /angina pain and shortness of breath), (incision pain, fluttering heart beat) (fatigue, leg swelling)

- **Third session**: covered the rest of the third, fourth part of the booklet (sleep disturbance, loss of appetite) (anxiety, depression) instructions for medication, movement, return to work, activity, follow up schedule, healthy diet.

Evaluation Phase

Effect of an educational program for patients post CABG on the compliance with symptoms management strategies evaluated the researcher by comparing the change in the studied patients experienced discomfort symptoms and their compliance to symptoms management strategies pre, 6 months post program implementation by using tools 2 and 3 in studied patients this phase took around 3 months.
<table>
<thead>
<tr>
<th>Demographic Data</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 - &lt;30</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>30 - &lt;40</td>
<td>27</td>
<td>18</td>
</tr>
<tr>
<td>40 - &lt;50</td>
<td>43</td>
<td>28.7</td>
</tr>
<tr>
<td>50 - 60</td>
<td>65</td>
<td>43.3</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td><strong>48.6 ± 9.89</strong></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>63</td>
<td>42</td>
</tr>
<tr>
<td>Female</td>
<td>87</td>
<td>58</td>
</tr>
<tr>
<td>Level of Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate (not read and write)</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Primary school</td>
<td>38</td>
<td>25.3</td>
</tr>
<tr>
<td>Secondary school</td>
<td>55</td>
<td>36.7</td>
</tr>
<tr>
<td>University</td>
<td>27</td>
<td>18</td>
</tr>
<tr>
<td>Social status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>20</td>
<td>13.3</td>
</tr>
<tr>
<td>Married</td>
<td>123</td>
<td>82</td>
</tr>
<tr>
<td>Divorced</td>
<td>4</td>
<td>2.7</td>
</tr>
<tr>
<td>Widow</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Working Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not working</td>
<td>55</td>
<td>36.7</td>
</tr>
<tr>
<td>Working</td>
<td>95</td>
<td>63.3</td>
</tr>
<tr>
<td>Nature of work (N=95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Need mental effort only</td>
<td>35</td>
<td>36.8</td>
</tr>
<tr>
<td>Need muscle effort only</td>
<td>20</td>
<td>20.1</td>
</tr>
<tr>
<td>Need mental and muscle effort</td>
<td>40</td>
<td>42.1</td>
</tr>
<tr>
<td>The place of residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural area</td>
<td>61</td>
<td>40.7</td>
</tr>
<tr>
<td>Urban area</td>
<td>89</td>
<td>59.3</td>
</tr>
<tr>
<td>Monthly income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enough</td>
<td>48</td>
<td>32</td>
</tr>
<tr>
<td>Not Enough</td>
<td>102</td>
<td>68</td>
</tr>
</tbody>
</table>
**Results**

Table (1) revealed that mean age of the patients were $48.6 \pm 9.8$, 58% with age from 50-60 years old, regarding gender 58% of them were female and only 18% of them had university education, concerning social status, 82% of the studied patients were married. In addition, 63.3% of the patients had work, 59.3% of them from urban area and 68% of them had not enough income.

Table (2): Comparison between the studied patient regarding to total level of the experienced discomfort symptoms post CABG according to three dimensions pre- and post-educational program (n=150).

<table>
<thead>
<tr>
<th>Items</th>
<th>Pre</th>
<th>Post</th>
<th>T.test</th>
<th>X2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild N (%)</td>
<td>Moderate N (%)</td>
<td>Severe N (%)</td>
<td>Mild N (%)</td>
<td>Moderate N (%)</td>
</tr>
<tr>
<td>Perception of symptoms</td>
<td>14 (9.4)</td>
<td>56 (37.3)</td>
<td>80 (53.3)</td>
<td>117 (78)</td>
<td>25 (16.7)</td>
</tr>
<tr>
<td>Evaluation of symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of symptoms</td>
<td>16 (10.6)</td>
<td>40 (26.7)</td>
<td>94 (62.7)</td>
<td>114 (76)</td>
<td>30 (20)</td>
</tr>
<tr>
<td>Severity of symptoms</td>
<td>16 (10.6)</td>
<td>45 (30)</td>
<td>89 (59.4)</td>
<td>119 (79.3)</td>
<td>27 (18)</td>
</tr>
<tr>
<td>Response to Symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interference with physical activity</td>
<td>18 (12)</td>
<td>46 (30.7)</td>
<td>86 (57.3)</td>
<td>117 (78)</td>
<td>27 (18)</td>
</tr>
<tr>
<td>Interference with enjoyment of life</td>
<td>22 (14.7)</td>
<td>42 (28)</td>
<td>86 (57.3)</td>
<td>112 (74.7)</td>
<td>32 (21.3)</td>
</tr>
</tbody>
</table>

Table (2) showed that, there was a highly statistical significant decrease in the patients total level of the experienced discomfort symptoms at three dimensions post CABG post-educational program where ($p<0.01$). As evidence, 53.3% of the patients who had sever level of perception of symptoms pre, where changed to 5.3% post. Moreover, (62.7% and 59.4% respectively) of the patients who had sever level of frequency and severity of symptoms pre, where changed to (4% and 2.7% respectively) post. In addition, (57.3% respectively) of the studied patients, who had sever level of interference with physical activity and enjoyment of life pre, where
changed to (4 % and 4 % respectively) post the educational program.

Figure (1): Percentage distribution of the studied patients regarding to total level of experienced discomfort symptoms post CABG pre- and post-educational program (n=150).

Figure (1) showed that, (60%) of studied patients had sever level of total experienced discomfort symptoms pre educational program , where changed to (3.3 %) post educational program.

Table (3): Comparison between the studied patients regarding to their compliance level with management strategies for the experienced discomfort symptoms post CABG pre- and post the educational program (n=150).

<table>
<thead>
<tr>
<th>Items</th>
<th>Pre</th>
<th>Post</th>
<th>T.test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compliance</td>
<td>Non-Compliance</td>
<td>Compliance</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Management Strategies for Shortness of breath</td>
<td>22</td>
<td>14.7</td>
<td>128</td>
</tr>
<tr>
<td>Management Strategies for chest pain</td>
<td>25</td>
<td>16.7</td>
<td>125</td>
</tr>
<tr>
<td>Management Strategies for incisional sternal pain</td>
<td>30</td>
<td>20</td>
<td>120</td>
</tr>
<tr>
<td>Management Strategies for fluttering heart rate</td>
<td>24</td>
<td>16</td>
<td>126</td>
</tr>
<tr>
<td>Management Strategies for Fatigue and Tiredness</td>
<td>18</td>
<td>12</td>
<td>132</td>
</tr>
<tr>
<td>Management Strategies for leg swelling</td>
<td>22</td>
<td>14.7</td>
<td>128</td>
</tr>
<tr>
<td>Management Strategies for sleep disturbance</td>
<td>14</td>
<td>9.3</td>
<td>136</td>
</tr>
<tr>
<td>Management Strategies for losing appetite</td>
<td>28</td>
<td>18.7</td>
<td>122</td>
</tr>
<tr>
<td>Management Strategies for Anxiety</td>
<td>16</td>
<td>10.7</td>
<td>134</td>
</tr>
<tr>
<td>Management Strategies for Depression</td>
<td>12</td>
<td>8</td>
<td>138</td>
</tr>
</tbody>
</table>
Table (4) showed that, there was a marked improvement in the studied patients compliance level toward management strategies for the experienced discomfort symptoms post CABG post program with highly statistically significant difference pre- and post the educational program where (p=<0.01).

Figure (2): Percentage distribution of patients total compliance level toward management strategies for the experienced discomfort symptoms post CABG pre and post the educational program (N=150).

Figure (2) presented that 12.7% of studied patient were complaint towards management strategies for the experienced discomfort symptoms post CABG pre the educational program, where changed to 83.3% post the educational program.

Table (4): Relation between and total level of compliance level toward management strategies for the experienced discomfort symptoms post CABG and demographic characteristics among studied patients post the educational program.

<table>
<thead>
<tr>
<th>Items</th>
<th>Total level compliance level</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compliance (n=125)</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>20 - &lt;30</td>
<td>15</td>
<td>12</td>
<td>0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 - &lt;40</td>
<td>27</td>
<td>21.6</td>
<td>0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 - &lt;50</td>
<td>40</td>
<td>32</td>
<td>3</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 - 60</td>
<td>43</td>
<td>34.4</td>
<td>22</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>53</td>
<td>42.4</td>
<td>10</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>72</td>
<td>57.6</td>
<td>15</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

X2  | P-Value |
---|---------|
18.62 | .000** |
2.360 | .106 |
Table (4): Relation between total level of compliance level toward management strategies for the experienced discomfort symptoms post CABG and demographic characteristics among studied patients post the educational program.

<table>
<thead>
<tr>
<th></th>
<th>Illiterate</th>
<th>Primary school</th>
<th>Secondary school</th>
<th>University</th>
<th>Total</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of Education</td>
<td>10</td>
<td>33</td>
<td>55</td>
<td>27</td>
<td>130</td>
<td>14.90</td>
<td>.006**</td>
</tr>
<tr>
<td>Marital Status</td>
<td>0</td>
<td>123</td>
<td>1</td>
<td>1</td>
<td>135</td>
<td>14.30</td>
<td>.005**</td>
</tr>
<tr>
<td>Working status</td>
<td>45</td>
<td>80</td>
<td></td>
<td></td>
<td>125</td>
<td>1.330</td>
<td>.194</td>
</tr>
<tr>
<td>Place of residence</td>
<td>53</td>
<td>72</td>
<td></td>
<td></td>
<td>125</td>
<td>1.360</td>
<td>.190</td>
</tr>
<tr>
<td>Monthly income</td>
<td>45</td>
<td>80</td>
<td></td>
<td></td>
<td>125</td>
<td>22.63</td>
<td>.000**</td>
</tr>
</tbody>
</table>

Table (4) presented that, there was highly statistically significant relation between the studied patients’ compliance total level toward management strategies for the experience discomfort symptoms post CABG post the educational program and their demographic characteristics as age, level of education, marital status and monthly income at (P= < 0.01). While, there was no statistically significant relation with their gender, working status and place of residence at (P= > 0.05).

Discussion

Part I: Patient’s demographic and medical data:

The current study demonstrated that mean age of patients was 48.6 ± 9.8, 58% with age from 50-60 years old, regarding gender more than half were females and only less than one fifth of them had university education, concerning social status, more than three quarters of patients were married. These results explained as increasing at age and female’s gender had high risk for cardiac disease than young people and male gender. These results were in cohort with the study performed by (5), about Association of microvesicles with graft patency in patients undergoing CABG, at Italy with sample size 60 patients and found the mean age of them was 64±8 and the majority of them were males. In addition, less than two thirds of the studied patients had work, more than half of them from urban area and more than two thirds of them had not enough income. These results attributed to setting of the selected study was university hospital which it wavers part of the cost of the surgery. These results were in cohort with the study conducted by (3), entitled Quality of Life among Post CABG Patients, at India with sample size 101 patients and found that more than half of patients were employee and had low income.
II–Patients’ cardiac symptoms survey:

Regarding to the patient total level of experienced discomfort post CABG pre- and post-educational program, there was highly significant difference where (p=<0.01). As evidence, less than two thirds of the patients who had sever level of the total experienced discomfort symptom preprogram, where changed to the few of them post program. These results may be due the designed educational program developed by the researcher based on need of the studied patient, it showed highly effective intervention for releasing experience discomfort symptom post CABG. These results were in agreement with the study conducted by (1)-about effects of implementing clinical pathway on pain and anxiety for patients undergoing cardiac surgery, at Egypt with sample size 50 patients and found that the intervention group had significantly lower level of anxiety and pain than the control group before and after surgery at p value <0.01**.

Part III: Compliance with symptoms management strategies (SMS)

According to the patient total compliance towards SMS, there was highly significant difference pre and post where (p=<0.01). As indicated, less than one fifth of the studied patients were complaint towards SMS preprogram, where changed to more than half of patients post program. These results explained as intervention program included valuable knowledge about importance of patient compliance to the health team instructions. These results were in cohort with the study performed by (6)-entitled Effects of tele monitoring on patient compliance with self-management recommendations and outcomes of the innovative tele monitoring enhanced care program for chronic heart failure: randomized controlled trial, at Australia with sample size 104 patients and found significant improvement at participant compliance with weight monitoring, although the withdrawal rate was high.

Also, this study finding supported with the study conducted by (8)-about the effects of a comprehensive rehabilitation and intensive education program on anxiety, depression, quality of life, and major adverse cardiac and cerebrovascular events in unprotected left main coronary artery disease patients who underwent CABG who detected that intensive education program had high significant improvement on patients’ compliance with guidelines of health team.

Part IV: Relations & correlations:

The study finding revealed that, there was highly statistically significant relation between the studied patients total’ compliance towards SMS pre-post educational program and their demographic characteristics as age, level of education, marital status and monthly income at (P= < 0.01). While, there was no statistically significant relation with their gender, working status and place of residence at (P= > 0.05). These results were in agreement with the study performed by (2)-about Frequency and predictors of non-adherence to lifestyle modifications and medications after CABG: A cross-sectional study, with sample size was 265 patients and stated that unemployment and expensive were the predictors to non-compliance to medication. Meanwhile, these result was inconsistent with the study performed by (13)-about Factors Affecting Compliance with Therapeutic Regimen for Patients with CABG Suggested Nursing Guidelines, at Egypt with sample size was 72 patients and reported that patient related factor had no significant relation with their compliance score at p value >0.05.

Conclusion

Educational program had highly statically significant positive effect on the studied patients experienced discomfort symptoms post CABG and their compliance towards the management strategies.

Conflict of Interest: Not present any conflict

Funding: Self-funding, without any external source.

Ethical Clearance: Ethical clearance obtained from ethical committee of faculty of nursing and Cardio Thoracic Academy at Ain Shams Hospital before conducting the study work.
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9. Mahdy AY, Ali GA. Effect of Discharge Planning on Patient with Cardiac Surgeries Regarding Compliance towered Therapeutic Regimen. 2014; Vol.5 No.2 : 305-360


Administrative Factors and Key Success Factors Affecting the Surveillance, Prevention and Control of Coronavirus Disease 2019 (COVID-19) among the Health Personnel at Sub-District Health Promoting Hospital

Gerattikun Kaenphrom1, Prachak Bouphan2, Krissana Aunthakot3, Surachai Phimha4

1Master of Public Health Student, 2Associate Professor in Department of Public Health Administration, Health Promotion, and Nutrition, Faculty of Public Health, KhonKaen University, Thailand, 3Kuchinarai District Public Health Office, Kalasin Province, Thailand, 4Lecturer of Department of Public Health Administration, Health Promotion, and Nutrition, Faculty of Public Health, KhonKaen University, Thailand

Abstract

This is a cross-sectional study aimed to identify administrative factors and key success factors affecting the surveillance, prevention, and control of COVID-19 among the health personnel at sub-district health promoting hospitals in KhonKaen Province. The sample size was 138 health workers working in Sub-district health promoting hospital KhonKaen Province, Thailand. Respondents were selected by applying systematic sampling method. A total of 905 people were selected from the study population for the quantitative study and 12 key informants were selected for the qualitative study. The qualitative study was carried out by using in-depth interview guidelines. The questionnaire was evaluated by 3 experts yielding an IOC value of more than 0.50 in all questions. The Cronbach’s alpha coefficient of the questionnaire was 0.92. Data was collected between 21st December 2020 to 9th January 2021. Data analysis was performed by descriptive statistics and inferential statistics as a significant level at 0.05.

The results of the study showed that key success in all levels of agencies involved were with a work plan to support the performance, the performance link between the hospital and link to a major hospital, and the administrative factors of time, money, and management affecting and could jointly predict the surveillance, prevention and control of COVID-19 (R² = 0.727, p-value < 0.001). In conclusion, five factors are identified to affect surveillance, control and prevention of COVID-19 among the public health officials. Therefore, there should be a plan to support the performance in assigning responsibilities, promoting participation in policy making, planning of time, budget management and regular and proper co-ordination for internal and external organizations.

Keywords: administrative factors, key success factors, control coronavirus disease 2019 (COVID-19)

Introduction

The outbreak of Coronavirus Disease 2019 (COVID-19) since December 2019, and consequent widespread, on March 11, 2020, the World Health Organization (WHO) declared the novel coronavirus outbreak as a pandemic after the virus spread to 213 countries and territories. The report of COVID-19 as of June 30, 2020, it is found more than 10,629,405 were confirmed cases, more than 514,901 deaths, 5,833,268 were cured, 4,821,236 were undergoing treatment, it was spread in Thailand since the beginning of January 2020[1].

Corresponding author:
Surachai Phimha
Address: Lecturer, Department of Public Health Administration, Health Promotion, and Nutrition, Faculty of Public Health, KhonKaen University, Thailand. E-mail: suraphi@kku.ac.th
In Health District 7, on September 17, 2020, 13 confirmed cases of COVID-19 were detected, 13 cumulative cases of infectious disease, one death and treatment, and 12 cases were missing[2].

As per the report of 1 May 2020, 6 confirmed cases of coronavirus 2019(COVID-19) and 613 surveillance physicians (PUI) were eligible for investigation in KhonKaenProvince[3]. The implementation of surveillance, control, and prevention of infectious diseases among public health workers in sub-district health promoting hospitalsto achieve this objective requires several factors. For the smooth work plan and its proper execution in terms of applying knowledge to control the disease in effective way, the administrative factors should play very immense role[4]. Ability to supervise and monitor the project are additional factors of health workers in the operational management[5].

From the problems, the researcher gets interested in studying administrative and key success factors that effect on surveillance and prevention of COVID-19 of public health officers in sub-district health promoting hospitals KhonKaenProvince. for the reliability of the study results we had applied a guideline about planning, developing and supporting, along with promoting surveillance, control, and prevention of COVID-19 infectious disease among public health workers in sub-district health promoting hospitals to be more effective sustainably.

Research Objectives

Objective of this study was to identify the administrative and key success factors that affect surveillance and prevention of COVID-19 of public health officers in sub-district health promoting hospital, KhonKaenProvince, Thailand.

Research Methodology

This is a cross-sectional descriptive study to collect quantitative and qualitative data. Population and sample size

Sample group of this study is public health professionals, who are working in sub-district health promoting hospital KhonKaenProvince, Thailand. There is a procedure for calculating the sample size. When the population is known and for the multiple regression analysis, sample size is calculated by using the formula for calculating the sample size of Cohen (1988)[6].

\[ N = \frac{\lambda(1-R^2_{AB})}{R^2_{AB}} + w \]  
\[ \lambda = \frac{\lambda_L - \lambda_U}{1/\lambda_L - 1/\lambda_U} \] 

According to the sample size calculation, it is found that “n” is equal to 138 respondents, who will be responding the questionnaire.

In-depth interview random sampling

A group of people who provided information on in-depth interview to verify quantitative data that obtained from public health workers who can provide information on the fast-moving surveillance practice of the sub-district health promoting hospital, KhonKaen Province (Key Informants 12 persons).

The research tools

Tools used for the data collection in this study is a questionnaire to confirm the quantitative data. It consists of two types of tools:

Set one is a questionnaire that consists of five parts: Part one is a question of personal characteristics which consists of gender, age, marital status, education level, position, income, length of time in disease control work including training on disease control. Part two is of 30 administrative factors which consist of budgets, personnel, materials, equipment, time management, and technology. Part three is 30 key success factors that consist of executives at all levels who support and follow up, support agencies at all levels have a support program, taking action in an area where ready and voluntary participation, making understanding and encouraging cooperation, developing the image of the service facility and connect with sub-district health
promotion hospital and community hospitals. Part four is 30 questions about prevention, control, along with prevention of COVID-19 which consists of screening and monitoring for patients at checkpoints, nursing homes, and communities, infection prevention, contact tracing and containment, and risk communication. Implementing social and legal measures. Coordination and management of information. Use rating scale five levels in part two, three and four[7].

The scoring criteria for comments are as follows: The highest level is five points. The high level is four points. The intermediate level is three points. Low level is two points. The lowest level is one point. Part 5 is three open-ended questions about problems, obstacles and suggestions about surveillance and prevention of COVID-19 of public health officers in sub-district health promoting hospital, KhonKaen Province.

Set two is an In-Depth Interview guideline that the information was found from the quantitative research data. The least average questions are used for in-depth interviews to confirm the data, support and explain the quantitative data. The researcher defined the issue in three parts: Part one is an in-depth interview guide about administrative factors. Part two is an in-depth interview guide about key success factors. Part three is an in-depth interview guide about surveillance, control and prevention of COVID-19.

Quality testing of the tools

Quality testing of the tools about content validity. The researcher led questionnaires and in-depth interviews offered three experts to check the correctness and validity. Then, questions were processed to find the Item Objective Congruence(IOC)[8].It was found that all items had IOC greater than 0.50. Checking the reliability of the tools [9] after improving them, try out was done with public health officials in sub-district health promoting hospital, Roi Et Province, Thailand that has similar area in operational service, and management characteristics. In this study, 30 people were given the reliability from Cronbach’s alpha coefficient of the whole questionnaire equal to 0.92, Administrative factors are 0.76, key success factors are 0.92, and surveillance, control, and prevention of COVID-19 infectious disease (COVID-19) are 0.94.

Data Analysis

Descriptive statistics used to analyze the data are percentage, mean, median, standard deviation. Inferential statistics used to analyze the data are Correlation Coefficient of Pearson, regression analysis and stepwise multiple regression analysis. In-depth interviews to verify quantitative data by Content analysis.

Research results

1. Personal characteristics

Personal characteristics of public health workers performing the work of surveillance, control and prevention of COVID-19 in the sub-district health promoting hospital, KhonKaen Province found that the majority of the sample was female, totaling 86(62.3%), most of them aged between 31-40 years, totaling 51(37.0%), average age is 39.72 years (S.D.=10.08 years, Min = 23 years old, Max = 59 years old). Mostly, the marital status amounted to 90 people (65.2%). Most of them are graduated with a bachelor’s degree or equivalent, 106 people (76.8%). There are 51 public health technical officer (37.0%) with an average income of 29,154.31 Baht (S.D.= 12,972.48 Baht). They have a duration of working about the surveillance and preventive work was 10 years or more, 62 people (44.9%) with a median of 10 years (Min = 1 year, Max = 39 years). Most of them have received training in an epidemiological course, a total of 111 respondents (80.4%).

2. Administrative and key success factors affecting to surveillance and control and prevention of COVID-19

The analysis results showed that independent variable that affects the surveillance, control and prevention of COVID-19 among public health professionals working in sub-district health promoting hospital, KhonKaen Province as follow: Key success Factors: supporting organizations at all levels have a support plan (p-value = 0.013), and connecting between
sub-district health promoting hospital and community hospitals (p-value < 0.001). Management factors: Time (p-value = 0.003), Budget (p-value < 0.001), and Management (p-value = 0.043). Therefore, five independent variables can forecast about surveillance, control and prevention of COVID-19 of Public Health Workers in sub-district health promoting hospital as 72.7 percentage (Table 1). Multiple Linear Regression equation which is a prediction equation is as follow:

\[
Y = 1.230 + (0.223)(\text{Key success factors: Supporting organizations at all levels have a support plan})
\]

\[
+ (0.250)(\text{Key success factors: Connecting between sub-district health promoting hospital and with Community Hospitals})
\]

\[
+ (0.242)(\text{Management factors: Time})
\]

\[
- (0.139)(\text{Management factors: Budget})
\]

\[
+ (0.160)(\text{Management factors: Management})
\]

**Conclusion and Discussion**

The results of the study showed that key success factors about supporting organizations at all levels have a support plan, connecting working between sub-district health promoting hospital and community hospitals, Management factors in Time, Budget, and Management can forecast about surveillance, control and prevention of COVID-19 of Public Health Workers working in sub-district health promoting hospitals, KhonKaen Province as 72.7 percentage.

Key success factors about supporting organizations at all levels have a support plan, connecting working between sub-district health promoting hospital and community hospitals. They are an essential part of the overall service coverage and efficiency. Supporting organizations at all levels have a support plan and make a surveillance action plan about control and prevent disease both short-term, medium-term, and long-term. This study’s results are consistent with the results of the study found that supporting organizations at all levels have a support plan effect on surveillance, control and prevention of infectious diseases of public health officials \[10-11\]. In addition, key success factors about connecting working between sub-district health promoting hospital and community hospitals affect on surveillance, control and prevention of public health officials \[12-13\]. However, the situation in COVID-19 working is different from other infectious disease therefore, the result of this study inconsistent with the results of the study found that supporting organizations at all levels have a support plan no effect on surveillance, control and prevention of infectious diseases of public health officials \[14-15\].

Management factors: Time, budget and management will be accomplished must take the time appropriately, organize work planning each day, each week, order of work by importance. As well as support the creation of a more efficient management process. Management is an essential process for resource management. Management to achieve the organization’s objectives goals base on effectively: planning, budgeting and organizing. This study’s results are consistent with the results of the study found that management factors; time, budget, and management affect on surveillance, control and prevention of infectious diseases of public health officials \[11,16\]. It also found that time management factors, budget management factors, and management factors affect the working of public health officials \[17-20\]. The management factors were applied by different directors and area problem thus the result of this study is inconsistent with the results of the study found that management factors; time, and budget no affect on surveillance, control and prevention of infectious diseases of public health officials \[21-22\].

Results of in-depth interviews represent the surveillance, control and prevention of COVID-19 of public health workers in sub-district health promoting hospital is interviewee about suggestions founded that the lowest mean is entry and exit to follow about the risk groups in areas where some people don’t have smartphones to report working data. Equipment isn’t enough, such as protective clothing. Therefore, the
management organization should provide sufficient supplies to support the work. In case of necessity, a raincoat can be used instead of protective clothing to prevent direct contact with secretions.

In conclusion, key success factors about supporting organizations at all levels have a support plan, connecting working between sub-district health promoting hospital and community hospitals, Management factors in Time, Budget, and Management affect on surveillance, control and prevention of COVID-19 of public health officials. Therefore, there should be a plan to support the performance in assigning responsibilities, promoting participation in policy making, planning time, budget, management and connecting working for internal and external organizations.

Table 1. Statistics about stepwise multiple regression analysis of surveillance, control and prevention of infectious diseases COVID-19

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>Beta</th>
<th>T</th>
<th>P -value</th>
<th>R</th>
<th>R2</th>
<th>R2adj</th>
<th>R2 change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Key success factors: Supporting organizations at all levels have a support plan</td>
<td>0.223</td>
<td>0.244</td>
<td>2.508</td>
<td>0.013</td>
<td>0.769</td>
<td>0.592</td>
<td>0.589</td>
<td>0.592</td>
</tr>
<tr>
<td>2. Key success factors: Connecting between sub-district health promoting hospital and with Community Hospitals</td>
<td>0.250</td>
<td>0.359</td>
<td>5.113</td>
<td>&lt; 0.001</td>
<td>0.815</td>
<td>0.664</td>
<td>0.659</td>
<td>0.072</td>
</tr>
<tr>
<td>3. Management factors: Time</td>
<td>0.242</td>
<td>0.270</td>
<td>3.054</td>
<td>0.003</td>
<td>0.831</td>
<td>0.690</td>
<td>0.683</td>
<td>0.026</td>
</tr>
<tr>
<td>4. Management factors: Budget</td>
<td>-0.139</td>
<td>-0.207</td>
<td>-3.752</td>
<td>&lt; 0.001</td>
<td>0.847</td>
<td>0.718</td>
<td>0.710</td>
<td>0.028</td>
</tr>
<tr>
<td>5. Management factors: Management</td>
<td>0.160</td>
<td>0.182</td>
<td>2.040</td>
<td>0.043</td>
<td>0.852</td>
<td>0.727</td>
<td>0.716</td>
<td>0.009</td>
</tr>
<tr>
<td>Constant</td>
<td>1.230</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Suggested for next research

There should be a study of factors affecting the participation in the surveillance and control of the COVID-19 of public health workers in the district health promotion hospital.

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Ethical Clearance: The researcher collected research data after being certified by the Human Research Ethics Committee of KhonKaen University on December 14, 2020 number HE 632257. Data collection was done between December 21, 2020 to January 9, 2020.

Conflict of Interest: No conflicts of interest to declare.
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Total Quality Management (TQM) and Sports (Development, Concepts and Definitions)

Ghazwan Aziz Mohsen1, Bashar Banwan Hasan1, Haidar Mahmood Allawi1
1Ph.D. Faculty of Physical Education and Sports Science, Wasit University, Iraq

Abstract

Sports entrepreneurship among countries is to achieve a good sporting performance in sportive competitions. At the present time, sports are considered as one of the essential parts in our life, which are sought out by the governments, due to the main role that sports take at the national and international levels in the political, economic, cultural and social levels.

The sports level has been improving over the past several decades, this improving coinciding with the changes of the sport concept that is generally recognised as system of activities which are based in physical athleticism or physical dexterity. The concept of the athlete has changed into a wide concept that is become a unit of feeling, excited, affected and tired.

Sports has got the attention of persons who interested in scientific, medical, social, political and economic matters, which made sports have different specializations such as administration and management.

Key Wards: Total Quality, Concept of Administrative, (TQM) and Sports.

Introduction

The development of administrative thought study is important as it helps to explain the past, practice and the different thinking styles that prevailed in it. Hence, this is due to the positive in understanding the present, and the changes that are happening such as: problems and challenges.

So, the prediction, the requirements and challenges of the future may be effect on the development of administrative thought that leads to decrease the deviations as well as to reduce the problems that may be effect on the productivity of the organization.

The development of sports management thought is related to the development of administrative thought at the level of the state as a whole and in all the various parts; It could even be said that sport in its various fields reflects the extent to which administrative thought has evolved, but in the Arabic countries such as Iraq, these countries interested in sports. So, the management is considered as a secondary element. The development of administrative thought has shown in sports a little while ago, especially in Arab world, which may be due to those who chronicling the management thought. Recent legislations and laws also made sport in the Arab world an area full of innovators and volunteers in form, and the issue is obligatory.

Management is considered as one of the practices that is as old as human, because the management is a part of knowledge. So, it is appeared in this century. As a result of the efforts of some intellectuals and scholars such as Taylor, Henri Fayol, Charles and Charles Babbage who try to put the scientific bases of administrative management; whose studies has stated that there is a scientific management that is used to achieve the aims, which every organization aims to.

The administration is also part of the world civilizations that have flourished; it is not possible to talk about administrative thought without referring to the
administrative capacity of ancient civilizations, because modern administrative thought did not appear except by being affected by what preceded it.

Researchers in the heritage of Sumerian civilization found written documents, indicating that the Sumerians exercised a color of administrative control, as the priests of their temples had a tight tax system, which they collected and managed large quantities of worldly goods, the Sumerian priests followed the writing system by recording all the data on all the transactions of the various priests responsible for the vast wealth of the religious establishment. Thus, the creation of Sumerian writing was for administrative control, not for religious rituals, i.e. it is found for administrative needs.

So, Management was the practice of man in the past, as they used to do planning; i.e., setting plans and making special decisions in the event of war, except that it did not appear as a science until the end of the last century, when man was interested in it as a thought and as a social philosophy of life We cannot talk about management as a science without getting to know management in ancient civilizations.

Based on all of the above, in this study we are going to talk about overall quality, as well as the development of administrative thought in general, and then the development of this thought in the field of sports.

Section One (Total Quality)

1. The Concept of TQ

Total quality means “a method that aims to cooperate and participate from all employees of the sports authority, with the aim of improving services and activities, thereby achieving the satisfaction of the beneficiaries of the activities, and achieving the objectives of the authority itself” 1.

To achieve the overall quality of the sports body, a number of components must be met:

1. Correct performance: it is intended to implement the work procedures correctly, from the first practice, and in time to complete it.

2. The use of information systems to solve problems: it means implementing the work procedures correctly, from the first practice, and in time to complete it.

3. Focusing on both processes and results: i.e. attention to the way of work, the way it is implemented, and the scrutiny of its continued development, i.e. attention to the administrative processes in the authority besides its results.

4. Human resources development: it means working to improve the performance of the individuals working in the sports body, through attention to training, to keep pace with changes and development in the field of career specialization, and to improve the conditions surrounding the work 2.

2. The Concept of Administrative

The word administrative comes from Latin, which made up of two syllables (ad=to), (ministrate =save) that means to save or to present a service 3.

The word management comes from the English verb “manage”, which also comes from the Italian maneggiare (to handle, especially tools or a horse), which derives from the two Latin words manus (hand) and agere (to act) 4.

Management (or managing) is the administration of an organization, whether it is a business, a not-for-profit organization, or government body 5.

The concepts that have been outlined for us have been made possible by many researchers in this field, Henri Fayol (1841-1925) stated: “to manage is to forecast and to plan, to organise, to command, to co-ordinate and to control” 6.

In another way of thinking, Mary Parker Follett (1868–1933), allegedly defined management as “the art of getting things done through people”. She described management as philosophy 7.
Jamal al-Din Laweisat defines it as: “is the coordination of different resources through planning, guidance and control processes, in order to reach established objectives”.

According to Amar Buhash, administrative is “the executive body charged with enforcing state laws and providing the necessary services to citizens, within the framework of the established laws and the objectives set by the political, economic and social leadership”.

Hassan Shelton and Hassan Mouawad also defined it as “the process of carrying out the work by others by planning, organizing, directing and controlling their efforts”.

Al-Hawari defined it as: “That member responsible for achieving the results for which any institution or body existed”.

To achieve results, management must essentially commit to:
1. Choose the best elements to achieve the planned results.
2. Use those elements, the best possible use.
3. Continuity in balancing short-term and long-term requirements.

3. Administrative and Other Sciences:

Science is an integrated structure, consisting of overlapping parts that are affected by each other, and affect each other; for example, psychology uses medical sciences, and the close relationship that exists between the humanities and each other, is evidence of influence and influence; The science of administration, history, economics, and law, they have close ties, as well as sport is associated with a lot of medical, psychological and social sciences.

4. The Administrative Level

After the industrial revolution, enterprises expanded and enterprises developed significantly, which increased the number of administrators, and this was a reason for their ranking at top to bottom levels; these levels are:

1. Top-Level Management

Top-Level Management is at the top of the organizational pyramid, with the organization’s policy being developed at its level; It has a group of administrators who develop the organization’s policy and general plans, in order to achieve the goal to be reached.

Top-Level Management is the mind of the organization: The mental management skills are the basis before human and technical skills, which is the ones that formulate policies, rules and laws, with objectives, setting plans, supervising them and following up on their implementation.

2. Middle Level Management

Middle manager is a link between the senior management and the lower (junior) levels of the organization. Due to involvement into day-to-day running of a business, middle managers have the opportunity to report valuable information and suggestions from the inside of an organization. Moreover, the middle manager is a channel of communication within the organization, as they pass on major decisions of executives and the main goals of an organization to lower levels of employees. This contributes to better coordination between workers and makes a company more united.

3. Supervision Management

The supervisory Management represents the various members of the organization, who hold the executive functions, and who oversee the performance, i.e. deal with the executor.

Section Two (The Development of Total Quality Management (TQM) and Sports)

1. The History of Sportive Thought

The development of sportive management thought is no different from that of administrative thought in general, except in the field of sports here, as it is full of
various activities and different levels of administrative work. The development of sports management thought is closely linked to the development of administrative thought at the state level as a whole and in its various sectors, but in developing countries, interest in management in the field of sports is lacking.

The administration is a secondary element, and attention to it has only emerged a short time ago, and this is due to historians of sports administrative thought, although the lack of law and the multiplicity of legislation and the change of senior administrations have made sport in the Arab world an area full of innovators and enthusiasts of volunteerism, which has led to their lack of interest in developing sports administrative thought and recording its history. Therefore, the management scientists, with great regret, did not give great importance to management in the field of sports, in order to apply their principles and theories, so they left for personal experience and the desire to achieve special benefit and political affiliations.

The department in the field of sport has also existed for a long time, as this is evident in the holding of the Old and Modern Olympic Games, as it is clear that they were sporadic efforts by individuals and groups, coordinating the administration among themselves to achieve the goal of organizing and establishing the modern Olympic Games, which began in 1896 and have continued to date once every four years; the presence of organizers, promoters, administrators and trainers is evidence of the concept of sports management. The Department is a guaranteed activity with key components, carried out by individuals who are able to use the available resources to guide staff towards specific objectives. Therefore, more analysis and characterization of the components of management are needed. From that characterization, the definition of sports management would be acceptable. Accordingly, sports management is defined as: “a process of planning, leadership and control, the efforts of the members of the sports organization, and the use of all resources to achieve the specified objectives”. (Badawi (Op. Cit.), p. 17)

Sports management was also defined as “skills associated with planning, organization, guidance, follow-up, budgets, leadership and evaluation within a body that provides a sports service, physical or recreational activities. Since the lack of expertise of sports bodies, the organization of the management of a tournament, a competition, a stadium, or a team started the sports management program, Ohio University began master’s studies in sports management in 1966, it was the first study in America, and then many other universities began to organize studies in this field”.

But the current situation is quite different: work in sports management requires the study of theories, arts, work and management, sports planning, potential in the sports sectors, administrative communication and laws, regulations and administration in the sports field, administrative leadership in the field of sports, organizational behaviour and supervision, guidance, follow-up and sports marketing.

2. The Concept of Sportive Management

With the diversity of sources of management knowledge between experience and study of public administration science, it has become difficult to define a specific definition of sports management since scientists differed in the definition of management, because of their differing views of definition. However, we mention some of the definitions of sports management developed by sports management students.

In this sense, the Department is a guaranteed activity with key components, carried out by individuals who are able to use the available resources to guide staff towards specific objectives. Therefore, more analysis and characterization of the components of management are needed. From that characterization, the definition of sports management would be acceptable. Accordingly, sports management is defined as: “a process of planning, leadership and control, the efforts of the members of the sports organization, and the use of all resources to achieve the specified objectives”. (Badawi (Op. Cit.), p. 17)

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Accordingly, the administrator is the person who directs the efforts of others with him/her to achieve the agreed objectives, using administrative processes and management skills, with optimal recruitment of capabilities and capabilities: planning, implementing,
following up and evaluating all sports activities.

3. The Importance of Administrative in Sports

The sports field of work absorbs large numbers of graduates of sports education to work in the management of sports bodies and institutions.

Although the talent for leadership is one of the requirements of administrative work in the field of sports, as in the management of the best areas. However: refinement, qualification, training, and study are important as a place to refine this talent, which can be said to be: the study of sports management is necessary to achieve success in administrative work, whatever the field in which it occurred 19.

All physical and sports activities are a collective effort; management is essential for the development of community spirit, advance planning of any programme, participation, coordination and cooperation in action, which requires accurate knowledge of the potential of management. Therefore, sports management is one of the most important components of modern scientific sports development; it helps to increase sports achievement and its development in quantity and quality.


The characteristics of effective scientific sports management are as the following 20:

1. Inclusion: that means the need for management to cover all aspects and areas of work in the sports body, within the limits of its competences.

2. Integration: that means intended to take over each department or part of the sports body for specialized functions, taking into account that all departments or parts of the administrative process of the sports body as a whole are completed, taking into account coordination to achieve the desired results.

3. Futurism: that means the sports management must work not only for the present, but also for the future through goals, aspirations and aspirations in a time to come, and in the course of its work to do so, it must look to the past and learn lessons from it. Here, the importance of predicting the future is evident as a fundamental duty of sports management.

4. Open-mindedness: It means that the sports management of the authority is characterized by openness to the environment in which it operates, so that it is affected and affected.

Conclusion

The development of sports management thought is related to the development of administrative thought at the level of the state as a whole and in all the various parts; It could even be said that sport in its various fields reflects the extent to which administrative thought has evolved, but in the Arabic countries such as Iraq, these countries interested in sports.

So, the management is considered as a secondary element. The development of administrative thought has shown in sports a little while ago, especially in Arab world, which may be due to those who chronicling the management thought. Recent legislations and laws also made sport in the Arab world an area full of innovators and volunteers in form, and the issue is obligatory.

On the basis of all of the above, sports management uses the elements and theories of management, and benefits from the development of administrative thought, in order to achieve the best social, educational, cultural, economic, and educational aims of the society, as well as the sports field, which is a catalyst in the success of other organizations: economic, industrial, commercial, government, political and educational institutions.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Physical Education and Sports Sciences and all experiments were carried out in accordance with approved guidelines.
References


Association between Metacognition and Obesity in Male Individuals of South Indian Population

Gokul.K1, A.Sangeetha2, Kumaresan M3, Samuel Sundar Doss4, Hemachandrika C5

1II MBBS Student, Department of Physiology, Saveetha Medical College, Chennai, Tamilnadu, India, 2Assistant Professor, Department of Physiology, 3Assistant Professor, Department of Anatomy, 4Tutor, Department of Physiology, 5Professor, Department of Physiology, Saveetha medical College and hospital, Chennai, Tamilnadu India

Abstract

Introduction: Metamemory is the introspective knowledge of one’s brain memory capability and process involved in memory self-monitoring. This self-awareness of memory has important implications for how people learn and use memories. Aim: The aim is to assess the metamemory in male obese individuals. Materials and Method: A study was conducted among 165 male obese category at random south Indian population. The participants were asked to complete a self-reported questionnaire on Metamemory. The Multifactorial Memory Questionnaire (MMQ), developed to assess separate dimensions of memory ratings that are applicable to clinical assessment and intervention, includes scales of Contentment (i.e., affect regarding one’s memory), Ability (i.e., self-appraisal of one’s memory capabilities), and Strategy (i.e., reported frequency of memory strategy use). Result: Association between corresponding subscale scores and obesity indicates a weak correlation between BMI, satisfaction (r = -.11) and strategy (r = -.11). In addition, the MMQ subscales and the total score showed good internal consistency (αs = 0.81-0.84). Conclusion: The results would conclude that overweight and obese male individuals had poor metamemory scores. The study would also help the overweight and obese individuals to identify any early stage of cognitive impairment and create an awareness to delay or prevent any further metacognitive dysfunction.

Keywords: Metamemory, obese, dementia, physical activity, Cognitive performance, Cingulate gyrus

Introduction

Metamemory refers to knowledge and monitoring of one’s own memory. Metamemory monitoring can be done prospectively with respect to subsequent memory retrieval or retrospectively with respect to previous memory retrieval. [1]. This self-awareness of memory has important implications for how people learn and use memories. For example, when a person asserts that he or she is good at remembering faces, but poor at remembering names, that person is making a statement concerning metamemory knowledge. Metamemory awareness refers to our feelings or experiences of our own memory. For example, if a person feels certain that he or she will remember later something just learned now, that person is having a metamemory experience [2-5]. Metamemory is a subarea of Meta cognitions. Accordingly refers to people’s self-monitoring and self-control of their own memory process and strategies that can aid memory. Due to a sedentary lifestyle, more and more people are becoming obese nowadays. In addition to health-related problems, obesity can also impair cognition and motor performance [6].

Corresponding author :
Dr. A. Sangeetha
Assistant Professor, Physiology, Saveetha Medical College & Hospital,
E-mail: sangeethasrmc@yahoo.com,
Contact: +919444686966
Obesity is related to several diseases (e.g., diabetes, stroke, and high blood pressure; [7] and it leads to early death [8] and cognitive decline[9]. What are the cognitive causes to enforce, maintain and to eliminate obesity are at a center of an extensive research effort [10,11]. It is increasingly evident that obesity negatively impacts human health and the prevalence of obesity is increasing world-wide [10]. Both overall obesity (body mass index (BMI) >30 kg/m²) and fat distribution (waist-hip-ratio (WHR) >1.0 in men and >0.85 in women indicative of abdominal fat accumulation) have been linked to cardiometabolic diseases and death in observational studies [12-15]. Compared to BMI, central adiposity has a stronger association with the risk of developing cognitive impairment and dementia in women [16]. Therefore it is important to evaluate the state of metamemory in obese female individuals. The state of metamemory in overweight or obese person will surely result in better understanding of the cognitive condition by the physicians. Therefore, the purpose of this study is to investigate and understand the relation between the decrease or increase in metamemory in obese individuals.

**Materials and Method**

**Ethical Consideration:**

The study proposal was approved by the board of the Saveetha medical college and hospitals (IRB No. SMC/IEC/2020/03/029). The purpose and objective of the study was clearly explained to the participants through an information sheet. It was emphasized that their participation was optional and the confidentiality of data was assured. The participants were requested to sign a consent form attached with the questionnaire, to ensure their willingness to participate in the study.

**Study setting and design:**

This was a cross-sectional, descriptive correlational study. This standard questionnaire was done by 165 male obese individuals who volunteered to participate in this study. A convenience sample of participants from 19 to 55 years old female individuals was recruited from Medicine OP, Saveetha Hospitals. The questionnaire was administered through face-to-face contact by the investigator with potential participants. Potential participants who expressed interest in the study were screened for eligibility based on the inclusion/exclusion criteria. Inclusion criteria were as follows: ages from 19 to 55; BMI ranges between 30 to 40; ability to read, speak, and understand english. Participants with comorbidities like diabetes mellitus, hypertension and hyperlipidemia were excluded. Individuals with cognitive impairment (Alzheimer’s disease) were also excluded. After the inclusion/exclusion criteria were applied, 165 participants have received paper copies of the study’s survey instrument. The questionnaire included about age, height and weight, hence BMI calculated BMI = Weight/Height in meter². The participant’s BMI ranges are between 30 and 40. Individuals falling under obese I & II category were included.

**Procedure:**

**Perceived memory:**

The multifactorial memory questionnaire is a standard metamemory questionnaire (MMQ) which helps to assess a Metamemory of a person. It consists of three scales measuring separate aspects of metamemory. Items are rated on a 5-point Likert scale (0 = strongly agree, 1 = agree, 2 = undecided, 3 = disagree, 4 = strongly disagree) based on the test’s takers experiences. The three MMQ scales and their respective metamemory domains include: MMQ-Satisfaction (formerly called MMQ-Contentment). This scale measures satisfaction, concern, and overall appraisal of one’s own memory. Each of 18 statements is rated based on degree of agreement. The score range is 0 to 72, with higher scores indicating a higher degree of satisfaction. MMQ-Ability. This scale measures self- perception of everyday memory ability. Respondents rate how often they experienced each of 20 common memory mistakes over the previous two weeks. The score range is 0 to 80, with higher scores indicating better self-reported memory ability. MMQ-Strategy. This scale measures the use of practical memory strategies and aids in day-to-day life. Respondents rate how often they used each of 19 memory strategies.
over the previous two weeks. The score range is 0 to 76, with higher scores indicating greater use of memory strategies. Based on questionnaire data total score ranges are measured. Using a method formula; Prorated Score = Number of possible items X (Obtained score/ Number of completed items).

Data Analysis

Statistical analysis was done using SPSS Version 25.0. Descriptive variables were reported (Mean with standard deviation, Percentage) for all demographic variables. Pearson’s correlation analysis was used to assess correlations between BMI and the survey scores (Satisfaction, ability and strategy) and Cronbach’s alpha was calculated to measure internal consistency among the individual scores (17). The significance level was set at 0.05.

Results

Among the 165 participants, the mean and SD for age, height & weight were calculated (Table 1). Mean and SD for BMI and MMQ Subscale scores (Satisfaction, ability and strategy) are given in Table 2. Age and BMI were correlated with MMQ subcomponents (Table 3). The internal consistency of subscale scores are measured by cronbach’s alpha to check the reliability. There was a weak negative relationship between age and MMQ subscales (Satisfaction, ability and strategy). This relationship suggests that in obese male individuals, increasing age is associated with decreased satisfaction and strategy. Based on the MMQ subcomponent scores the study participants were found to have more worries about their memory (MMQ-contentment), reported significantly more instances of forgetfulness (MMQ-ability), and use less memory aid strategies in their day-to-day activities (MMQ-strategy) (Table 2 & 3). BMI had a weak negative correlation with MMQ-contentment ($r = -.11$) or MMQ-ability ($r = -.19$) or MMQ-strategy ($r = .11$). In our evaluation with a sample of 165 middle-aged and older obese male individuals analyses using Cronbach’s alpha indicated good internal consistency for the Satisfaction ($\alpha = .84$), Ability ($\alpha = .81$), and Strategy ($\alpha = .83$) scales (Table 3).

Table 1: Demographic characteristics of the participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>45.12</td>
<td>10.2</td>
<td>22 - 67</td>
</tr>
<tr>
<td>Height (cms)</td>
<td>155.88</td>
<td>4.1</td>
<td>142 - 165</td>
</tr>
<tr>
<td>Weight (kgs)</td>
<td>90.73</td>
<td>9.7</td>
<td>55 - 109</td>
</tr>
</tbody>
</table>

Table 2: Summary statistics for BMI and MMQ raw scores:

<table>
<thead>
<tr>
<th>Scale</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>32.23</td>
<td>4.1</td>
<td>0.39</td>
</tr>
<tr>
<td>Satisfaction</td>
<td>47.87</td>
<td>8.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Ability</td>
<td>51.41</td>
<td>10.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Strategy</td>
<td>52.44</td>
<td>10.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>
Table 3: Correlations between demographic characteristics and cognitive variables & internal consistency of MMQ subscales

<table>
<thead>
<tr>
<th>Scale</th>
<th>Age</th>
<th>BMI</th>
<th>Cronbach’s α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satisfaction</td>
<td>r = - .11</td>
<td>r = - .01</td>
<td>.84</td>
</tr>
<tr>
<td>Ability</td>
<td>r = - .19</td>
<td>r = - .03</td>
<td>.81</td>
</tr>
<tr>
<td>Strategy</td>
<td>r = - .11</td>
<td>r = - .07</td>
<td>.83</td>
</tr>
</tbody>
</table>

**Discussion**

In the present study, Obesity in male individual adults showed a negative correlation on all the metacognitive components. When the BMI values were correlated with metamemory components it showed a weak negative correlation. Simply, the study has indicated that male with overweight and obese reported more worries about their memory, more forgetfulness, and more use of strategies to ameliorate memory difficulties. Based on BMI data, individuals who are overweight or obese, fall in the lowest quartile of global cognition, verbal fluency, delayed recall, immediate logical memory, and intelligence (18). Other than BMI, other adiposity measures are also related to cognitive performance and brain changes. Visceral adiposity is inversely correlated with verbal memory and attention. High visceral adiposity is associated with smaller hippocampus and larger ventricular volume (19). There is also a negative correlation between waist-to-hip ratio and hippocampal volume and a positive correlation between waist-to-hip ratio and white matter hyperintensities (20).

Global loss and regional alterations in gray matter volume occur in obese male subjects, suggesting that male subjects with a high BMI are at bigger risk for future declines in cognitive skills or other brain functions (21). Statistical parametric mapping has revealed a significant negative correlation between BMI and metabolic activity in prefrontal cortex (Brodmann areas 8, 9, 10, 11, 44) and cingulate gyrus (Brodmann area 32) but not in other regions (22-24). These results further indicate the urgency of creating awareness on obesity in the society. A host of previous literature has suggested that exercise can improve both obesity-related cognitive and motor declines. As more and more people develop obesity in young age, introducing exercise intervention early would result in the greatest benefits towards good health (6).

**Conclusion**

Obesity has become a worrying health and social issue. The current study also has shown that obese and overweight individuals had poor metamemory scores. Obesity affects cognition mainly through altering the brain structures and functions and motor performance. The study would help the obese individuals to identify any early stage of cognitive impairment and create an awareness to delay or prevent any further metacognitive dysfunction. Regular physical activity and exercise benefits both cognition and motor behaviours.

**Limitations:**

The small sample size from a single area of the country also limits generalizability. The current study had taken individuals who were obese for past 2 years. BMI measurements were independent of the quantity of total body fat and a number of potential confounders, including age and household income. The study did not differentiate metamemory values between overweight and obesity. Physical activity was not measured. Future research is needed to investigate relationships between these metacognition variables, objective neuropsychological tests, and functional MRI imaging.
Conflict of Interest: No conflict of interest

Funding Source: Self-funding

References
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Profile of Rodenticide Poisoning at Vijayanagar Institute of Medical Sciences (VIMS), Ballari District, Karnataka, India: Retrospective Analysis of Cases from 2016 to 2020

Gururaj Biradar1, Charan Kishor Shetty2, Pavanchand Shetty H3, V Yogiraj4

1Assistant Professor, Department of Forensic Medicine, VIMS Bellary, India, 2Assistant Professor and Unit Head, Department of Forensic Medicine, University Sultan Zainal Abidin (UniSZA), Malaysia, 3Associate Professor, Department of Forensic Medicine Kasturba Medical College, Mangalore, MAHE Manipal, India, 4Professor and Head, Department of Forensic Medicine, VIMS Bellary, India

Abstract

Background: Poisoning is the major health problem in developing countries like India. Rodenticides are the substances used to kill rats. Rodenticides are one of the commonest substances used for poisoning in India one of the reasons is that it is easily available and cheaper than other pesticides. They differ from chemical composition and toxicity profile. Prognosis mainly depends on chemical content of poison.

Methods: A retrospective descriptive 6-year review of data from 597 rodenticide poisoning autopsies performed by the Department of Forensic Medicine at Vijayanagar Institute of Medical Sciences (VIMS), Ballari, Karnataka, India between January 1, 2015, and December 31, 2020, was carried out. Data were obtained from judicial requisitions, autopsy reports, toxicology, and histology results. Different variables like the gender, age-wise distribution of cases, marital status, profession, antemortem laboratory investigations, and manner of death were analysed. The data were analysed using Statistical Package for Social Sciences (SPSS) version 27.0.

Results: Out of 597 autopsy cases, majority of the cases 492 (82%) were male, 105 (18%) were female. Most common age group was 51 to 60 years (45.23%) cases, followed by 41 to 50 years (26.19%) cases. Most victims were married 469 (79%) and by profession were farmers 292 (48.91%) cases. Majority of the cases were mostly reported in the night 454 (76.04%). Antemortem laboratory investigations data like prothrombin time (PT), activated partial thromboplastin time (APTT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin were elevated in majority of the cases. Manner of death was suicidal 497 (83%) cases.

Conclusions: Rodenticide poisoning is quite common and treatable, most of the time it is suicidal in nature. Public should be educated regarding consequences of poisoning. Family support and proper counselling for high-risk groups may decrease these incidences.

Key words: Rodenticides, Agriculturist, Suicide, Epidemiological Profile, Coagulation markers.

Introduction

Rodenticides, commonly referred to as “rat poisons,” are chemical agents aimed at eliminating small rodents [1-5]. The common targets for their use are household rodents i.e., rats/mice, squirrels, gophers, etc. Controlling rodents is imperative as they are vectors for spread of disease, destroy crops/grains, and multiply...
India being a primarily agrarian country, rodenticides are widely used and freely available. They are available in various formulations as powders, pastes, pellets, cereal baits, or blocks[7].

Rodenticides are classified based on their toxicity as:[1]

- **Highly toxic**: Median lethal dose (LD 50) ranging from 0 to 50 mg/kg body weight.e.g., Strychnine, Thallium, Elemental phosphorous, Metal phosphides, Sodium monofluoroacetate, Arsenic, Alpha-naphthyl thiourea.

- **Moderately toxic**: LD 50 ranging from 50 to 500 mg/kg.e.g., Cholecalciferol, Warfarin.

- **Less toxic**: LD 50 ≥ 500 mg/kg.e.g., Superwarfarins— brodifacoum, bromadiolone, chlorophacinone, difenacoum, and diphacinone, Bromethalin, Red squill.

Poisoning due to rodenticides is relatively rare compared to organophosphorus compounds but rodenticide poisoning is an important health problem with a high case fatality rate especially with metal phosphides[8]. In India it is a major health problem due to its easy availability, over the counter or on e-commerce websites, and a lack of antidotes for rodenticides[1-8]. Improving public awareness regarding their lethality and strict monitoring of sales and usage of rodenticides could help to avoid indiscriminate use and poisoning[9-11]. The knowledge on the epidemiology and clinical manifestations of rodenticide poisoning is essential not only to the clinical practitioners but also to the forensic pathologist because of their predominant haemorrhagic manifestations which can sometimes mimic injuries caused by blunt force trauma[11-16]. The present study was undertaken with objectives of studying the epidemiological Profile of rodenticide poisoning which could help the health and various other agencies take some precautionary measures against rodenticide poison sale and consumption.

**Methods**

A retrospective descriptive 6-year review of data from 597 rodenticide poisoning autopsies performed by the Department of Forensic Medicine at Vijayanagar Institute of Medical Sciences (VIMS), Ballari, Karnataka, India between January 1, 2015, and December 31, 2020, was carried out. Data were obtained from judicial requisitions, autopsy reports, toxicology, and histology results. Judicial requisitions contained the identity of the victims and a brief description of the circumstances of death. All autopsy cases were subjected to comprehensive toxicology testing for pesticides, drugs, and alcohol. Specimens including blood, urine, and stomach contents were collected from each victim at autopsy. Rodenticide poisoning was confirmed in these cases by analysing the substance using thin-layer chromatography and gas chromatography–mass spectrometry at the Regional forensic science laboratory (RFSL). Antemortem laboratory investigations data like prothrombin time (PT), activated partial thromboplastin time (APTT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin was collected from the hospital records. Different variables like the gender, age-wise distribution of cases, marital status, profession, antemortem laboratory investigations and manner of death were analysed. The data were analysed using Statistical Package for Social Sciences (SPSS) version 27.0. This study was approved by the ethics committee of Vijayanagar Institute of Medical Sciences (VIMS), Ballari, Karnataka, India.

**Results**

A total of 597 medico-legal autopsies of rodenticide poisoning cases were conducted between 1st January 2015 and 31st December 2020. Majority of the victims 492 (82 %) were males (Fig. 1). The age of the victims ranged from 11 to 82 years, with a peak incidence in the fifth decade of life 152 (25.46%) while not a single case occurred in the first decade (Fig. 2). Majority of the victims were married 469 (79%) cases (Fig. 3). When we investigate the occupation of the victims 292 (48.91%) cases were agriculturist closely followed by unemployed 163 (27.30%) cases (Fig. 4). Most of the incident of rodenticide poisoning happening at night 454 (76.04%) cases. (Fig. 5). When laboratory investigations were...
analysed, prothrombin time (PT), activated partial thromboplastin time (APTT), were elevated in 498 cases whereas aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin were raised in 350 cases (Fig.6). Manner of death in 497 (83%) cases were suicidal closely followed by 100 (17%) cases of accidental poisoning there was case of homicidal poisoning.

**Figure 1: Sex wise Distribution of Rodenticide poisoning victims (n=597)**

![Sex wise Distribution of Rodenticide poisoning victims](image1)

- Male: 492 (82%)
- Female: 105

**Figure 2: Age wise distribution of Rodenticide poisoning victims (n=597)**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-20 yrs</td>
<td>20</td>
</tr>
<tr>
<td>21-30 yrs</td>
<td>123</td>
</tr>
<tr>
<td>31-40 yrs</td>
<td>122</td>
</tr>
<tr>
<td>41-50 yrs</td>
<td>140</td>
</tr>
<tr>
<td>51-60 yrs</td>
<td>152</td>
</tr>
<tr>
<td>61-70 yrs</td>
<td>37</td>
</tr>
<tr>
<td>&gt;70 yrs</td>
<td>3</td>
</tr>
</tbody>
</table>
Figure 3: Marital Status of Rodenticide poisoning victims (n=597)

- Married: 469 (79%)
- Unmarried: 128 (21%)

Figure 4: Occupation wise distribution of victims of rodenticide poisoning (n=597)

- Employed: 68
- Unemployed: 163
- Student: 35
- Agriculturist: 292
- Housewife: 39
Figure 5: Distribution of cases of Rodenticide poisoning according to time of incident (n=597)

Figure 6: Ante mortem laboratory investigations in victims of rodenticide poisoning (n=597)
Discussion

In the present study males 492 (82%) outnumbered females. Our study correlates with other study in which incidence was high among males [16]. But our study does not correlate with a study done by Suneetha, et al 2016 [17] where they reported female preponderance. Most of the cases 152 (25.46%) were in the age group of 51-60 years however only 123 (23.95%) cases in this study were 11 to 30 years old, contradicting other studies where majority of the cases where below 25 years old [7], those studies attributed those findings to increased peer pressure, competitiveness, stress, and heavy expectations from family and loved ones. According to the American Association of Poison Control Centre’s (AAPCC) 2009 annual report, about 70% of the rodenticide exposures occurred in children younger than 6 years [3], whereas no children were exposed to rodenticides in the present study. Most of the rodenticide poisoning cases 469 (79%) were married which was comparable to other studies [7]. This finding can be due to abuse, marriage related issues, dowry related issues, extramarital affairs, divorce, impotence or infertility illness, unemployment, or other family problems. Rodenticide poisoning was commonly seen among farmers 292 (48.91%) cases, closely followed by unemployed 163 (27.30%) as these groups are more vulnerable groups and easily exposed to the poisoning agents. Similar findings were reported by Suneetha et al and Morrissey et al [17,18]. Most cases of rodenticide poisoning were reported at night 454 (76.04%). But according to other studies the popular time for ingestion of poison was the daytime (6 am to 6 pm) [19-21]. In our study altered laboratory findings such as an increase in APTT and PT can be attributed to inhibition of vitamin K (1)-2,3 epoxide reductase and thus the synthesis of vitamin K and subsequently clotting factors II, VII, IX and X. These findings correlate with many other studies [10-15]. Similarly, increased AST and ALT levels are due to hepatic accumulation of these compounds. Furthermore, the intent for poisoning in the greatest number of the patients in this study was suicidal 497 (83%) cases, followed by accidental poisoning 100 (17%) cases, which was comparable to other studies [22,23,24,25]. Three was no homicidal poisoning reported in this study. However, a study done in France showed 70% cases were accidental in nature [26].

Conclusion

Epidemiological profile of rodenticide poisoning cases was discussed in our study. As majority of the cases of rodenticide poisoning were suicidal community awareness programs, and campaigns, poison control centres and suicide help lines, targeted towards the youth, especially women, can help significantly reduce the incidence of poisoning. Moreover, rodenticides should be clearly labelled, locked, and safely stored at homes to prevent accidental poisoning among children and dissuade suicidal poisoning among adults. Finally, legislation and guidelines restricting sales of pesticides to those without farmlands should be strictly implemented to prevent non-farmers from having access to these chemicals.

Source of Funding - Self

Conflict of Interest – Nil

References

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Socialization Program for Prevention and Early Detection of Congenital Hearing Loss in the Families of Deaf Schoolchildren

Gwenny Ichsan Prabowo¹, Citrawati Dyah Kencono Wungu¹, Retno Handajani², Nyilo Purnami³, Fis Citra Ariyanto⁴,⁵

¹Associate Professor, ²Professor, Department of Physiology and Medical Biochemistry, ³Associate Professor, Department of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, ⁴Staff, Hearing Vision Ltd – Darmo General Hospital, Surabaya, Indonesia, ⁵Postgraduate, Faculty of Nursing, Universitas Jember, Jember, Indonesia

Abstract

Objective: socializing hearing loss examination and early detection to patients and their families in deaf type B schools. Methods: A community service program in the form of socialization was performed to the family of patients with hearing loss in deaf school type B, Surabaya, Indonesia. Pretest and posttest were conducted to determine the initial understanding and post socialization knowledge of these people. We also asked the participants to fill on a questionnaire regarding the possible causes of the hearing loss (family history, drug use, history of disease, and history of head trauma). Results: Based on the summary of pretest and posttest from the participants, an increase in participants’ knowledge of hearing lost was found. This activity was attended by 90 family members of 37 patients with hearing loss. Pre and post test results. The results of the questionnaire showed that 94.59% of the patients came from Javanese ethnicity. As many as 21.62% of patients had a family history of hearing loss, and even 2 patients had a father, mother, and sibling with hearing loss. A total of 18.92% had a history of using ototoxic drugs, 16.22% had a history of maternal Rubella infection during pregnancy, and 2.7% had a history of head trauma. Conclusion: Socialization program was effective to increase knowledge of congenital hearing loss for family of deaf schoolchildren. The result of the questionnaire showed that deaf schoolchildren had several risk factors for the occurrence of hearing loss. Similar program can be performed in communities in other areas to increase prevention and early detection of hearing loss in Indonesia.

Keywords: Socialization, hearing loss, deaf schoolchildren

Introduction

Hearing loss is a commonly found sensory disorder, around 1/1000 births. Hearing loss can be caused by genetic factors, environmental factors and the interaction of these two factors. Genetic factors play a role in about 50% to 75% of the cause of hearing loss(⁵, ²). Hearing loss can occur by sensorineural disturbances (damage to the inner ear, for example: damage to cranial nerves VIII, brain stem or cortex cerebri) or by disturbances in the conductive system (for example: outer ear anomalies or middle ear)(⁶). Hearing loss greatly affects the development of language skills as it can interfere with development in children’s social life and education. Hearing loss related to genetic factors (congenital hearing loss) can be found in two forms: syndromic disorders and non-syndromic disorders. About 70% of congenital hearing loss is in the form of non-syndromic disorder⁷,⁸.

Corresponding author:
Gwenny Ichsan Prabowo
Department of Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Jalan Mayjend Prof. Dr. Moestopo No. 6-8, Airlangga, Gubeng, Surabaya, East Java 60286, Indonesia
Mail: gwenny-i-p@fk.unair.ac.id
Factors that contribute to hearing loss can come from: genetic, environmental or the interaction between the two factors. There are two forms of hearing loss that are influenced by genetic factors: syndromic and non-syndromic hearing loss. According to Guy Van Camp et al., 50% of hearing loss is hereditary and about 70% of congenital hearing loss is in the form of non-syndromic hearing loss (most often due to sensorineural disorders). Congenital hearing loss is very complex, as it is influenced by various genes and can be either autosomal recessive, autosomal dominant, X-linked or mutations in mitochondrial genes. Eighty percent of congenital hearing loss is autosomal recessive and is often seen prelingual\(^5,\)\(^6\) Environmental factors that contribute to hearing loss are: prenatal and postnatal (eg asphyxia, prematurity), exposure to infection (eg rubella virus, cytomegalovirus) and the use of cytotoxic drugs\(^6\).

An understanding of the mechanisms associated with the onset of congenital hearing loss plays an important role in early detection and its therapeutic aspects. Genetic analysis of congenital hearing loss is an important means of tracking hereditary diseases in the patients as early as possible so that they can be treated immediately, besides tracking the presence of hearing loss in family members of sufferers and can be used as a premarital screening\(^7\). Considering the lack of knowledge of the public about risk factors of hearing loss and the low awareness of the community to get early detection, we conducted socialization on the family of deaf schoolchildren for early awareness and detection of hearing loss to increase knowledge and reduce the incidence of congenital hearing loss in Indonesia, especially in people with family history of hearing loss.

**Methods**

This socialization program targeted 90 family members of deaf schoolchildren in deafschool type B, Surabaya. The program consisted of socialization of congenital hearing loss, risk factors and early detection of hearing loss in children, screening of hearing loss in infants, and managements of children with hearing loss. Socialization was conducted by expert otolaryngologists. Pretest and posttest before and after socialization were performed to determine the increase of knowledge of the participants. Both pretest and posttest had similar questions, thus a paired t-test was performed and the results were analyzed by SPSS version 23. Representatives of each deafchild family member were asked to fill on questionnaire consisted of the child’s information: age, sex, family history, race, use of ototoxic drugs, history of disease, and history of head trauma. The questionnaire used to assess the participant’s knowledge was first performed validity and reliability beforehand. The validity value of the questionnaire was 0.76-0.92 and declared reliable with a value of \(\alpha = 0.83\).

**Results**

At this program, from the results of the pretest assessment, the total average score was 62 of 100 (61.89 ± SD 14.37), with results: 50% had poor knowledge (<50 score), 32% had moderate knowledge (50-70 score) and 18% had good knowledge (>70 score). In the results of the posttest assessment, the total average score was: 87 (87.11 ± SD 7.68), with results: 90% had good knowledge and only 10% had moderate knowledge, and no participant had poor knowledge (Table 1). From the results of the evaluation of the pre-test and post-test results, it can be said that this program at deafschool type B could significantly increase participants’ knowledge about the prevention and early detection of congenital hearing loss (\(p < 0.001\)). During the discussion, participants actively asked questions and gave responses to the problems raised.

In this program, representatives of family members of 37 deaf schoolchildren with hearing loss were asked to fill out a questionnaire about the child with hearing loss. The results of the questionnaire is displayed on Table 3. The result had almost the same distribution between children aged <10 years and ≥10 years and between female and male. The patient tribe is dominated by Javanese (94.59%). A total of 21.62% of patients had a family history of hearing loss, with 50% of them having sibling with hearing loss, and 25% of them had a
family history of complex hearing loss (father, mother, and sibling). A total of 18.92% of patients had a history of use of ototoxic drugs, with 85.71% being the drug ibuprofen. As many as 16.22% of patients had a history of maternal infection during pregnancy in the form of Rubella infection. Only 2.7% had a history of head trauma.

Table 1. Pretest and posttest results of the participants

<table>
<thead>
<tr>
<th>Questions</th>
<th>Pretest</th>
<th>Posttest</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the definition of hearing loss?</td>
<td>86 (96)</td>
<td>90 (100)</td>
</tr>
<tr>
<td>Since when human beings can hear?</td>
<td>51 (57)</td>
<td>73 (81)</td>
</tr>
<tr>
<td>What are the causes of hearing loss?</td>
<td>78 (87)</td>
<td>90 (100)</td>
</tr>
<tr>
<td>Do people with hearing loss always have speech disorder?</td>
<td>30 (33)</td>
<td>74 (82)</td>
</tr>
<tr>
<td>When should we suspect if our child has hearing loss?</td>
<td>29 (32)</td>
<td>60 (67)</td>
</tr>
<tr>
<td>How many parts do our ears have?</td>
<td>38 (42)</td>
<td>73 (81)</td>
</tr>
<tr>
<td>What is congenital hearing loss?</td>
<td>38 (42)</td>
<td>66 (73)</td>
</tr>
<tr>
<td>What should you do if your child has hearing loss?</td>
<td>71 (79)</td>
<td>85 (94)</td>
</tr>
<tr>
<td>When is the right time for screening of congenital hearing loss?</td>
<td>60 (67)</td>
<td>85 (94)</td>
</tr>
<tr>
<td>Is hearing loss a contagious disease?</td>
<td>80 (89)</td>
<td>88 (98)</td>
</tr>
</tbody>
</table>

Table 2. Pretest and posttest results of the participant’s pair t test

<table>
<thead>
<tr>
<th>Participant</th>
<th>t</th>
<th>CI 95%</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretest</td>
<td>40.86</td>
<td>58.88 – 64.90</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Posttest</td>
<td>107.61</td>
<td>85.50 – 88.72</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 3. Characteristics of deaf schoolchildren in this study

<table>
<thead>
<tr>
<th>Age</th>
<th>Percentage (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10 y.o</td>
<td>48.65</td>
</tr>
<tr>
<td>≥ 10 y.o</td>
<td>51.35</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>48.65</td>
</tr>
<tr>
<td>Male</td>
<td>51.35</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Javanese</td>
<td>94.59</td>
</tr>
<tr>
<td>Others</td>
<td>5.41</td>
</tr>
<tr>
<td>Family history of hearing loss</td>
<td>21.62</td>
</tr>
<tr>
<td>Ototoxic drugs usage</td>
<td>18.92</td>
</tr>
<tr>
<td>History of maternal infection during pregnancy</td>
<td>16.22</td>
</tr>
<tr>
<td>History of head trauma</td>
<td>2.7</td>
</tr>
</tbody>
</table>
Discussion

There are 360 million persons in the world with disabling hearing loss, in which 32 (9%) millions of these are children. The prevalence of hearing loss in children is greatest in South Asia, Asia Pacific and Sub-Saharan Africa\(^{(8)}\). The prevalence is also high in developing and underdeveloped countries. Hearing loss can occur at various ages. It becomes a public health problem because the population in both developed and developing countries is around 6-8%\(^{(1)}\). The results of the 2013 Basic Health Research showed that the prevalence of hearing loss in children aged 5-14 years was 0.8%. From these data, it is known that the prevalence of respondents with hearing loss in women tends to be slightly higher than that of men (2.8-2.4%), however the research does not differentiate by age. This result is somewhat different from our data which shows that girls with hearing loss have a higher prevalence than boys\(^{(9)}\).

In this study, 21.62% of schoolchildren deaf students had a family history of hearing loss, there were even 2 children with multiple family history of hearing loss, in which all of the main family members were affected. Hearing loss can be caused by genetic factors, environmental factors and the interaction of these two factors. Genetic factors play a role in about 50-75% of the cause of hearing loss\(^{(1, 10)}\). Hearing loss related to genetic factors (congenital hearing loss) can be found in two forms: syndromic disorders and non-syndromic disorders. About 70% of congenital hearing loss is in the form of nonsyndromic disorder\(^{(11)}\). Previous research conducted by Purnami et al showed the prevalence of mutations in the gap junction protein beta-2 (GJB2) gene was 13.64% in schoolchildren deaf schoolchildren in Surabaya, Indonesia\(^{(12)}\).

In this study, 18.92% of children had a history of using ototoxic drugs, namely Ibuprofen and Streptomycin. A systematic review conducted by Kyle et al showed a significant increase in the risk of hearing loss in patients taking Ibuprofen (RR = 1.13; CI = 1.06-1.19), even though Ibuprofen is an over-the-counter medicine that is often given to children\(^{(13)}\). Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) for the treatment of inflammation, mild-to-moderate pain and fever in children, and is the only NSAID approved for use in children aged ≥3 months\(^{(14)}\). The use of streptomycin is also often associated with hearing loss. Streptomycin is an aminoglycosides class of antibiotics which can cause cochlear or vestibular damage\(^{(15)}\).

In this study, 16.22% of children had a history of pregnant women with Rubella. Rubella is a viral infection that can cause Congenital Rubella syndrome (CRS). CRS is a syndrome consisting of several congenital disorders such as deafness, cataracts and heart defects. Deafness is the most common manifestation in CRS cases and 70-90% suffer from sensorineural type deafness. The mechanism of hearing loss due to the rubella virus can be caused by hypoxia, which occurs when the endothelial damage in the cochlea is followed by cell death in the organs of the Corti and the stria vascularis\(^{(16)}\). Apart from Rubella, other infections in pregnant women such as mumps, measles, meningitis, and cytomegalovirus can also cause congenital deafness\(^{(8)}\).

The World Health Organization (WHO) estimates that around 60% of childhood hearing loss could be avoided through prevention measures\(^{(8)}\). This program has benefits for the community to reduce the incidence of congenital hearing loss in Indonesia, especially for families with risk factors. Screening and detection of risk factors in children with congenital hearing loss also helps better management of congenital hearing loss patients in the community.

Conclusion

Socialization program could increase public awareness about hearing loss, especially for family who had children with hearing loss. Most deaf schoolchildren involved in this program had risk factors for hearing loss that could be prevented for the other family members. In general, there was an increase in the knowledge of the participants about hearing loss. Additional support from the community is needed and similar programs need to be carried out in other places, especially in families who had members with hearing loss to increase public
Awareness and reduce the incidence of hearing loss, especially congenital hearing loss in Indonesia.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** This work was supported by Universitas Airlangga, Surabaya, Indonesia.

**Ethical approval:** All procedures performed in studies involving human participants were in accordance with the declaration of Helsinki the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (510/Panke.KKE/2017).

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**References**


Measurement of Some non-routine Markers in Patients with Chronic Kidney Disease

Haider Ali Laibi¹, Abdulkareem M. Jawad²

¹Research Scholar, ²Assistant Professor, Medical and Health Technical College, Southern Technical University, Iraq

Abstract

Chronic kidney disease has an indirect effect on global disorder and death by raising the risks of morbidity and mortality. This study included (60) patient diagnosed chronic renal failure; it also included (25) healthy subjects as control. In study population age ranged from (14-65) years old who visited Al-Basrah Teaching Hospital in Basrah throughout the period from October 2020 to February 2021. Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure human serum soluble alpha klotho proteins, irisin and cystatin C. The results showed that there is a high significant difference (P value < 0.01) in the levels between CKD patients and control for the following: Soluble-a-klotho protein; Irisin (ng/ml); Cystatin-C (ng/ml). There was also a high significant decrease in the levels of irisin (ng/ml) among different stages of chronic kidney. The same table revealed a high significant increase (P value <0.01) in the levels of Cystatin-C (ng/ml) and Soluble-a-klotho protein (ng/ml) among different stages of chronic kidney and respectively. The study concluded that there is a remarkable decrease in the level of serum soluble alpha klotho proteins and irisin and increase in serum cystatin C in patients with chronic kidney disease; and these differences are positively correlated with the glomerular filtration rate.

Keywords: CKD, soluble alpha klotho proteins, irisin, cystatin C

Introduction

Chronic kidney disease has an indirect effect on global disorder and death by raising the risks related with at least five other main killers: cardiovascular diseases (CVD), diabetes mellitus (DM), hypertension, human immunodeficiency virus (HIV) and malaria. For instance, the Global Burden of Disease (GBD) has been estimated to cause 1.2 million deaths a year, of them 19 million disability-adjusted life-years (DALYs) and nearly the same million years of life died from cardiovascular diseases were straightly resulted from reduced glomerular filtration rates. It has also been estimated that, in 2015, 1.2 million people died from kidney failure, an elevate of 32% since 2005. In 2010, an estimated 2.3–7.1 million people with end-stage kidney disease died without access to chronic dialysis. Additionally, each year, around 1.7 million people are thought to die from acute kidney injury. Finally, therefore, an estimated 5–10 million people die yearly from kidney disease. According to some epidemiological data, the common shortage of knowledge and the frequently poor experience to laboratory facilities, such numbers may be underestimate the real deaths resulted from kidney disease.

Patients of chronic kidney disease (CKD) clients may complain from cardiovascular or cerebrovascular disorder, and their deaths may be imputed to either consequences. Changed kidney function is often found in clients with hypertensive and ischemic heart disease, both of which are correlated with elevated cardiovascular
disorder and death. About 30 percent of clients with diabetes have diabetic nephropathy, with elevated risk found in some ethnic groups. (3).

Traditionally, renal function has been evaluated having levels of plasma and/or serum creatinine or urea. Unluckily, both markers are not reliable and less sensitive in intensive care clients. Cystatin C reveals few non-GFR determinants when comparing to creatinine and is more sensitive and specific to find out early reduces of GFR. At recent, new functional assays are investigated, called the furosemide stress test (FST) and renal functional reserve (RFR). An elevate in urine output to >100 ml/h is indicator of a GFR >20 ml/min and mainly certainly excludes progression to AKI stage III and need for RRT (4).

Materials and Methods

This study included (60) patient diagnosed chronic renal failure; it also included (25) healthy subjects as control. In study population age ranged from (14-65 years) old. Who visited Al-Basrah Teaching Hospital in Basrah. All patients in this research were examined by specialist physicians in Al-Basrah Teaching Hospital throughout the period from October 2020 to February 2021. Several patients have been excluded from the study because of acute and chronic diseases. All patients affected by hormonal disturbances were excluded from the study. Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure human serum soluble alpha klotho proteins, irisin and cystatin C.

Results

Table (1) Differences in the measurement of non-routine markers between control group and CKD group

<table>
<thead>
<tr>
<th>Non-routine Markers</th>
<th>Control Group (N= 30)</th>
<th>CKD Patients (N= 60)</th>
<th>T Test P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Soluble-a-klotho protein (ng/ml)</td>
<td>5.2</td>
<td>1.35</td>
<td>2.18</td>
</tr>
<tr>
<td>Irisin (ng/ml)</td>
<td>19.94</td>
<td>10.35</td>
<td>5.64</td>
</tr>
<tr>
<td>Cystatin-C (ng/ml)</td>
<td>0.57</td>
<td>0.44</td>
<td>0.98</td>
</tr>
</tbody>
</table>

HS : High Significant at P≤0.01 ; SD : Standard Deviation
Table (2) ANOVA table for Differences in the measurement of non-routine markers among different stages of chronic kidney disease

<table>
<thead>
<tr>
<th>Non-routine Markers</th>
<th>Stage 2 (N= 5) Mean ± SD</th>
<th>Stage 3 (N= 25) Mean ± SD</th>
<th>Stage 4 (N= 3) Mean ± SD</th>
<th>Stage 5 (N= 27) Mean ± SD</th>
<th>F Test P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble-a-klotho protein (ng/ml)</td>
<td>3.12 ± 0.83 AB</td>
<td>2.73 ± 1.13 AB</td>
<td>2.25 ± 1.08 A</td>
<td>0.86 ± 1.35 B</td>
<td>3.53 (0.02 S)</td>
</tr>
<tr>
<td>Irisin (ng/ml)</td>
<td>8.95 ± 2.52 AB</td>
<td>6.44 ± 2.48 A</td>
<td>5.16 ± 3.35 A</td>
<td>3.25 ± 0.99 B</td>
<td>17.74 (0.000 HS)</td>
</tr>
<tr>
<td>Cystatin-C (ng/ml)</td>
<td>0.49 ± 0.15 AA</td>
<td>0.62 ± 0.3 A</td>
<td>1.35 ± 0.29 A</td>
<td>1.4 ± 0.17 B</td>
<td>35.2 (0.000 HS)</td>
</tr>
</tbody>
</table>

HS : High Significant at P≤0.01 ; S : Significant at P≤0.05 ; SD : Standard Deviation ; GFR : Glomerular Filtration Rate ; ANOVA : Analysis of Variance , Different letters refer to significant difference among stages

Table (1) is about differences in the measurement of non-routine markers between control group and CRF group. This table shows that there is a high significant difference (P value < 0.01) in the levels between CKD patients and control for the following : Soluble-a-klotho protein (T test = 8.03 ; P value = 0.000) ; Irisin (ng/ml) (T test = 9.39 ; P value = 0.000); Cystatin-C (ng/ml) (T test = 3.96 ; P value = 0.000) .

Table (2) is about ANOVA table for Differences in the measurement of non-routine markers among different stages of chronic kidney disease . This table shows that there is a high significant decrease (P value <0.01) in the levels of irisin (ng/ml) among different stages of chronic kidney (F test = 17.74 ; P value = 0.000) . The same table revealed a high significant increase (P value <0.01) in the levels of Cystatin-C (ng/ml) and Soluble-a-klotho protein (ng/ml) among different stages of chronic kidney (F test = 35.2 ; P value = 0.000) and (F test = 3.53 ; P value = 0.02) respectively .

Discussion

Klotho protein in mammals is present in 2 different isoforms: a membrane-bound protein and as a soluble form . Full-length Klotho is a single-pass transmembrane protein that functions as an obligatory co-receptor for fibroblast growth factor-23 (FGF-23) which promotes phosphate excretion and inhibition of calcitriol synthesis (5) .

The serum levels of soluble Klotho may be determined by age, and renal function . Although a decrease in urinary Klotho levels in a relatively small number of CKD patients was reported previously , Some studies clearly demonstrated for the first time that not only the serum Klotho concentration, but also the amount of 24 hr urinary excreted Klotho correlated with the eGFR among the patients with various degrees of CKD. Besides the finding that the serum Klotho concentration was negatively correlated with age, which is consistent with previous findings, it was also confirmed that there was a similar trend between the amount of urinary excreted Klotho and the patient age. However, the clinical significance of age on the serum and urinary Klotho levels among the overall patients with CKD should be interpreted carefully, since our
results demonstrated that the eGFR, but not age, was one of the pivotal predictive variables for the amount of urinary excreted Klotho in various CKD patients, while only the serum calcitriol was identified as a predictor of the serum soluble Klotho levels (6).

The kidneys, parathyroid gland, and choroid plexus of the brain have been identified as the sites where the Klotho is predominantly expressed. This concept is strongly supported by the recent demonstration of the Klotho expression in human vascular tissues, including vascular smooth muscle cells, which is reduced during the course of CKD and can be restored by exogenous calcitriol. In an animal model of chronic kidney disease, in wildtype mice, Hu et al. showed very low renal, plasma and urinary levels of Klotho. Klotho is also one of the novel components in this complex systems biology between the kidney, skeleton and cardiovascular system. In chronic kidney disease, the expression of membrane-bound Klotho, an obligatory co-receptor for fibroblast growth factor-23 (FGF-23) signaling, is reduced and this limits FGF-23-stimulated signal transduction (5).

Cystatin C plays role in many biological processes, such as degradation of cellular proteins, regulation of enzymes and many pathologic processes. It is present in all body fluids and is important in arterial wall remodeling and atherogenesis. Cystatin C is synthesized by all nucleated cells of body and the rate of its production is constant. Results from other studies have also indicated that serum concentrations of cystatin C are not influenced by muscle mass, diet or sex. It has no extrarenal routes of elimination, with clearance from the circulation only by glomerular filtration (Khan et al., 2014). Cystatin C is cleared from the circulation by only renal filtration and is freely filtered at the glomerular filtration barrier. Its serum concentration is not influenced by non-renal factors. Its small size enable it to be freely filtered at the glomerulus. The use of serum cystatin C to estimate GFR is based on the same logic similar to that for blood urea nitrogen and creatinine. But because it is neither reabsorbed nor secreted by renal tubules, it is suggested to be closer to the ideal endogenous marker (7).

Cystatin C is generated at a mostly constant rate, is freely filtered, reabsorbed and catabolized in the proximal tubule of the nephron. It is not affected by body habitus and muscle mass and as such, it is thought to be a more accurate measure of kidney function compared to serum creatinine. Limitations to its routine use in clinical practice has been primarily cost considerations where, for example, it costs up to ten times that of a serum creatinine assay and it can be affected by thyroid disease, adiposity, and underlying inflammation (8).

The results of the present study have shown that the level of serum irisin is significantly reduced in in CKD patient as compared with healthy subjects, this result agrees with Wen et al., (2013) and Ebert et al., (2014). Irisin, a novel polypeptide hormone, is proteolytically processed from fibronectin type (9-10). III domain containing protein 5 (FNDC5), which is highly expressed in skeletal muscle and heart (11). As sarcopenia is a frequent finding in CKD and irisin is a myocyte-secreted protein, it can be hypothesized that sarcopenia directly contributes to lower irisin levels in end-stage renal disease (9). Low physical activity in patients with CKD may interfere with its levels, as recent studies demonstrated that physical exercise induced the expression of peroxisome proliferator-activator receptor coactivator (PGC) 1 and its downstream membrane protein, fibronectin type III domain-containing 5 (FNDC5), which is cleaved to form irisin in skeletal muscle (11).

Conclusions

There is a remarkable decrease in the level of serum soluble alpha klotho proteins and irisin and increase in serum cystatin C in patients with chronic kidney disease; and these differences are positively correlated with the glomerular filtration rate.

Ethical Clearance: Taken from Southern Technical University ethical committee

Source of Funding: Self

Conflict of Interest: Nil
 References


Correlation between Total Ige and Cytokines (Interleukin-24 and Interleukin-4) in Patients with Allergic Urticaria

Hajır Salam Sabeeh Alghannami1, Özcan Özkan2, Fatih Bakır3

1Lecture, 2Prof., 3Assoc. Prof., Çankırı Karatekin University, Graduate School of Natural and Applied Sciences, Department of Microbiology

Abstract

Allergic disease refers to a variety of disorders including seasonal allergies, atopic dermatitis, urticaria, life-threatening anaphylaxis reactions to food, and allergic asthma. Curiously, the incidence of allergic diseases has increased dramatically in the past decades and continues to rise in developed countries. In this study, 20 patients with a pre-diagnosis of acute urticaria among the patients admitted to Çankırı State Hospital and 20 normal patient sera were used for control purposes. Total IgE - Interleukin 4 - Interleukin 24 serum levels were determined by ELISA method. IgE, IL4 and IL24 were determined as 19.62ng / L, 155.55 ng / L and 155.51 ng / L, respectively, in patient serum samples by ELISA method. The difference between the patient and control groups was not significant (p> 0.05). On the other hand, in the statistical analysis of the patient group, it was determined that the relationship between IgE and IL24 was strong (p = 0.01). The correlation between IgE and IL4 was found significant (p = 0.01). However, it was found to be significant between IL4 and IL24. Our results support that the levels of IgE, IL-24, IL-4 in their patients are associated with the disease.

Keywords: Allergic Urticaria; IgE; Interleukin-24; Interleukin-4

Introduction

Allergic diseases; It refers to various disorders including seasonal allergies, atopic dermatitis, urticaria, food-borne anaphylaxis reactions, and allergic asthma. There has been a dramatic increase in the incidence of allergic diseases in the last decade, and this increase continues to increase in developed countries (1). It was first suggested 75 years ago that blister production in patients with urticaria may have an underlying allergic mechanism. The efficacy of H1-antihistamines in patients with urticaria will support this hypothesis, although there is no apparent external allergen (2). This led Rorsman to think that urticaria might be an autoallergenic (i.e., type I hypersensitivity to allergens per se) reaction. IgE involvement in patients with urticaria has been suggested from two sources. First, chronic spontaneous urticaria (CSU) is associated with high levels of total IgE and increased levels of IgE directed against, for example, thyroid antigens and double-stranded DNA. The second is the efficacy of omalizumab (anti-IgE) (3). In patients with other autoimmune conditions such as systemic lupus erythematosus, the disease is complex and characterized by the production of autoantibodies against a wide variety of self antigens. In eight samples, it has been suggested that in patients with systemic lupus erythematosus, the double-strand-specific IgE antibodies DNA stimulate plasmacytoid dendritic cells, an immune cell dependent on viral defense, to secrete significant amounts of IFN-α (4). Pleiotropic cytokines interleukin4 (IL-4) and IL-13 cause the protection of the Th2 lymphocyte profile and increase the initial IgE levels (5). Murine models demonstrated the critical nature of IL-13 independent of IL-4. IL-13 is produced predominantly by T cells; In addition, human basophils and mast cells can also secrete IL-13. Regulation of IL-13 production is completely different from IL-4 (6). This has been explained by the fact that stimulation of T cells with anti-CD28 and phorbol myristate acetate (PMA)
resulted in optimal induction of IL-13 with virtually no IL-4 secretion. Local tissue expression of IL-13 is found in the nasal mucosa of patients with allergic rhinitis and in patients with allergic asthma after pulmonary allergen challenge.

It has been shown in bronchoalveolar lavage fluid, whereas normal controls were unable to express IL-13. Increased spontaneous expression of IL-13 was observed in mononuclear cells (PBMC) in peripheral blood samples from patients with atopic dermatitis, but IL-4 mRNA was not found. Little is known about the contribution of IL-4 and IL-13 to IgE production in patients with allergic rhinitis and asthma. It has been shown in bronchoalveolar lavage fluid, whereas normal controls were unable to express IL-13.

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We hypothesize that CSU is an autoimmune disease in which IgE recognizes several autoantigens possibly primarily of dermal origin. Their low level diffusion into the bloodstream will be consistent with the basopenia seen in patients with CSU. In addition, increased local production periods of such allergens will explain local exacerbations of CSU. To test this hypothesis, large-scale screening of autoreactive IgE in serum of patients with CSU has been used to investigate potential targets of IgE autoantibodies (IgE-AAbs) in those with this disease. IgE found in blood sera from patients with idiopathic anaphylaxis (IdA) and healthy subjects was used as a control. The relationship of primary IgE autoantibody has been further investigated both in vitro and clinically.

**Material and Method**

This study was conducted retrospectively in the blood serum of 20 patients (10 females and 10 males) and 20 normal patients (14 females and 6 males) for control purposes who were admitted to the Dermatology Outpatient Clinic of Çankırı State Hospital between August 2020 and January 2021. The study was planned regardless of age and gender. The approval of the Ethics Committee of Lokman Hekim University dated 11.12.2020 and numbered 2020/100 was obtained for the study.

- Determination of Total IgE - Interleukin 4 - Interleukin 24 Levels

In this study, Total IgE - Interleukin 4 - Interleukin 24 levels were carried out by ELISA method in the Biochemistry Laboratory of Çankırı Private Karatekin Hospital. For this purpose, Human Receptor 1 for The Fc Region of IgE ELISA kit [(Biassay Tecnology Laboratory - Cat.No E66212Hu) Standard Curve Range: 5ng / L-100ng / L, Sensitivity: 0.27ng / L], Human Interleukin 4 ELISA kit [(Biassay Tecnology Laboratory - Cat.No E00092Hu) Standard Curve Range: 5 ng / L-1000 ng / L, Sensitivity: 2.53 ng / L], Human Interleukin 4 ELISA kit [(Biassay Tecnology Laboratory - Cat.No E2162Hu) Standard Curve Range: 5 ng / L-1500 ng / L, Sensitivity: 2.56ng / L]. All materials in the kit were kept in the dark until they reached room temperature before analysis. The application was made in accordance with the manufacturer’s test protocol. All reagents, standard solutions and samples were prepared according to the instructions. Standards included in each analysis kit were used. The samples used in the study were calculated according to the layout plan and placed in the frame. 50μl of standard was added to standard wells and 40μl of samples were added to test wells. 10μl of anti-FcεRIβ antibody was added to sample wells (Figure 1,2).
50μl streptavidin-HRP was added to the sample and standard wells and mixed thoroughly by making circular motions. The plate was covered and incubated at 37 °C for one hour in the dark. After removing the cover, the plate was placed in an automated ELISA washer and washed five times with wash buffer. It was placed in the ELISA washer and washed again five times with wash buffer. After washing, 50 μl of substrate A and 50 μl of substrate B solution were added to the wells, respectively. After covering the plate tightly, it was left in the dark at 37 °C for 10 minutes. After standing, 50μl of test stop solution was added to each well. The plate was placed in the ELISA reader (Robonic Readwell touch) within 10 minutes and the optical density (OD) value was read at 450 nm wavelength.

Statistical Analysis

Statistical analysis was evaluated in the Statistics Department of Çankırı Karatekin University. The Mann-Whitney U test was used to compare the groups that did not conform to normal distribution in the investigation of the compliance of the data to normal distribution, the difference between the control and experimental groups. Determination of the direction and size of the relationship (correlation) between variables was examined using Spearman Rho coefficients for variables with normal distribution, and for variables that did not conform to normal distribution. SPSS Statistics 21.0 program was used in the implementation of the analyzes. A p <0.05 value was accepted as the criterion for statistical significance.
Result

In the serum samples included in the study, the patients who were diagnosed with allergic urticaria were equally (50%) distributed in males (n = 10) and females (n = 10). In the control group, male (30%) and female (70%) normal serum samples were used. In this study, total IgE, IL 4 and IL 24 levels in patient and control serum samples by ELISA method are given in Table 1. Figure 1. Total IgE, IL 4 and IL 24 ELISA test results (ng / L) in patient (H) serum samples.
While IL24 level could not be detected in 55% of patient serum samples, this rate was determined as 40% in IL4. When the number of samples was n <50, Shapiro Wilk test was applied. Since p <0.05 according to the Shapiro-Wilk test, the data are not normally distributed. Therefore, Mann Whitney test was used to compare the means of two independent groups. According to the analysis results, no significant difference was found between the mean of the patient and control groups. Since the data are not normally distributed, the relationship between variables was calculated with the Spearman correlation coefficient. According to the value of the correlation coefficient, the result being +1 indicates that there is a strong positive relationship between the two variables, while -1 indicates a strong negative relationship. Correlation coefficient to 0 As the relationship gets closer, the strength of the relationship weakens, and zero indicates that there is no relationship between the two variables ((0.90-1.00: Very strong, 0.70-0.89: Strong, 0.50-0.69: Moderate, 0.30-0.49: Low, 0.16-0.19: Weak and < 0.16: Very weak). In patient samples, there was a strong positive correlation between IgE and IL4 (P <0.05), a strong positive correlation between IgE and IL24 (P <0.05), and a moderate positive correlation between IL4 and IL24. has been seen to be There was no significant difference between samples and control groups (p> 0.05). On the other hand, in the statistical analysis of the patient group, the relationship between IgE and IL24 was found to be strong (p = 0.01). The correlation between IgE and IL4 was found to be significant (p = 0.01). However, it was observed that there was a significant difference between IL4 and IL24 (p = 0.01).

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>IgE (ng/7L)</th>
<th>S:E.M</th>
<th>IL4 (ng/7L)</th>
<th>S:E.M</th>
<th>IL24(ng/7L)</th>
<th>S:E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>19,62*</td>
<td>5.97</td>
<td>151,55*</td>
<td>45.55</td>
<td>155,51*</td>
<td>48.46</td>
</tr>
<tr>
<td>K</td>
<td>18,60</td>
<td>6.44</td>
<td>264,60</td>
<td>191.17</td>
<td>183,01</td>
<td>81.74</td>
</tr>
</tbody>
</table>

Discussion and Conclusion

Urticaria is a chronic, not just acute, skin disease that affects about 5% of the general population. Interleukins have been reported to play a role in the pathophysiology of urticaria. Antigen-specific IgE responses to T cell dependent antigens such as allergens can only be generated by cognate interaction between B cells and T helper (Th2) cells. Interleukin-4 plays a central role in the differentiation of antigen-stimulated naïve T cells into T helper (Th) 2 cells, which are the main sources of IL-4. Interleukin-4 also suppresses Th1 cell growth and directs the transition of human B cells to IgE. The fact that the number of patients was low due to the Covid19 pandemic during the period of your study was limited to the sampling. In the scope of retrospective study in archived patient sera, etiological and demographic data were excluded from the study. It was aimed to investigate the relationship between IgE, IL-24-IL-4 in serum of patients pre-diagnosed in the dermatology clinic investigated IL-4, IL-6, IL-8, IL-10 in 51 patients with urticaria in the acute period and the increase in IL-4 level was found to be statistically significant reported that high IgE-anti-IL-24 values were associated with disease activity in patients with chronic spontaneous urticaria, while IgE-anti-IL-24 levels increased in eighty percent of patients. As a result, IL-24 has been defined as a common, specific and
functional autoantigen of IgE antibodies in patients with chronic spontaneous urticaria. (14) reported that IL23, IL10 and IL4 levels were significantly lower in the patient group with chronic spontaneous urticaria compared to the control group. In addition, they found that the IL-4 level of the patient group with positive and negative autologous serum skin test was significantly lower than the control group. In our study, the relationship between IL24 and IgE was found to be strong in patients with a pre-diagnosis of acute allergic urticaria. There was no difference in serum IgE patterns between the patients and the healthy control groups.

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Conflict of Interest: there has been no conflict of interest of any kind with the authors of this work.

Ethical standard: The study was formally approved the research plan by the ethical committee board at the Babylon health directorate.

Informed consent was taken from all the participant patients before being enrolled in the study. All data and materials are available

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Swimming Improves Memory Function and Decreases N-Methyl-D-Aspartate in Ageing Rats

Hanik Badriyah Hidayati1, Purwo Sri Rejeki2, Lilik Herawati2, Susi Wahyuning Asih3, Suhartati Suhartati4, Siti Khaerunnisa2

1Lecturer, Department of Neurology, Faculty of Medicine Airlangga University – Dr. Soetomo General Hospital, 60132; 2Lecturer, Department of Physiology and Medical Biochemistry, Faculty of Medicine, Airlangga University, 60132; 3Lecturer, Department of Community Nursing, Faculty of Health Science, University of Muhammadiyah Jember, 68121; 4Professor, Department of Medical Biochemistry, Faculty of Medicine, University of Wijaya Kusuma Surabaya, East Java, Indonesia

Abstract

A single Memory impairment substantially reduces the quality of life in the elderly. It is associated with the alteration of neurotrophic (NT) factors, such as brain-derived neurotrophic factor (BDNF) and glutamate receptor N-methyl-D-aspartate (NMDA). Exercise is often used to reduce cognitive impairment. Previous studies show that the benefits of aerobic exercises on such impairments are correlated with increasing BDNF and preventing the production of NMDA. However, some results remain controversial. Thus, the association between exercise and Memory was addressed by examining increases in BDNF and the reduction of NMDA in ageing rats. The study used a randomized, post-test-only controlled group of 30 male one-year-old ageing Rattus norvegicus divided into three groups, namely, K0 (control) and K1 and K2 (aerobic swimming exercise). K1 and K2 animals differed in the frequency of exercise, which is three and four sessions per week, respectively. Memory was assessed using Y-maze performance. BDNF and NMDA were analyzed using enzyme-linked immunosorbent assays. A significant improvement in memory function and reduction in the NMDA level were observed in K1 and K2 group rats (p = 0.001; p = 0.041). No significant impact on the BDNF levels was observed (p = 0.387). Swimming may boost Memory by reducing the NMDA level but not by increasing BDNF. Swimming is a promising method for preventing or delaying memory loss in degenerative brain diseases. Further investigation is needed to fully understand underlying mechanisms.

Keywords: Memory; NMDA; BDNF; ageing rat; swimming

Introduction

Life expectancy has continuously increased during the last decades, which has led to increasing numbers of elderly individuals 1. In fact, the elderly are the fastest-growing segment of the United States population 2. The latest projections suggest that the number of individuals older than 60 years will increase from 810 million in 2012 to 2 billion by 2050 worldwide (United Nations Department of Economic and Social Affairs, 2012) 1.

The elderly are at a high risk of developing cognitive impairments 2. Previous studies show that cognitive impairment is associated with chemotherapy and age 2-4. Many elderly individuals experience difficulty performing complicated daily tasks 5. Physical and mental abilities markedly decline with age and hence reduced quality of life 1,6. Quality of life is an important concern in ageing societies 7. Various recent studies indicate that cognitive impairment, among degenerative brain diseases, are particularly common for elderly...
individuals\textsuperscript{2,6}.

Memory, the accumulated and stored aggregate of an individual’s experience, plays an important role in the overall cognitive function\textsuperscript{3}. Memory has multiple dimensions, including the ability to store, retain, and retrieve information\textsuperscript{2}. Memory problems include a tendency toward misplacing items, difficulty remembering appointments, and forgetting details of conversations and events\textsuperscript{2}. These and other memory issues substantially impact the quality of life of afflicted individuals\textsuperscript{3}.

The hippocampus, an important brain region for learning and memory, is the primary site of neurogenesis in adulthood and retains the greatest brain neuroplasticity\textsuperscript{3}. Brain-derived neurotrophic factor (BDNF) is expressed ubiquitously in the brain\textsuperscript{8}. BDNF, a fundamental neurotrophic factor in learning and memory\textsuperscript{8}, plays a pivotal regulatory role in the development, cognition, and plasticity of the hippocampus\textsuperscript{1,3,4,9}. BDNF is key to maintenance, growth, neuronal survival, differentiation, neurotransmitter release, dendritic remodeling, axon growth, the formation of neurons, regulation of hippocampal structure, synaptic transmission, synaptic modulation, promotion of synaptic growth and plasticity\textsuperscript{3,8,9}. BDNF exerts neurotrophic anti-apoptotic regulation in neurons by binding with tyrosine receptor kinase B, also known as tropomyosin-related kinase B (TrkB)\textsuperscript{3,9}.

BDNF is secreted at pre- and post-synaptic sites. Synaptic BDNF is secreted in response to activities and activates pre- and post-synaptic TrkB receptors. BDNF is important for long-term potentiation (LTP). BDNF mediates the regulation of excitatory synapses during early LTP. Presynaptic BDNF increases the exocytosis of glutamate-containing synaptic vesicles. Post-synaptic BDNF–TrkB signaling induces NMDA receptor (NMDAR) phosphorylation\textsuperscript{8}.

A key aspect of quality of life is health\textsuperscript{7}. Exercise, a major component of efforts to maintain and improve health, is an effective intervention for addressing degenerative brain diseases\textsuperscript{3,7}. Exercise may relieve stress and anxiety that can lead to deteriorating mental and physical health. Regular exercise is widely reported to help maintain and enhance a cognitive function, activate neurotransmitters, induce gene expression, and promote neuroprotection\textsuperscript{3}, but underlying mechanisms are not yet clear. Several studies have concluded that exercise can be a therapeutic strategy to increase the BDNF level and that a sedentary lifestyle may impair BDNF signaling\textsuperscript{9}.

BDNF and NMDA play important roles in hippocampal synaptic plasticity\textsuperscript{8}. Therefore, the current study aims to determine whether a cognitive impairment, neuroplasticity in the hippocampus.

**Materials and Methods**

**Setting**

This work was conducted in the animal research laboratory of the Department of Medical Biochemistry of Airlangga University, Surabaya, East Java, Indonesia. This study received approval by the Animal Care and Use Committee, Veterinary Faculty of Airlangga University with certificate number 745-KE.

**Animal**

Thirty-one-year-old male rats (\textit{Rattus norvegicus}), with an average weight of ±300 g, were used. Animals were acclimatized for one week and allowed free access to food and water \textit{ad libitum}. The exercise period lasted for eight weeks. Behavioral analyses were conducted upon completion of all exercise sessions. Brains (cerebral hemisphere) were removed and homogenized using a sonicator, and BDNF and NMDA were measured using enzyme-linked immunosorbent assays (ELISA)\textsuperscript{3}.

**Grouping**

The animals were randomly divided into three groups (\(n = 10\)):

- Group 1 (K0): control group, non-swimming
- Group 2 (K1): swimming exercise sessions three times/week for eight weeks
- Group 3 (K2): swimming exercise sessions five
times/week for eight weeks

2.1.3. Exercise Protocol

The rats in Groups 2 and 3 used a pool made for animal exercise sessions. Animals swam for 10 min during the first week, 20 min during the second week, and 30 min in weeks three through eight.

Y-Maze

The rat performance was assessed in a Y-maze after the exercise period. The performance in the maze measured the willingness of the rats to explore a new environment. The Y-maze allowed only two choices: right and left pathways. Food was placed at the end of one path as an incentive. An animal was placed in the middle of the maze and allowed to choose one of the paths. The rats were guided manually to the target if the food was not found after 90 seconds. A sign was provided in the maze to indicate the location of food. During the memory test, signs were eliminated to test whether the rats recalled the food location. The time of placement in the maze to achieving the target was measured. Re-entry into the path with food was taken as an indication that the animals recalled their previous maze experience. Entry to the path without food was taken as a sign of a lack of recall.

Determining the Level of NMDA and BDNF

The samples were collected from the left or right hemispheres of the hippocampus and analyzed using ELISA. The samples were homogenized using a sonicator suspended in a lysis buffer and centrifuged at 15,000 x g for 15 min at 4 °C. Supernatants were retained for the analysis. The samples were immediately frozen at −20 °C. BDNF and NMDA levels were measured using a Rat CREB Kit (category no. E0039Ra, Bioassay Technology Laboratory), Rat NMDA Kit (category no. E0999Ra, Bioassay Technology Laboratory) and Rat BDNF Kit (category no. E0476Ra, Bioassay Technology Laboratory).

Statistical Analysis

Normally distributed data were analyzed by one-way analysis of variance, followed by the least significant difference tests. Non-normal distributions were analyzed by the Kruskal–Wallis test, followed by Mann–Whitney U test. Statistical significance was set at p < 0.05. Data analysis using SPSS version 23.

Results

Table 1. BDNF, NMDA, and Memory in each group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K0</td>
<td>K1</td>
</tr>
<tr>
<td>BDNF</td>
<td>4.17 ±0.64</td>
<td>4.68±1.03</td>
</tr>
<tr>
<td>NMDA</td>
<td>19.77±1.67</td>
<td>15.59±5.12</td>
</tr>
<tr>
<td>Memory</td>
<td>52.31±22.73</td>
<td>19.85±8.2</td>
</tr>
</tbody>
</table>

*Significant with p<0.05
The BDNF, NMDA, and Memory results for each group are presented in Table 1. The BDNF levels between groups did not differ significantly ($p = 0.387; p > 0.05$). The NMDA levels were significantly different between Groups K0 and K1 ($p = 0.016$). Significant differences were not observed between Groups K0 and K2 ($p = 0.055$) and Groups K1 and K2 ($p = 0.749$).

Memory performance from K0 to K2 increased with increasing amounts of exercise. Between Groups K0 and K1 and Groups K0 and K2, significant differences in the maze recall were observed ($p = 0.001$ and $p = 0.000$, respectively). No significant difference was found between Groups K1 and K2 ($p = 0.962$).

**Discussion**

In this study, the cognitive function improved in elderly rats after eight weeks of regular exercise. Exercise has been shown to improve Memory at different ages, along with other beneficial effects \(^1\,^3\,^4\,^10\,^11\). Physical activity is needed for the elderly in exercising the muscles of the elderly from the condition of movement that tends to reduce function \(^14\). The treatment of cognitive impairments has emphasized the regulation of maintenance, growth, survival, and formation of neurons. Besides, physical activity is known to increase the BDNF level, which binds TrkB \(^3\,^9\), thus inducing an anti-apoptotic state \(^3\). Some authors have suggested exercise as a treatment for cognitive impairments. At the cellular level, mitochondria endow hippocampal neurons with neuroplasticity in several ways: control of Ca\(^{2+}\) uptake, redox signaling, developmental and synaptic plasticity, and cell survival and death. Furthermore, mitochondrial permeability transition pores, which are closely related to apoptosis, regulate various physiological functions at the cellular level, and play a key role in learning and synaptic plasticity \(^3\).

Exercise relieves stress and anxiety and thus augments mental and physical health \(^3\,^11\). Physical activity causes brain neuronal and biochemical changes that activate neurotransmitters and induce gene expression, which promotes proliferation and survival of neurons, maintain and improve cognitive function and confer neuroprotection against brain damage \(^3\). Exercise has also been associated with enhanced capacity of mitochondria for calcium toleration, with further consequences on the control of mitochondrial-driven apoptotic cell death \(^12\). Exercise also helps preserve brain function during ageing by enhancing BDNF signaling \(^4\).

Nagahara et al. found that BDNF treatments can decrease Aβ-mediated cell death, counter cognitive dysfunction, and synapse loss and even retard the cognitive decline in nonhuman primates and APP transgenic mice \(^9\). The effects of BDNF on neurogenesis, neuronal survival, and synaptic plasticity appear to be mediated by signal transduction pathways involving Akt kinase, Ca\(^{2+}\)/calmodulin and mitogen-activated protein kinases. The gene targets of BDNF signaling pathways include the anti-apoptotic protein Bcl-2, NMDA glutamate receptor subunits and neuronal nitric oxide synthase \(^4\). High levels of BDNF correlate with a low risk of developing dementia \(^1\). BDNF signaling can promote healthy ageing and protect the brain against age-related neurodegenerative disorders \(^4\).

Erickson et al. (2011) investigated relationships between chronic aerobic physical activity and peripherally assessed concentrations of BDNF, hippocampal volume, aerobic fitness (VO\(_2\) max) and cognitive performance (spatial Memory) in a 1-year RCT with older adults. This study demonstrates that changes in aerobic fitness from pretest to posttest were positively correlated with changes in hippocampal volume and the increase in hippocampal volume experienced in the physical activity group was positively correlated with better spatial memory performance and greater change in BDNF concentration \(^15\). Umm et al. (2008) demonstrated that treadmill running increases the BDNF level \(^1\). BDNF signaling is probably the key mechanism for triggering brain neuroplasticity \(^16\,^17\) and anti-apoptotic cell death that leads to the improvement of cognitive function \(^4\,^16\).

In this study, BDNF and NMDA were used to assess changes in brain chemistry associated with improved Memory after regular swimming exercise. BDNF plays
a pivotal role in neurogenesis, neuronal survival and synaptic plasticity in the brain. The lowest BDNF mean level was recorded in the hippocampus of rats in Group K0 (4.17 ± 0.64). This group did not exercise. BDNF gradually increased from Groups K0 to K2 along with the increasing frequency of swimming sessions. However, no statistically significant interaction between exercise and BDNF level was found. A protective effect of exercise against apoptosis demonstrated by the increased BDNF levels in a frequency-dependent manner was not demonstrated by a statistically significant increase in BDNF concentration.

Elderly with exercise swimming can improve their memory function compared to that without exercise. The molecular mechanism is suggested by inhibiting NMDA expression; thus, it could prevent intracellular calcium build up that results in the activation of Ca²⁺/calmodulin dependent protein kinase II (CaMKII) and c-AMP response element-binding (CREB) pathway. This pathway related to the maintenance of synaptic plasticity through increased BDNF expression.

BDNF is related to the NMDAR. One BDNF signaling pathway targets the NMDA glutamate receptor subunits. BDNF stimulates NMDAR autophosphorylation. Autophosphorylation and function of CaMKII protect synaptic function. An in vivo investigation showed that Met carriers reduced the hippocampal volume and changed synaptic plasticity, especially NMDAR-dependent LTP. These effects resulted in a loss of hippocampal-related Memory.

A meta-analysis found that physical activity positively influences cognitive function in patients with dementia, independent of the clinical diagnosis and frequency of the aerobic exercise program. In this study, swimming stimulated the BDNF levels but showed no significant interaction. The greater the time between the placement of animals into the maze to finding food, the worse the animals’ Memory. The slowest time to reach the target was observed in rats in Group K0 (52.31 ± 22.73 sec) and the quickest in Group K1 (19.85 ± 8.2 sec). In Group K2, rats were essentially as quick at solving the maze as Group K1 animals (20.69 ± 6.69 sec). Thus, exercising either three or five times a week had a similar positive impact on recall. Statistically, the results from Group K0 rats were significantly different and higher than those from animals in Groups K1 and K2 (p = 0.001, p = 0.000, respectively).

The results of the current study are consistent with those of previously published works, which show that exercise can help improve memory and neurodegenerative diseases. Investigators have used various methods and parameters in different settings to assess the impacts of exercise, and outcomes are always similar; that is, exercise improves the cognitive function.

Conclusions

The results of the current study help confirm that exercise can improve cognitive function and prevent and delay degenerative brain diseases in the elderly. Exercise may produce its effects via inhibiting the excessive increase of NMDA. Additional investigations on the relationship between BDNF and cognitive function are warranted.

Declarations

Acknowledgements

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AVAILABILITY OF DATA AND MATERIALS

All data generated in the present study are included in this article.

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Conflicts of Interest: The authors declare that they have no competing interests.

Ethics Approval and Consent to Participate

This study received approval by the Animal Care
and Use Committee, Veterinary Faculty of Airlangga University with certificate number 761-KE.

**References**


Human Carbon Footprint as the Basis for Determining Healthy Indoor Volume Dimensions

Harida Samudro¹, Sarwoko Mangkoedihardjo²
¹Researcher, Department of Architecture, State Islamic University of Malang, Malang, Indonesia, ²Professor, Department of Environmental Engineering, InstitutTeknologiSepuluhNopember, Surabaya, Indonesia

Abstract
This paper was presented to determine the volume unit of indoor based on the human carbon footprint. The purpose of this approach was to formulate the ideal dimensions of an indoor, which was able to maintain human health. The basis for determining the dimensions was adapted to tropical conditions. For users in non-tropical areas, slight adjustments to temperature and ventilation may be required. Some examples of indoor dimension evaluation and planning were presented in this paper. In general, it was obtained that the indoor dimension could be found using the volume unit of a healthy room of $3.0 \text{ m}^3$/person/hour. The volume unit was linear with the number of users and time of existence in the room. This contributed significantly to people, who can easily and quickly find out the dimensions of space according to their needs.

Keywords: Carbon footprint; indoor; volume unit; public space; personal room

Introduction
Living organisms are physically composed of organic matter, which easily breaks down into other forms. This organic material can basically be represented as glucose, and integrated into the material as biomass. The simple evidence is physical growth, which indicates an increase in biomass. Along with that growth is breathing, which gives off mainly carbon dioxide gas. The process of converting biomass into carbon dioxide is evidence that biomass is organic matter that is easily biodegradable.

Naturally, organic matter is a complex chemical compound containing carbon, symbolically represented as C. Except for the gases carbon dioxide ($\text{CO}_2$) and methane ($\text{CH}_4$) and bicarbonate ions ($\text{HCO}_3^-$) in solution, all of which are categorized as inorganic materials. On earth, carbon is the most important element and influences the earth’s climate. The presence of carbon is in the atmosphere (the gases carbon dioxide and methane, which are known as greenhouse gases), the geosphere (hydrocarbon carbonate minerals), the biosphere (organic matter), and the hydrosphere (bicarbonate ions) (1–3). Especially for greenhouse gases in the atmosphere, which are the breathing medium for living organisms, there are safe levels for life, namely $\text{CO}_2$ of 360 ppm and $\text{CH}_4$ of 1.7 ppm (4). The content and/or the proportion of the two gases in the atmosphere is dynamic, which can be influenced by the dynamics of living organisms, especially the activities of human life (5,6).

In humans, the process of converting organic matter is known as respiration, which exhales a mixture of carbon dioxide, nitrogen, oxygen, water vapor and various volatile materials (7–9). Most of the time, the activities of human life are indoors, at least 8 hours of sleep rest as a time considered capable of maintaining human health. The activity room can be a personal...
space, for example a bedroom, family room, kitchen room, and others. The activity space is also in a format for the public, for example offices, shopping, tours, and the like. Every human being living in any place emits greenhouse gases and leaves a carbon footprint on the environment.

The carbon footprint can have a negative impact, both on the environment and on humans themselves\(^{(10–16)}\). This leads to the need for space, which is able to overcome the negative impact of these gases. So far there is no known way to determine the size of space, which is based on the results of human life activities, namely carbon from human life processes. Therefore, this paper has examined the human carbon footprint approach for determining the dimensions of spaces, both private and public places. The purpose of this approach is to provide an indoor spatial dimension capable of maintaining human health while in it.

**Methods**

The method of calculating carbon footprint products is based on the reactions of human life and consumption of various living necessities. The general reaction of life is expressed as follows:

- Under aerobic conditions: \( n\text{ME} + C_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + n\text{ME} \) \(^{(1)}\)

- Under anaerobic conditions: \( n\text{ME} + C_6\text{H}_{12}\text{O}_6 \rightarrow 3\text{CO}_2 + 3\text{CH}_4 + n\text{ME} \) \(^{(2)}\)

In these 2 equations, \( n\text{ME} \) are the many types (\( n \)) of materials and energy (\( \text{ME} \)) that are used for human life. For example, various kinds of drinking water, various kinds of food, various kinds of building materials, various kinds of energy sources, various kinds of vehicles, and various kinds of products.

Various kinds of gas products resulting from life activities (\( \text{CO}_2, \text{CH}_4, \text{SOx}, \text{NOx}, \text{HFC} \), etc.) can be equivalent to \( \text{CO}_2 \) gas and expressed in units of emission/person/time (for example: kg/person/day). The equivalence of greenhouse gases to carbon dioxide is measured by the global warming potential (GMP). In practice, the GWP of \( \text{CH}_4 \) is variable over the source of the generation\(^{(17–19)}\), however, to illustrate the magnitude of the effect of heating, 1 mol of \( \text{CH}_4 \) is 25 mol of \( \text{CO}_2 \) equivalent. Thus, equation 2) can be stated as follows:

- Under anaerobic conditions: \( n\text{ME} + C_6\text{H}_{12}\text{O}_6 \rightarrow 78\text{CO}_2 + n\text{ME} \) \(^{(3)}\)

Equation 3) is appeared to give a warning that a room with insufficient oxygen leads to an increase in room temperature as a result of the carbon dioxide equivalent increasing sharply. This is important to consider in determining room ventilation.

**Results and Discussion**

**Basics of calculation**

**Healthy concentration of carbon dioxide**

The outdoor \( \text{CO}_2 \) concentration is in equilibrium with ambient air, which is about 360-380 parts per million (ppm)\(^{(4,20)}\). Likewise, in the room that is balanced with the outside. This concentration is a level that supports the health of human life. However, indoor \( \text{CO}_2 \) concentrations can fluctuate and exceed these healthy concentrations, but humans still do not experience health problems. There are a number of concentrations that can be tolerated, but as a sustainable healthy concentration and for maximum protection for human life, the indoor \( \text{CO}_2 \) level is as follows:

\[
\text{Healthy indoor } \text{CO}_2 \text{ concentration} = 360 \text{ ppm} \quad (4)
\]

**Individual carbon dioxide emissions**

The average annual carbon emission from energy demand for household use is 6.744 kg \( \text{CO}_2 \)\(^{(21)}\). In general, people do indoor activities for more than 19 hours a day\(^{(22)}\). In these activities, humans need energy 34 MJ/person/day for the basic needs of life, such as eating and drinking; and various activities, such as transportation and works. Thermodynamic energy stated that 1 mol of glucose equals 2.8 MJ. Stoichiometric glucose requirement is 34/2.8 or 12 mol glucose/person/day. Emission of \( \text{CO}_2 \) is 12*6 mol CO\(_2\) = 72 mol CO\(_2\) = 72 * 44g = 3.2 kg CO\(_2\)/person/day\(^{(23)}\). About 25-40% of
them are sourced from human life, namely 0.8-1.2 kg CO$_2$/person/day. The calculations used are considered appropriate that confirmed to Prairie & Duarte$^{(24)}$ for the average human body weight of 70 kg, which releases 1.4 kg CO$_2$/person/day. It is also confirmed respirometrically 0.9 kg CO$_2$/person/day$^{(25)}$. For general calculations, the average individual CO$_2$ emission units is as follows:

$$\text{CO}_2\text{ emission units} = 1.0 \text{ kg CO}_2/\text{person/day} \quad 5)$$

**Carbon dioxide density**

The density of indoor air is in the same equilibrium as the outside air. The density of air is variable with temperature. The higher the temperature, the lower the air density. For tropical climatic conditions, the air temperature is in the range 20-35°C, which indicates air density in the range 1.12-1.20 kg/m$^3$.$^{(26,27)}$ While the density of carbon dioxide is about 50% higher than dry air$^{(28)}$. For general calculations, the average CO$_2$ density is taken as follows:

$$\text{CO}_2\text{ density} = 1.75 \text{ kg/m}^3 \quad 6)$$

**Room ventilation**

According to eq. 3) means that the ventilation system must be sufficient to maintain the aerobic conditions of the room. The ventilation becomes the gate for air transportation between indoor and outdoor spaces. In that case, there is a release of CO$_2$ from the room to the outside air. For a general calculation, the approximate CO$_2$ release from the room is as follows:

$$\text{CO}_2\text{ release} = 95\% \quad 7)$$

**Space volume calculation**

**Public space**

Two examples of defining indoor dimensions are given for workspaces and lecture halls. Its implementation can be applied to a large number of public meeting rooms.

Example 1: Workspace carbon footprint for indoor dimensional planning

- For 1 person, the CO$_2$ emission unit is eq.5): ($1.0$ kg CO$_2$/day)/(24 hours) = 0.04 kg CO$_2$/hour.
- The CO$_2$ volume is the CO$_2$ emission unit divided by CO$_2$ density eq.6): ($0.04$ kg CO$_2$/hour)/(1.75 kg/m$^3$) = 0.023 m$^3$/hour.
- Gas CO$_2$ in a healthy room eq.4) divided by normal working hours: (360 ppm) / (8 hours) = 45 ppm/hour.
- Total CO$_2$ in the room (95% release, eq.7) is [(100) / (100-95)] * 45 ppm/hour = 900 ppm/hour. This is to be converted into volume by volume = (900/1,000,000)/hour = 0.0009/hour.
- Volume of space = (0.023 m$^3$/hour) / (0.0009/hour) = 25.56 m$^3$/person.

Thus, the volume of space of healthy room works 8 hours for 1 person is rounded to 25 m$^3$, which is free of placement of length, width and height, and this follows the comfort of the architectural design of a workspace.

Example 2: Classroom carbon footprint to predict the length of the meeting in a certain dimensional indoor

- Assumed a classroom for 50 people, the CO$_2$ emission unit is eq.5): (50)*(1.0 kg CO$_2$/day)/(24 hours) = 2.08 kg CO$_2$/hour.
- The CO$_2$ volume is the CO$_2$ emission unit divided by CO$_2$ density eq.6): (2.08 kg CO$_2$/hour)/(1.75 kg/m$^3$) = 1.19 m$^3$/hour.
- Volume of room (assumed the dimensions of the existing space) = 20 m * 10 m * 3 m = 600 m$^3$.
- Room CO$_2$ concentration is the CO$_2$ volume divided by volume of existing room = (1.19 m$^3$/hour) / (600 m$^3$) = 0.0020*1,000,000 (this is the number to convert into parts per million, ppm) = 2,000 ppm/hour.
- Gas escaping out of the room is 95% (eq.7), then the indoor gas becomes(5%)*(2,000 ppm/hour) = 100 ppm/hour. Therefore, the maximum time of meeting for healthy conditions is (360 ppm) / (100 ppm/hour) = 3.6 hours.
Personal room

Often and many people ask about the size of a healthy personal space for bedrooms, living rooms, kitchens, and homes as a whole. Several examples below provide the answer.

Example 3: Bedroom carbon footprint, an evaluation of the size of the existing space:

- For 1 person, the CO₂ emission unit is eq.5): (1.0 kg CO₂/day)/(24 hours) = 0.04 kg CO₂/hour.
- The CO₂ volume is the CO₂ emission unit divided by CO₂ density eq.6): (0.04 kg CO₂/hour)/(1.75 kg/m³) = 0.023 m³/hour.
- Room volume (assumed with existing dimensions) = 3m x 3m x 3m = 27m³.
- Room CO₂ concentration = (0.023 m³/hour) / (27 m³) = 0.000852* 10⁶ (this is the number to convert into parts per million, ppm) = 852 ppm/hour.
- Gas escaping out of the room = 95% (eq. 7), then the indoor CO₂ becomes 43 ppm/hour.

These results can then be adjusted for the number of people and the length of time in the bedroom, such as:
- 1 person may stay in the bedroom for 360/43 = 8.4 hours.
- 2 people may stay in the bedroom for 360/86 = 4.2 hours.

Example 4: Bedroom carbon footprint, a new plan for a spouse for 8 hours stay at room:

- For a spouse (2 person), the CO₂ emission unit is eq.5): (1.0 kg CO₂/day)/(24 hours) = 0.08 kg CO₂/hour.
- The CO₂ volume is the CO₂ emission unit divided by CO₂ density eq.6): (0.08 kg CO₂/hour)/(1.75 kg/m³) = 0.046 m³/hour.
- Gas CO₂ in a healthy room eq.4) divided by normal working hours: (360 ppm) / (8 hours) = 45 ppm/hour.
- Total CO₂ in the room (95% release, eq.7) is [(100) / (100-95)] * 45 ppm/hour = 900 ppm/hour. This is to be converted into volume by volume = (900/1,000,000)/hour = 0.0009/hour.
- Volume of bedroom for a spouse= (0.046 m³/hour) / (0.0009/hour) = 51.11 m³ (rounded to 50 m³).

Example 5: House carbon footprint, a new plan for 24 hours (longest time on a holiday):

- For 1 person, the CO₂ emission unit is eq.5): (1.0 kg CO₂/day)/(24 hours) = 0.04 kg CO₂/hour.
- The CO₂ volume is the CO₂ emission unit divided by CO₂ density eq.6): (0.04 kg CO₂/hour)/(1.75 kg/m³) = 0.023 m³/hour.
- Gas CO₂ in a healthy room eq.4) divided by normal working hours: (360 ppm) / (24 hours) = 15 ppm/hour.
- Total CO₂ in the room (95% release, eq.7) is [(100) / (100-95)] * 15 ppm/hour = 300 ppm/hour. This is to be converted into volume by volume = (300/1,000,000)/hour = 0.0003/hour.
- The indoor volume unit is (0.023 m³/hour) / (0.0003/hour) = 76.67 m³ (rounded to 75 m³), which is for 1 person.

By knowing the indoor volume unit, it is easy to find out the volume of the house for a number of residents who live in the house during a healthy holiday. As an illustration for a family of 4 residents, they need a house with volume 300 m³. In dimensions, the house is 1 floor with a fixed height of 3 m, so a length and width of 10m are required.

Conclusion

Based on the calculation examples above, the volume of space is linear with the number of users and the time of existence. It is known that the volume unit of a healthy room is rounded to 3.0 m³/person/hour. The space volume calculation based on carbon footprint is applicable to all indoor functions, both for personal and public activities.
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References


Diarrhea Prevalence in East Java, Indonesia: Does Access to Sanitation and Health Behavior Ecologically Related?

Hario Megatsari¹, Agung Dwi Laksono², Anita Dewi Moelyaningrum³

¹Lecturer, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia, ²Researcher, National Institute of Health Research and Development, the Indonesia Ministry of Health, Jakarta, Indonesia, ³Lecturer, Faculty of Public Health, University of Jember, Jember, Indonesia

Abstract

Diarrhea is still a problem in East Java in Indonesia. The study aims to analyze the ecological relationship between sanitation access and health behavior with diarrhea prevalence in East Java, Indonesia. The research conducted the ecological analysis using secondary data from the Ministry of Health of the Republic of Indonesia report in 2018. The study takes all regencies/cities in East Java Provinces as samples. Apart from the diarrhea prevalence in children under five, four other variables analyzed as independent variables were access and clean water sources, the percentage of coverage of access to healthy latrines, the proportion of proper handwashing behavior, and the proportion of defecating behavior in healthy latrines. The study analyzed data using a scatter plot. The study results found that the higher the percentage of coverage of access and clean water source in regency/city, the lower the prevalence of diarrhea in children under five in that regency/city. The higher the percentage of access to healthy latrines in a region, the lower the majority of diarrhea in children under five. Meanwhile, the higher the proper handwashing behavior proportion in an area, the lower the prevalence of diarrhea in children under five in that area. Moreover, the higher the proportion of defecating behavior in healthy latrines in a region, the lower the prevalence of diarrhea in children under five. The study concluded that sanitation access and health behavior were related to the diarrhea prevalence in children under five.

Keywords: diarrhea, sanitation access, health behavior, ecological analysis, secondary data.

Background

Diarrhea is described as the passing of three or more loose or liquid stools per day, or more frequently than is typical for the person¹. East Java, as one of the provinces in Indonesia with the second largest population after DKI Jakarta Province, has a diarrhea prevalence rate that is close to the national prevalence rate, which is 6.5%². Based on the East Java Provincial Health Office data in 2019, the diarrhea service coverage from 2015-2019 experienced a downward trend, wherein 2019 the figure was 74.10%, indicating that people who had diarrhea have not been fully served by health facilities³.

Diarrhea diseases are some of the major contributors to global child mortality, resulting in 8 per cent of all child deaths under 5 years of age⁴. Diarrhea diseases, such as cholera, may be endemic, with continuous transmission, or epidemic, like during an outbreak⁵. Research conducted by Baral et al in 2020, states that the average cost spent by patients suffering from diarrhea with outpatient is US $ 36.56 and inpatient is US $ 159.90. Another judgment from the study is direct medical costs for 79% (83% for inpatient and 74% for outpatient) of the total direct costs⁶.

Access to clean water is an indicator of the Sustainable Development Goals (SDGs), which is also an effective way to prevent diarrhea cases⁷. Research conducted by
Otsuka in 2019, states that the management of drinking water at home and meaningful personal hygiene practices for children is essential for the sustainability and promotion of children’s health in urban slum areas in Indonesia. Other research conducted by Bidkhori in 2019 stated that the diarrhea-associated deaths in children under 5 years are influenced by factors such as Use of Improved Drinking-Water Sources (UIDWS) and Use of Improved Sanitation Facilities (UISF), and this rate can be reduced by making UISF and UIDWS available for people and especially people of rural areas.

Behavioral aspects with behavior are closely related. Research conducted by Auliaailahi in 2020 states that there is a relationship between healthy life behavior and cases of diarrhea in Indonesia. Another research conducted by Aftab et al in 2018, stated that many children may not receive prescribed medication for diarrhea and pneumonia on time. Taking into account the concerns of caregivers, an appropriate supply of medicines to lady health workers, enhanced facility-level care could strengthen care-seeking practices and child health outcomes.

The study aimed to analyze the ecological relation between access to sanitation (clean water and latrines), health behavior (defecating and washing hands), and the prevalence of diarrhea in East Java, Indonesia.

Materials and Methods

The author designed the study using an ecological analysis approach. Ecological studies focus on comparisons between groups, not individuals. The data analyzed is aggregate data at a particular group or level, which in this study is the provincial level. An ecological analysis variable can be aggregate measurements, environmental measurements, or global measurements.

The author conducted the study using secondary data from the 2018 Indonesia Basic Health Survey chapter of East Java Province. The study involved all regions in East Java (38 regencies/cities).

In this study, the dependent variable is the prevalence of diarrhea in children under five. Meanwhile, the study analyzed two groups of independent variables, namely sanitation access and health behavior. The sanitation access consists of two variables: the percentage of access and clean water sources and the percentage of coverage of access to healthy latrines. Healthy latrine is a type of latrine with a goose neck seat and uses a septic tank for disposal. The health behavior consists of two variables: The proportion of proper handwashing behavior and the proportion of defecating behavior in healthy latrines.

The study used univariate and bivariate analysis to examine the data. The review uses a scatter plot to perform bivariate analysis. The analysis employs a fit-line to assess the relationship between diarrhea prevalence in children under five and independent variables. The research used SPSS 21 program in the entire review process.

Results

Table 1 shows the diarrhea prevalence in children under five and other variables in East Java, Indonesia, in 2018. Table 1 indicates a very high variation between regencies/cities. The diarrhea prevalence in children under five was the lowest, 1.2% (Kediri City), while the highest prevalence was 17.9% (Bondowoso Regency).
Table 1. Statistic descriptive of the diarrhea prevalence in children under five and other variables in East Java, Indonesia, in 2018

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>The prevalence of diarrhea in children under five</td>
<td>38</td>
<td>1.2</td>
<td>17.9</td>
<td>9.121</td>
<td>4.0220</td>
</tr>
<tr>
<td>Percentage of coverage of access and clean water sources</td>
<td>38</td>
<td>54.9</td>
<td>100.0</td>
<td>90.889</td>
<td>9.4529</td>
</tr>
<tr>
<td>Percentage of coverage of access to healthy latrines</td>
<td>38</td>
<td>34.4</td>
<td>92.1</td>
<td>72.561</td>
<td>14.4047</td>
</tr>
<tr>
<td>The proportion of proper handwashing behavior</td>
<td>38</td>
<td>33.7</td>
<td>79.1</td>
<td>54.074</td>
<td>11.8987</td>
</tr>
<tr>
<td>The proportion of defecating behavior in healthy latrines</td>
<td>38</td>
<td>51.2</td>
<td>99.6</td>
<td>87.426</td>
<td>11.2948</td>
</tr>
</tbody>
</table>

Sanitation Access

Figure 1 showscatter plot of the prevalence of diarrhea in children under five and the percentage of coverage of access and clean water source in East Java Province in Indonesia in 2018. The figure indicates hubungan kedua variabel tersebut memiliki kecenderungan yang negatif. It means bahwa semakin tinggi the percentage of coverage of access and clean water source in a regency/city, maka semakin turun the prevalence of diarrhea in children under five in that regency/city.
Source: The 2018 Indonesia Basic Health Survey chapter East Java

Water supply is basic sanitation that must be fulfilled properly. The fulfillment of basic sanitation remains the best protection against diarrhea. Basic sanitation includes water supply, healthy latrine, waste and water disposal. Several previous studies at the individual level also found consistent results. Sources of clean water were found to be the factor that can reduce the incidence of diarrhea. Clean water supply continuously can reduce acute diarrhea among under-five children in slums. Clean water supply is a crucial factor for life. Clean water is needed by each individual for drinking and daily activities. For this reason, the supply of clean water plays a decisive role both in the survival of life and in preventing water-borne diseases such as diarrhea. The results showed that improvement in drinking water was associated with a decrease in the risk of diarrhea. Water supply associate with the incidence of acute diarrhea among children under five. The results show that improved water supply could reduce diarrhea in under-5 children by 11%.

Figure 2 shows scatter plot of the prevalence of diarrhea in children under five and the percentage of coverage of access to healthy latrines in East Java Province in Indonesia in 2018. The result shows the relationship between the two variables has a negative tendency. It informs that the higher the percentage of coverage of access to healthy latrines in a region, the lower the prevalence of diarrhea in children under five in that region.
The high level of access to healthy latrines in society makes feces as a polluting factor more easily localized. Feces is the main polluting factor that is often found in the environment. The distribution of feces in the environment needs to be controlled because it can become a medium for transmitting fecal-oral diseases such as diarrhea. Several studies have stated that the E. coli bacteria from feces often contaminate the environment like water sources. The data shows that there are 14% of countries in Europe and 52% of countries in Africa faecally contaminated. Several water sources in Indonesia are also proven to be contaminated. E. coli that comes from feces; Escherichia coli is a germ that exists in the digestive tract, so if the environment contains Escherichia coli, it can indicate that environmental contamination has occurred. Latrine cleanliness must be build up. Latrine can significantly reduce the incidence of diarrhea. One of the factors that influence the localization of feces depends on the type of toilet. Latrine type that is close with wastewater disposal, hand washing, and storage of water has a risk of diarrhea incidence \( p < 0.05 \). The presence of healthy latrine, water shortage in household consumption/water access at the individual level, water shortage in households had a statistically significant association with diarrhea occurrence in the Dangla district. Healthy latrine, include the availability of handwashing facilities around latrine, hand washing practice at the critical time for handwashing, storage of water were the determinant factors of diarrheal diseases.

Health Behavior (Hand Washing)

Figure 3 shows a scatter plot of the prevalence of diarrhea in children under five and the proportion of proper handwashing behavior in East Java Province in Indonesia in 2018. The scatter plot informs the relationship between the two variables has a negative tendency. It concludes that the higher the proportion of proper handwashing behavior in an area, the lower the prevalence of diarrhea in children under five in that area.

These findings further reinforce the results of previous studies that examined the same themes at the individual level. The habit of handwashing using soap in running water can reduce the risk of diarrhea. This is in line with previous research which said that The main sources of drinking water, domestic waste disposal place, and use of soap for handwashing were the most important factors for the prevention of childhood diarrhea. Hand washing practice have sig associate with diarrhea.
Figure 4 shows the bivariate analysis of the prevalence of diarrhea in children under five and the proportion of defecating behavior in healthy latrines in East Java Province in Indonesia in 2018. The figure informs that the relationship between these two factors has a negative tendency. It concludes that the higher the proportion of defecating behavior in healthy latrines in a regulation/city, the lower the prevalence of diarrhea in children under five in that regulation/city. In line with handwashing behavior, defecating behavior in healthy latrines is also a protective factor from the incidence of diarrhea.

Policymakers in East Java Province can use the results of this study to determine the target regency/city to be accelerated to reduce the prevalence of diarrhea in children under five. This regency/city is an area with low coverage in four respects, namely access and clean water sources, access to healthy latrines, proper handwashing behavior, and defecating behavior in the healthy latrine. The behavior of diarrhea prevention associated with knowledge \(^{28}\) so the level of knowledge in diarrhea prevention should be increasing to behavioral change.

The author conducted a study with an ecological analysis approach to capture the superficial phenomena needed by policymakers to reduce the prevalence of diarrhea in children under five in East Java, Indonesia. On the other hand, this study cannot capture specific local phenomena related to others’ behaviors. Some of them are behaviors that relate to the habit of feeding...
practices in children under five that was informed in previous studies 29–31.

**Conclusions**

Based on the research results, the study concluded that sanitation access and health behavior were related to the diarrhea prevalence in children under five. The percentage of access to clean water sources, the rate of coverage of access to healthy latrines, the proportion of proper handwashing behavior, and the proportion of defecating behavior in healthy latrines, were negatively related to the diarrhea prevalence in children under five.

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**Source of Funding:** Self-funding

**Ethical Clearance:** The study was conducted by utilizing secondary data from published reports. For this reason, the study not required ethical clearance in the implementation.

**Conflicting Interests:** Nil

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A Prospective Study to Determine the Timing of Complete Fusion of Spheno-Occipital Synchondrosis Using Computed Tomography

Hariprasad T. Nambiar¹, Aysha Mehanaz¹, Nitika C. Panakkal², Suresh S³, Visakh T²
¹Intern, ²Assistant Professor, Department of Medical Imaging Technology, Manipal College of Health Professions, Manipal Academy of Higher Education, Manipal, ³Assistant Professor, Department of Radio-diagnosis and Imaging, Kasturba Medical College, Manipal Academy of Higher Education, Manipal

Abstract

Background: The Spheno-occipital synchondrosis (SOS) has a significant role in the field of forensic, medical and anthropological sciences for age analysis. Therefore, SOS can help estimating age depending on different stages of closure. Therefore, the present study aimed to evaluate the timing of complete fusion of Spheno-occipital synchondrosis using computed tomography.

Methods: A prospective study including 110 subjects was conducted in which 55 males and 55 females between the ages of 15-25. All CT examinations were performed on a 128 slice incisive CT, Philips and 16- slice big bore CT, Philips. The sagittal image was used for analysis the stage of spheno-occipital fusion. Based on the stage of fusion of SOS, patients were categorized into stage 0 indicating partial/no fusion and stage 1 indicating complete closure of the SOS. An experienced radiologist scored all images.

Conclusion: The mean age of male cases with complete SOS closure was 20.3 ± 3 years for males and 20 ± 3.1 years for females. The study results show that by age of 20, no individual will belong to stage 0 irrespective of gender and all individuals would have completely fused SOS.

Keywords: Spheno-occipital synchondrosis, computerized tomography, age determination

Introduction

The Spheno-occipital synchondrosis (SOS) is a hyaline cartilage growth center joining the sphenoid and occipital bones and is largely present during the development of skull base and later gets ossified throughout maturation (¹). This has a significant role in facial development and the thorough examination of skeletal ossification plays an important role in the field of forensic, medical and anthropological sciences for age analysis. Therefore, SOS can help estimating age depending on different stages of closure. The fusion stage can help determine if the individual is minor as the SOS is undergoing fusion or considered as adults if the fusion is complete. As per literature, the SOS fuses between 11-16 years of age, however this remains controversial, as there are variable reports in literature of SOS fusion in individuals as late as 25 years (²-¹⁰). This variation of fusion reported in studies can be as a result of different methods of analysis employed, variation in population, sample sizes or age ranges (¹¹).
The various methods of analysis include dry skull method, histological sections, conventional radiographs, computed tomography, and magnetic resonance imaging. Conventional radiographs provide superficial information and therefore it is difficult to visualize areas of the skull due to superimposing structures. Although, direct inspection methods are cost-effective and the most commonly used method in forensic anthropology (11), computed tomography are high resolution and provide opportunities to perform three-dimensional investigation. Therefore, they can detect the state of fusion earlier due to superior visualization of skull base and is considered to have a greater accuracy in determining closure (4). Although a lot of studies have utilized SOS for age estimation, it is a well-known fact that standards vary in different populations and ethnic groups, there is a need for anthropological data that is specific to population (4, 12). Therefore the present study was aimed to evaluate the timing of complete fusion of Spheno-occipital synchondrosis (SOS) using computed tomography.

Materials and Methods

A prospective study including 110 subjects was conducted in a tertiary care hospital, Dakshina Kannada region. The study approval was obtained from the institute research and ethical committee, Kasturba hospital. The study population included 55 males and 55 females between the ages of 15–25. The participants included in the study were referred for CT scan of brain, spine, and temporal bone as part of their diagnostic package. Patients with congenital anomalies, developmental disorders and recent trauma were not included in the study. Informed consent was obtained from all patients and all the CT examinations were performed on a 128 slice incisive CT, Philips and 16 slice big bore CT, Philips. The patients were positioned in supine and placed head first. The scan was performed at 120 kVp and automatic tube current modulation. The image were acquired in 5 mm thickness and later reconstructed into thinner sections (1mm). The image was reformatted to sagittal plane using Multi Planar reconstruction technique and scored by an experienced radiologist. Based on the stage of fusion of SOS, patients were categorized into stage 0 and stage 1. Stage 0 indicated partial or no-fusion of SOS and stage 1 indicated complete closure of the SOS as shown in figure 1. The mean age of patients coming under each grade was calculated and noted for both genders.

![Stage 0 – No fusion](image1)

![Stage 1 – completely closed](image2)

Figure 1: Stages of SOS fusion in the mid sagittal plane
Statistical Analysis

The statistical analysis was done using R analysis. Age estimation in both genders as per spheno-occipital synchondrosis fusion stage was described in terms of mean, standard deviation, minimum and maximum values. Status of complete closure was compared in males and females using independent t test. To test the association between age and stage logistic-regression was used.

Results and Discussion

Among the 110 subjects included in the study, four male subjects of age 15 and 16 years showed partially closed Spheno-occipital synchondrosis, with the rest of them categorized into stage 1 indicating complete closure of SOS. The average age of male participants with open/partially fused suture was 15.5±0.5 years. In stage 0, the highest age of partially closed suture was 16 years. From the 110 subjects, 106 individuals showed complete fusion of spheno-occipital synchondrosis in which 51 were males and 55 were females. The mean age of male cases with complete SOS closure was 20.3 ± 3 years for males and 20 ± 3.1 years for females. The lowest age of complete suture closure was at 15 years for both males and females (table 1).

<table>
<thead>
<tr>
<th>AGE (years)</th>
<th>Stage 0 (Open/Partially closed)</th>
<th>Stage 1 (Completely fused)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n)</td>
<td>Females (n)</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
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<tr>
<td>18</td>
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<td>-</td>
</tr>
<tr>
<td>25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>15.5±0.5</td>
<td>-</td>
</tr>
</tbody>
</table>
The study results showed no significant difference (p= 0.9) among gender in stage 1 of complete SOS closure. To evaluate the effect of age on fusion stages, logistic regression was performed. The test gave an odds ratio of 10.4 indicating that as the age increases by one unit the chance of getting stage one is 10 times more than the stage 0. The study results show that by age of 20, no individual will belong to stage 0 irrespective of gender and all individuals would have completely fused SOS.

The study on SOS development and its important role in age estimation is been studied over the years. By estimating the fusion of the joint, it is possible to determine the age of an individual that will be helpful in solving criminal activities like sexual offenses, child labour. The late stage of fusion of this particular joint has its own significance in forensic analysis as well as in medical and anthropological fields (5). The objectives of this research was to identify the time of complete-fusion of SOS in both males and females. As per the mean age of our study, we found there was complete SOS at 20.3 ± 3 years for males and 20 ± 3.1 years for female.

Similarly, a study conducted by Rajeshwar et al also showed similar results. His study included 198 samples within the central Indian population in which 117 were males and 81 were females. The study reported that SOS closure occurs at a mean age of 20.2 and 21.4 year among females and males respectively (8). The mean age of fusion can vary according to the population as well as genders. Although our study did not show a significant variation in mean age for SOS closure among genders, a notable amount of studies report variation in mean age of complete SOS closure between males and females (3, 7, 10, 11, 13). However, a study conducted by Richard B et al reported complete sphenoid occipital fusion by the age 17 years for both genders (4). He also reported that after an age of sixteen years there was no remarkable change in the progression of the fusion among genders. This was similar to the present study, which showed no significant difference of SOS fusion progress after an age of sixteen years in males and fifteen years in female.

Table 2 demonstrates summary of these research along with the present study showing age range, number of participants and mode of analysis for complete fusion of sphenoid-occipital synchondrosis.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Mode of Analysis</th>
<th>Sample size(n)</th>
<th>Location</th>
<th>Mean age of complete fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richard B</td>
<td>2010</td>
<td>CT</td>
<td>458 208</td>
<td>Australia</td>
<td>16-17 16-17</td>
</tr>
<tr>
<td>Alper Sinanoglu</td>
<td>2015</td>
<td>Cone Beam CT</td>
<td>90 148</td>
<td>Turkey</td>
<td>20 18</td>
</tr>
<tr>
<td>Rajeshwar Sambhaji</td>
<td>2018</td>
<td>Direct inspection</td>
<td>117 81</td>
<td>Central India</td>
<td>21.4 20.2</td>
</tr>
<tr>
<td>Ismail Ozgur</td>
<td>2013</td>
<td>CT</td>
<td>399 139</td>
<td>Turkey</td>
<td>20.3 18.2</td>
</tr>
<tr>
<td>Mitra Akhlaghi</td>
<td>2010</td>
<td>Direct Inspection</td>
<td>190 186</td>
<td>Tehran</td>
<td>21.1 19.7</td>
</tr>
<tr>
<td>Salina Hisham</td>
<td>2018</td>
<td>CT</td>
<td>336 164</td>
<td>Malaysia</td>
<td>20.84 19.78</td>
</tr>
<tr>
<td>Oguzhan Ekizoglu</td>
<td>2016</td>
<td>MRI</td>
<td>455 623</td>
<td>Istanbul, Turkey</td>
<td>18.4 17.7</td>
</tr>
<tr>
<td>Present study</td>
<td>2019</td>
<td>CT</td>
<td>55 55</td>
<td>Dakshina Kannada, India</td>
<td>20.91 20.84</td>
</tr>
</tbody>
</table>
The differences in the results can be due to methodological difference used for determination of SOS, also differences in population, selection of sample size, socio-economic status are factors that could influence the result. The present study evaluated the SOS closure using computer tomography. However, a lot of studies have been done to estimate age of SOS closure using direct inspection methods in cadavers, cone beam CT, MRI, X-ray etc. Our study is also in accordance with various studies in literature that report an interdependence of age with stage of closure. The study demonstrated that, by the age of 18 years the SOS is complete for both males and females as no notable progression occurs in SOS closure after 17 years of age. Therefore, an open suture or an incomplete SOS can indicate that the individual may be less than 18 years. Further population specific studies can be done to estimate age of unknown individuals.

The current study did have certain limitations, as it was a time-bounded study; the obtained sample size was small. The present study only investigated timing of closure of spheno-occipital synchondrosis, which as shown in the study occurs earlier than expected in females, therefore, further studies including a larger population involving younger children (7-14 years) is recommended.

**Conclusion**

As there is no significant progression in SOS fusion seen after an age of 15-16 years in both females and males, we can conclude that male subjects with open/semi closed suture will be below the age of 16 years and females with open/semi closed suture will be less than 15 years. In addition, the study indicates that SOS fusion stages increases with age. Therefore, it can be helpful in prediction of age.

**Ethical Clearance:** Institute ethical Committee, KMC, Manipal

**Conflict of Interest:** Nil

**Funding:** There no external funding for this research project as this project was conducted within the institute itself that provided us with all facilities required to conduct the project.

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Autism Spectrum Disorder A Review

Harshith N1, Kanakavalli K. Kundury2

1Assistant Professor, 2Department of Health System Management Studies, JSSAHER, Sri Shivarathreeshwara Nagara, Mysuru, Karnataka, India

Abstract

Autism Spectrum Disorder issue (ASD) infers a degree of conditions depicted by some level of debilitated social lead, correspondence and language, and an obliged degree of interests and exercises that are both unprecedented to the individual and did slowly. ASDs start in youth and will when everything is said in done proceed into energy and adulthood. An incredible piece of the time the conditions are clear during the hidden 5 years of life. It is assessed that overall one of every 160 youngsters has an ASD. This gauge speaks to a normal figure, and revealed commonness fluctuates significantly across contemplates. Some very much controlled investigations have, notwithstanding, revealed figures that are significantly higher. The predominance of ASD in some low-and centre salary nations is so far obscure People with ASD a great part of the time present other co-happening conditions, including epilepsy, incapacitation, weight and thought misfortune hyperactivity issue (ADHD). Intervention during early childhood is imperative to advance the ideal turn of events and prosperity of individuals with an ASD. Observing of kid advancement as a feature of routine maternal and youngster medicinal services is suggested.

Key Words: Autism Spectrum Disorder, debilitated, epilepsy, adulthood

Introduction

Autism Spectrum Disorder issue is a neurological and developmental issue that begins taking everything into account in youth and drives forward through all through a person’s life1. It impacts how an individual shows and teams up with others, permits, and learns. It joins what used to be known as Asperger condition and unavoidable developmental issues2.

A genuine formative issue that disables the capacity to convey and associate. Chemical imbalance range issue impacts the sensory system and influences the general intellectual, enthusiastic, social and physical wellbeing of the influenced person. The range and seriousness of side effects can fluctuate generally. Basic side effects incorporate trouble with correspondence, trouble with social communications, over the top interests and redundant practices.

Early acknowledgment, just as conduct, instructive and family treatments may decrease side effects and bolster advancement and learning.

It is known as a “run” issue since people with ASD can have a level of signs. People with ASD may have issues talking with you, or they evidently won’t take a gander at you without bouncing when you visit with them3. They may correspondingly have restricted interests and dreary practices. They may contribute a huge amount of noteworthiness overseeing things, or they may impart a relative sentence again and again. They may typically have all the stores of being in their “own existence.”

Corresponding Author
Harshith N
Assistant Professor, Department of Health System Management Studies, JSSAHER
Sri Shivarathreeshwara Nagara, Mysuru - 570 015, Karnataka, India, E mail:harshith.dhsms@jssuni.edu.in
Contact:8618423152
At well-kid tests, the social protection provider should check your youth’s new development. If there exhibit ASD, your child will have a broad evaluation. It may merge a party of bosses, doing various tests and evaluations to make an end.

**SIGNS AND SYMPTOMS**

People diagnosed with ASD have issues with social Communications and Interactions, restricted interests, and excess activities. The summary underneath gives a couple of cases of the sorts of practices that are found in people resolved to have ASD. Not all people with ASD will show all practices, anyway most of the children exhibits at least two symptoms.

**Social correspondence/affiliation practices may include:**

Coming to, Tending not to look at or take a gander at people, Once in some time sharing fulfillment in articles or activities by pointing or showing things to others, Neglecting to, or being surrendered to, respond to someone calling their name or to other verbal undertakings to get thought, Experiencing issues with the forward and in converse of conversation, Regularly talking at long last about a most esteemed subject without seeing that others are not interested or without permitting others to respond, Having outward appearances, redesigns, and advancements that don’t organize what is being communicated, Having a strange technique for talking that may sound sing-song or level and robot-like, Experiencing burden understanding another person’s viewpoint or being not set up to envision or appreciate others’ exercises.

**Prohibitive/dull practices may include:**

Reiterating certain practices or having puzzling practices. For example, rehashing words or verbalizations, a lead called echolalia, Having a mulling outrageous fervour over unequivocal subjects, for instance, numbers, nuances, or certifiable segments, Having incredibly focused interests, for instance, with moving articles or parts of things, Getting induced with slight changes in a bit by bit plan, Being in every way that really matters tricky than others to obvious data, for instance, light, unrest, dress, or temperature, Individuals with ASD may in like way experience rest issues and irritability. Notwithstanding the way that people with ASD experience various challenges, they may in like way have various characteristics, including, Having the decision to learn things in detail and review information for far reaching time frames, Being strong visual and hear-capable understudies Surpassing needs in math, science, music, or craftsmanship.

**Causes and Risk Factors**

While specialists have no idea about the particular explanations behind ASD, research suggests that properties can act near to impacts from nature to influence development in penchants that lead to ASD. Despite how experts are so far attempting to comprehend why a few people make ASD and others don’t, some danger factors include:

- Having a family with ASD
- Having more masterminded guardians
- Having certain inherent conditions—Down disorder, delicate X disorder, and Rett disorder are almost certain than others to have ASD

**Diagnosis**

An ASD assessment joins a reality orchestrating, which is used to show how much assistance kids need: Level 1 – kids need support, Level 2 – kids need stunning assistance, Level 3 – kids need monster help. These rankings reflect the way that a few people have smooth ASD appearances, and others have more authentic symptoms. The rating is given autonomously for the two zones of difficulty, so youngsters may have gathered reality rankings for social difficulties and dull practices.

**Medications and Therapies**

Treatment for ASD should begin as snappy as time grants after end. Early treatment for ASD is basic as sensible thought can diminish individuals’ difficulties.
while helping them learn new cut off points and piece of breathing space at any rate much as could be ordinary from their properties.

The wide level of issues standing up to people with ASD suggests that there is no single best treatment for ASD. Working before long with a force or therapeutic associations capable is a fundamental bit of finding the right treatment program11

Ethical Clearance: As the Manuscript is a Review article hence it does not require any ethical clearance

Source of Funding: Nil

Conflict of Interest : Nil

References


Knowledge of Healthcare Providers toward New Corona Virus at Directorate of Military Medical Affairs Units

Muhammed Hussein Ali¹, Wasnaa Jomaa Mohammed²

¹MScN(C), University of Baghdad, College of Nursing, Community Health Nursing Department, Baghdad, Iraq, ²Assistant Professor, University of Baghdad, College of Nursing, Basic Sciences Department, Baghdad, Iraq

Abstract

Background: Coronavirus is a rapidly spreading disease also known as COVID-19. The epidemic is caused by a new human coronavirus, previously known as (SARS-COV-2), new coronavirus disease it first appeared in December 2019 among patients who had symptoms of viral pneumonia in Wuhan, China. This study aims to determine healthcare providers’ knowledge related to coronavirus.

Methods: A descriptive design is carried throughout the present study Directorate of Military Medical Affairs Units (Al- Muthanna Military Hospital, Al-Hussein military Hospital, Al-Shaheed Mubder Military Clinic, Al-Naser Military Clinic, Military Medical School) for the period from January 28th, 2021 to February 25th, 2021. The study included a non-probability “purposive” sample of (223) health care providers. The questionnaire encompasses two main parts (Health Care Providers Socio-Demographic Characteristics, and Health Care Providers’ knowledge’s Tool).

Data were analyzed using the statistical package for social science. The descriptive statistical measures of frequency, percent, mean, Relative Sufficiency, Percentile Grand Mean of Score, Percentile Global Mean of Score, Pooled Standard Deviation, and Grand/or Global Relative range, standard deviation, and inferential statistical measures of T-test, Chi-Square test, Binomial test, Mann-Whitney test, Contingency Coefficients test, and Wilcoxon Signed Ranks test.

Results: The study results showed that half of the study participants depend on their information about the studied diseases on the “Center for Disease Control of the World Health Organization”, then 158 (70.9%) depend on government websites and official media, then 109 (48.9%) depend on news media about 148 (66.4%) rely on Social media. About 26(11.7%) rely on Journals” only.

Conclusion: The researchers concluded that the overall evaluation of healthcare providers’ knowledge is moderate.

Recommendation: The researchers recommend that there is a need to conduct further studies on larger sample at the national level with the need for an educational program to increase the knowledge of health care providers about Corona virus in military medicine.

Keywords: Coronavirus; Healthcare Providers, Knowledge

Introduction

Coronavirus is a rapidly spreading disease also known as COVID-19. The epidemic is caused by a new human coronavirus, previously known as (SARS-COV-2), new coronavirus disease it first appeared in
December 2019 among patients who had symptoms of viral pneumonia in Wuhan, China (1-2).

The first cases of pneumonia of unknown source were discovered in Wuhan, capital of Hubei Province, in early December 2019, the pathogen was identified as the new, enveloped beta-corona RNA virus. This is now called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is fairly similar to SARS-CoV. It was found linked to the Huanan seafood market in Wuhan, Hubei Province, China, where Aquatic animals were also sold before the outbreak (3-4).

The disease highly contagious, its main clinical manifestations include fever, dry, weakness, muscle pain and shortness of breath, which characterized by acute respiratory distress syndrome, septic shock, difficult-to-treat metabolic acidosis, bleeding and dysfunction. The incubation period for a novel coronavirus infection is about 2-5 days, after which symptoms begin to appear, however, this period varies according to the immune system and the age of the patient (5-6).

Health care systems in first world countries have failed to provide medical care due to the rapidly increasing numbers of infected patients, not to mention the developing or underdeveloped countries. In most cases, the initiative and management in the various countries appeared inconsistent, wasted, ill-equipped, and inadequate to contain the disease. Unprecedented in history, the active participation of everyone on earth, through testing, confinement, contact follow-up, social segregation, staying home, self-isolation, improving individual hygiene and using individual defense equipment, for example, blankets and gloves, is essential to containing COVID-19, prevent medical service workers from overcoming it, and give specialists time to establish treatment methodologies (7).

To control Outbreaks, China has taken some strict measures includes complete closure of public places, Public transportation and isolation of suspected cases. Authorities have locked down the entire Hubei province for months and days. Residents inside and outside Hubei Province are being asked to stay home and practice self-isolation to avoid any physical contact with others. Fight against the epidemic continues in China as well as around the world (8).

Many millions have sacrificed their self-sufficiency, well-being, position, jobs, transformation, and education. However, deliberate self-cooperation methods of responding to COVID-19 have led to difficulties in various countries due to changing information degrees and perspectives. As needed, the plan and implementation of antiviral activities is based on a complete and microscopic understanding of separate regions and within each country (7).

Around the world, the main challenges lie in providing the right services Caring for COVID patients and preventing the spread Infection among health care workers and the public General. Appropriate preventive measures for COVID-19 Infection are not effectively carried out both places and adherence to them is inconsistent (9).

In addition, implementation of the updated guidelines brings a host of communication challenges and guidance, availability of resources, access to equipment, and practice in using that equipment, simulation sessions and a willingness to participate in the application of those directions. These factors influenced by the knowledge and attitudes of health care providers regarding the disease and updated guidelines to be provided for their routine work (10).

Materials and Methods

A descriptive design was used to guide this study. The study included a purposive sample of 223 healthcare providers. The study was conducted at the Directorate of Military Medical Affairs Units (Al-Muthanna Military Hospital, al-Hussein military Hospital, Al-Shaheed Mubder Military Clinic, Al-Naser Military Clinic, Military Medical School) for period of from of January 28th, 2021 to February 25th, 2021. Data were collected through a self-reported questionnaire using the structured interview as a way of data collection. The questionnaire encompasses two main parts as follows:
**Part I:** healthcare providers’ sociodemographic characteristics: This part includes items of Workplace, gender, age, service Years, Occupation, level of education, have you participated in a course on methods of preventing the emerging corona virus, Duration of the course and socioeconomic status which are calculated through use Testing based on One-Sample Chi-Square test, and Binomial test.: where Non-significant at P>0.05, Significant at P<0.05 and Highly significant at P<0.01

**Part II:** healthcare providers’ knowledges: This part is comprised of 12 items that measure knowledge of healthcare providers’ knowledge which are measured as [(0.00 – 33.33) for Low (L) evaluation; (33.34 – 66.66) for Moderate (M) evaluation; and (66.67– 100) for High (H) evaluation].

The content validity and Pearson correlation coefficient reliability were determined through a pilot study.

**Data Analyses/Statistics**

Data were analyzed using the statistical package for social science (SPSS) ver. (21.0). The descriptive statistic measures of frequency, percent, mean, and standard deviation and inferential statistical measures of T-test, multiple linear regressions, person correlation coefficient, Chi-Square, and analysis of variance (ANOVA) were used.

**Results**

**Table 1. Participants’ distribution according to the source of information on the new Coronavirus**

<table>
<thead>
<tr>
<th>Source of information</th>
<th>Response</th>
<th>No.</th>
<th>%</th>
<th>C.S. P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>World Health Organization, CDC</td>
<td>No</td>
<td>112</td>
<td>50.2</td>
<td>P=1.000 (NS)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>111</td>
<td>49.8</td>
<td></td>
</tr>
<tr>
<td>Official government websites and media</td>
<td>No</td>
<td>65</td>
<td>29.1</td>
<td>P=0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>158</td>
<td>70.9</td>
<td></td>
</tr>
<tr>
<td>News media</td>
<td>No</td>
<td>114</td>
<td>51.1</td>
<td>P=0.789 (HS)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>109</td>
<td>48.9</td>
<td></td>
</tr>
<tr>
<td>Social media</td>
<td>No</td>
<td>75</td>
<td>33.6</td>
<td>P=0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>148</td>
<td>66.4</td>
<td></td>
</tr>
<tr>
<td>Journals</td>
<td>No</td>
<td>197</td>
<td>88.3</td>
<td>P=0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>26</td>
<td>11.7</td>
<td></td>
</tr>
</tbody>
</table>

(*) HS: Highly Sig. at P<0.01; Testing based on Binomial test.

There are significant differences between observed distribution with their expected outcomes in each source of information, except with “World Health Organization, CDC” source.

A half of participants rely on their information regarding Covid-19 on “World Health Organization, CDC”, followed by 158(70.9%) who rely on their information on “Official government websites and media”, followed by 109(48.9%) who rely on “News...
“Social media”, then followed with 148(66.4%) rely on their information regarding studied disease on “Social media”, followed by 26(11.7%) who rely on “Journals” only.

**Table 2. Participants’ distribution according to the (SDCv.) with comparisons significant**

<table>
<thead>
<tr>
<th>General (SDCv.)</th>
<th>Groups</th>
<th>No.</th>
<th>%</th>
<th>C.S. P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>182</td>
<td>81.6</td>
<td>P=0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>41</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>Age Groups (Years)</td>
<td>20 _ 29</td>
<td>116</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 _ 39</td>
<td>76</td>
<td>34.1</td>
<td>(\chi^2= 134.543) P=0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>40 _ 49</td>
<td>26</td>
<td>11.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 _ 59</td>
<td>5</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>31.42 ± 6.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td>Dresser</td>
<td>63</td>
<td>28.3</td>
<td>(\chi^2= 24.345) P=0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>Nurse</td>
<td>31</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pharmacist</td>
<td>30</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dentist</td>
<td>25</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Laboratory</td>
<td>37</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Physician</td>
<td>37</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td>Level of education</td>
<td>Fast course</td>
<td>63</td>
<td>28.3</td>
<td>(\chi^2= 248.816) P=0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>Diploma</td>
<td>12</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. Sc.</td>
<td>130</td>
<td>58.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M.Sc.</td>
<td>9</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ph.D.</td>
<td>9</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Years of Service</td>
<td>&lt; 1</td>
<td>24</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-5</td>
<td>111</td>
<td>49.8</td>
<td>(\chi^2= 143.076) P=0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>6-10</td>
<td>45</td>
<td>20.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11-15</td>
<td>37</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 16</td>
<td>6</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Have you participated in a course on methods of preventing the emerging corona virus</td>
<td>No</td>
<td>179</td>
<td>80.3</td>
<td>P=0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>44</td>
<td>19.7</td>
<td></td>
</tr>
<tr>
<td>Duration of the course Days</td>
<td>Non applicable</td>
<td>179</td>
<td>80.3</td>
<td>(\chi^2= 13.818) P=0.001 (HS)</td>
</tr>
<tr>
<td></td>
<td>1-5</td>
<td>16</td>
<td>36.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-10</td>
<td>24</td>
<td>54.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 10</td>
<td>4</td>
<td>9.09</td>
<td></td>
</tr>
</tbody>
</table>
Testing based on One-Sample Chi-Square test, and Binomial test.

Regarding gender, most of participants were males (n = 182; 81.6%), “Age Groups” are focusing at the first and second classes (i.e. 20 – 39) yrs., since they accounted 192(86.1%), with mean and standard deviation 31.42, and 6.92 yrs. respectively, “Occupational status” showed that “Dresser” staff were formed twice the number of the leftover providers, more than half of studied sample had “B.Sc.” degree, and they are accounted 130 (58.3%), “Years Service”, showed that half of studied providers had “1-5 years, and they are accounted 111(49.8%), most of them did not take the courses on Coronavirus disease, and finally who were taken the courses their cycle times were mostly short, since ranged from one to ten, and they are accounted days 40(90.91%).

<table>
<thead>
<tr>
<th>Table 4. Participants’ knowledge about Coronavirus at directorate of military medical affairs unit’s</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Items</strong></td>
</tr>
<tr>
<td>Clinical symptoms of the emerging Coronavirus are:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Early detection of infection with the emerging Coronavirus can help treat the infected:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
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<tr>
<td>People who become seriously ill with the emerging Coronavirus:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>The emerging corona virus is transmitted mainly through:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>The emergence of one of the symptoms of the emerging corona virus (such as fever, dry cough, shortness of breath) with contact with a confirmed infection within 14 days:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>The incubation period for the emerging corona virus is:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Replace the medical mask when:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>It is necessary to protect the eyes when dealing with people infected or suspected of being infected with the emerging Coronavirus, in order to:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>One of the important ways to prevent infection with the emerging corona virus:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Hand disinfection is done by using:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Medical protective equipment used on infected or suspected persons is disposed of as such:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>In the absence of places for isolation, it must take place:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Ev. = Evaluated (0.00 – 33.33) Low (L); (33.34 – 66.66) Moderate (M); (66.67– 100) High (H).

The observed responses of high evaluating assigned are accounted 4(33.33%) items, and responses of assigned a moderate evaluate are accounted 3(25.0%) items, while the leftover items were assigned a low evaluation, and accounted 5(41.67%), through using differentiated intervals, such that: [0.00 – 33.33) for Low (L) evaluation; (33.34 – 66.66) for Moderate (M) evaluation; and (66.67– 100) for High (H) evaluation.

For summarizes preceding results, it could be conclude that “Providers’ Knowledge towards” coronavirus at directorate of military medical affairs units items were non assigned at the established level in which that achieving the goal of this study, also results observed concerning estimates of the percentile grand mean of score due to knowledge main domain having a moderate evaluating

Discussion

The source of information for half of participants about Covid-19 is that they rely on their information on the “World Health Organization, CDC”, followed by governmental websites “official” and the media, followed by the media, the social media, and finally journals only.

This result may be due to the prevalence of the Internet and the ease of access to the WHO website, the Centers for Disease Control and Prevention, official government websites, the media, social media sites and magazines. This finding contradicts a study conducted by researchers from the United Arab Emirates, India, and Iran who stated that the main sources of participants’ information are official government websites Social media, with about 30% of them saying they used the news Media (TV/video, magazines, newspapers, radio) and social media (Facebook, Twitter, Whatsapp, YouTube, Instagram and Snapchat) for information about COVID-19. Moreover, nearly 40% of respondents occasionally discussed topics related to COVID-19 with family and friends.\(^{11}\)

Concerning participants’ knowledge, most have a moderate knowledge. This result may be due to the fact that the epidemic is modern and occurred suddenly, which caused confusion in most health systems in the world, not only Iraq, in addition to the lack of scientific resources related to the prevention of this disease and the frequent updating of information related to this disease.

This finding is consistent with a study conducted by Al-Thaqafi et al., 2020 in terms of lack of knowledge about the emerging corona virus\(^ {12}\).

Conclusion

The researchers concluded that the overall evaluation of knowledge healthcare providers is moderate.

Recommendations

The researchers recommend that more research be conducted at the national level on the size of a large sample of health care providers, with the need for an educational program to increase the knowledge of health care providers about Corona virus in military medicine.

Conflict of Interest: The researchers confirm that there is no any conflict of interest.

Source of Funding: This study is self-funded.

Ethical Clearance: The researchers obtained the ethical approval from the University of Baghdad, College of Nursing

References


Gene Expression and Antioxidant Effects of Cinnamon (Cinnamomum Zeylanicum, Breyne) in Alloxan-Induced Diabetic Rats

Hayder Ghazi Abdulshaheed¹, Haidar K.A. Alsaedi²

¹Associate Professor, Department Animal Production, Agriculture College, Al-Qadisyah University, Iraq,
²Assistant Professor, Department of Basic Science, Faculty of Dentistry, Al-Qadisyah University, Iraq

Abstract

Background and Aim: In this study rats, a systematic analysis was conducted to examine the antioxidant properties of cinnamon (Cinnamomum Zeylanicum, Breyne) in controlling high blood sugar levels, and a pharmacological comparison made to investigate whether or not it had a positive effect on the antioxidant system.

Materials and Methods: Alloxan 60 mg injected into the marginal ear veins of eighteen diabetic rats. After this diabetic animal study, the rats were randomly assigned to 4 groups. There were six animals in each of the 4 groups: Group 1(C): Positive control, Received orally 5 ml normal saline (0.9% NaCl) daily. Group 2(D): Negative control, Diabetes rats only, received single-dose alloxan 60 mg/kg body weight. Group 3(K): Diabetes rats, received cinnamon orally 35 mg/kg body weight dissolved in 2 ml normal saline. Group 4(I): Diabetes rats, was given 2 I.U/animal of insulin subcutaneously daily.

Results: All animal groups were treated for four weeks. Blood samples were taken from these groups weekly for biochemical analysis to estimate: Blood glucose, malondialdehyde (MDA), Glutathione (GSH). The results showed high glucose and MDA concentration associated with an increased oxidant stress alloxan induces on diabetic animals. The statistical analysis showed that a cinnamon significant (P<0.05) reduction in glucose, MDA, GSH when compared to positive control. The level of MDA, GSH was also significantly reduced (P<0.05) in all period comparison with the period before treated with Cinnamon extract. There was 4.3-fold more Ins I upregulated in the Cinnamon -treated diabetic rats 35 percent of the control group were heterozygous and three percent of the experimental group were mutant for that trait.

Conclusion: Furthermore, Cinnamon-treated diabetic rats (group K) showed a significant increase in the activities of both enzymatic and non-enzymatic antioxidants with concomitant overexpression of insulin I genes when compared to diabetic control rats (group D). Cinnamon contains antioxidants and antihyperglycemic effects at the end of the experiment.

Keywords: Cinnamon, Antioxidant capacity, Diabetes mellitus, INS I gene.

Introduction

As part of normal physiological metabolism, small amounts of ROS and other reactive oxygen species (ROS) and other reactive oxygen species, also known as ROSs or ROS/ROSs) are constantly generated. An oxygen intermediate reactant; When ROIs, Reactive Oxygen Species, are partially reduced, the result is that they are transformed into atmospheric oxygen (O2). For redox reactions, they generally lead to the formation of single oxygen or to the transfer of one or two or three electrons to a superoxide radical (which yields O2 and HO)¹. There is a class of antioxidants called “non-enzymatic” that simultaneously removes reactive and “nonenzymic” antioxidants. The oxidation of polyunsaturated fatty
acids compromises food quality and can lead to damage to cell membranes, heart disease, and cancer\textsuperscript{2}. The human body is damaged by oxidative stress, which increases the risk of disease development and accelerates its progress\textsuperscript{3}. Damage from free radicals and reactive oxygen species has been studied and has been proven to play a role in atherosclerosis \textsuperscript{4}. The antioxidants in food have been used to increase shelf life and reduce wastage and nutritional losses due to oxidation, which means the antioxidants in food have become quite popular as a countermeasure against spoilage \textsuperscript{5}. 2,4- and 6-Di-t-butyl-caffeatechinol are commonly used in the food industry as synthetic antioxidants. Regardless, there are serious questions about the carcinogenic nature of these substances, and natural alternatives are sought after\textsuperscript{6}. As a result, significant efforts are being made to determine natural antioxidants with the ability to fight cancer and age-related diseases. Various natural antioxidants are obtained from all parts of plants, for example, fruits, vegetables, roots, and bark\textsuperscript{7}. Plant phenolics can neutralize free radicals (quench free radicals), as well as single- and double-oxygen agents (radical scavengers). Studies show that the correlation between phenolic food and beverage intake and reduced risk of heart disease\textsuperscript{8}. Flavonoids, coumarins, coumarins, and polyunivalent hydroxyacids are the most commonly occurring plant phenolic antioxidants\textsuperscript{9}. Several techniques have been employed to assess the antioxidant ability of a given substance. It is more commonly used for its accessibility, efficiency, speed, and sensitivity when referring to chromogenic antioxidants. The presence of the antioxidant allows these two widely used radicals, ABTS and DPPH, to vanish. Both ABTS and DPPH are acquired radicals that are enzymatically or chemically formed\textsuperscript{10,11}. Antiallergic, antiulcerative, analgesic, antidiarrheumatic, and anodyne properties\textsuperscript{12}. Cinnamaldehyde and eugenol are present in the bark oil. In short, in many biological activities, antitumorigenic, and anti-cancer activities, cinnamaldehyde has been said to have strong vasodilatory, antifungal, and cytotoxic properties\textsuperscript{13,14,15}.

**Methodology**

**Experimental animal**

In the experiment mature male rats, Sprague-Dawley was used. Male rats had to grow accustomed to the environment of the animal house for 4 weeks before beginning the experiment. Rats were fed all and drinking water ad libitum. Room temperature was kept at 23± 2 °C.

**Preparation of n-butanol**

cinnamon (Cinnamomum Zeylanicum, Breyne) was extracted from the local store and graded by the State Seed Testing and Identification Board. Ministry of Agriculture supplied 1 kg of a celery plant. Methanol extract dried, rotavaporated (40 °C and 50 to 60 rpm). lyophilized by a dry freezer. The dried extract had been measured and put under extreme conditions of congelation. Three forms of polarity-dependent solvents have been found\textsuperscript{16}.

**Induction of diabetes in rats**

32 mature male Sprague-Dawley rats. rated between 150 and 200 g (50 days old) for diabetes injection\textsuperscript{17}. Rats were injected with alloxan 60 mg/kg body weight in the marginal vein of the ear\textsuperscript{18}.

**Design of Experimental**

The rats were accordingly divided into 4 groups were fed on each group = 6 rats: Group 1(C): Positive control, Received orally 5 ml normal saline (0.9% NaCl) daily. Group 2 (D): Negative control, Diabetes rats only, received single-dose alloxan 60 mg/kg body weight. Group 3(K): Diabetes rats, received cinnamon orally 35 mg/kg body weight dissolved in 2 ml normal. Group 4(I): Diabetes rats, was given 2 I.U/ animal of insulin subcutaneously daily.

**Collection of Blood Samples:**

Blood samples were taken weekly from the insertion site, and the blood was placed in test tubes the following week. At 3,000 revolutions per minute, the blood was
centrifuged to break up the clots. Before the serum being stored at 20 degrees Celsius, the extract was extracted and bottled before freezing. Testers collected a sample after there had been no change in glucose and other chemical components after two days without changing anything.

**Determination of Malondialdehyde (MDA) concentration:**

Thiobarbituric acid (TBA) coupled with lipid peroxidation (MDA). The blood was centrifuged at 3,500g for 30 minutes, with the red cells removed (New). The RBCs were cooled to the homogenization buffer (pH 7.4, 1 M), and FCCl (1.7% w/v) was added. A lot of the supernatant centrifugate (10% homogenate) was taken out of the final homogenate (100-fold) volume. Xo.5 ml of 30% TCA and 0.2% TBA were then poured into it. The tubes were submerged in water in an agitated for 30 minutes at 80 degrees Incubation was carried out in ice-cold water for ten minutes. These were then separated by centrifugation at SOQ in fifteen minutes. The supernatant’s absorbance was found to be 540 nanometers. The test results of the MDA concentration were obtained using the standard calibration curve of tetrapropylene. Protein was estimated by the method of extraction and partial hydrolysis.

**Determination of total Glutathione (GSH) activity**

The estimated GSH content was calculated using the method.

**Realtime-polymerase chain reaction**

per the manufacturer’s directions (Qiagen, Courtaboeuf, France). Total RNA was extracted from the gel and eluted with a 35-fold greater amount of RNase-free water than RNase. Residual genomic DNA was incubated with 15 units of the DNase I-free MgCl with 90 °C for 10 minutes to in 2 mM of magnesium chloride (MgCl) to inactivate the DNase I. 20 µl of the DNase-treated RNA solution containing 50 mM Tris-HCl (pH 8.3), 10 mM dithi0.5 mg of blood protein concentrate, 3 mM Mg acetylated deoxyNTP, 30 units of Moloney Murine-MLV RTase free RNA, 200 units of M-MLV reverse transcriptase (Promega, Madison, WI), and 3 U of RNase-free RNase H. It was difficult to normalize the reverse transcription to equalize total RNA in both the beginning and the results. For 10 minutes at 26 °C, then for 45 minutes at 42 °C, followed by 3 minutes at 90 °C, and then left to cool to return to room temperature. To inactivate the reverse transcriptase, an additional 300 units of the enzyme were incubated at 42°C for 45 minutes, after which it was shifted to 75° for the required time required to complete the process. DNA controls for the RT procedure and negative controls were run in parallel samples. The cDNA samples were stored at a temperature of -80 degrees Celsius. Using all the same cDNA samples throughout the study allowed the researchers to gain complete confidence in their results. Primers for ratInsulin-1were forward (fw), 5’-TCAGCAAGCAGGTCAATGCC-3’, and reverse (rv), 5’-GGTGCAGCCTGATCCACAATG-3’. PCR conditions were 35 cycles of initial denaturation y’’’C for 5rain,95°Cfor45s,60°Cfor 30 s,72°Cfor 1 min, and, finally, 72°C for 5 min; viability of the RT ;>rr;duci was controlled by a separate PCR withprimers specific for the housekeeping mRNA (GAPDH) (fw: 5’- TGAACGGATTTGGCCGTATTGGGC3’;rv:5’- TCTCCTGGGTGGCAGTGGCATGCGAT-3’). conditioning for 3 min, then 25 cycles of denaturation, annealing at 95° for 45 seconds, 60° for 45 seconds,
and annealing for 1 minute at 72°C, the PCR was used to prove a theory. At 72°C, and, followed by 5 minutes, then 102°C Using 1.5% agarose gel, the electrophoresisilatated PCR products were run through it Real-standardized PCR performed with the Quantiect Green PCR (Qiagen) involved the use of Opticon-2 PCR machine (MSKIN965), with plain white 965 on the controls (MJ Research). The appropriate annealing temperatures for each primer type were also established by using PCR gradients.

The Statistical Analyses

The study was conducted with a one-wise robust analysis of covariance (1-way) except for the Malondialdehyde “S” test. We used the program for statistical analysis. Thus, the results of the data were presented as X ± two standard deviations. seems to perform the least significant differences (group ANOVA, LSD) (2016).

Results

Bodyweight gain

On the third day, results of daily weight clarification revealed significant differences (P<0.05) between the diabetic and normal control groups, but the differences disappeared with time. In contrast, the statistical comparison revealed that there were no significant overall differences in the three groups of diabetics’.as in (fig.1).

![Graph showing bodyweight gain](image)

Fig.1: The action of cinnamon (CinnamomumZeylanicum, Breyne) on Bodyweight gain in alloxan—induced mature male rats with diabetics. The mean values are ±S.D. (p < 0.05) .
Blood glucose

On The Fifth day, glucose in the diabetic rats has been checked for relevance. The research found that male rats given cinnamon and treated with insulin fared best. However, the normal levels of blood glucose in the control rats is higher than that in the experimentally treated rats. It was shown on the other hand that rats fed cinnamon syrup only had significant ($p<0.05$) glucose levels in their blood but those fed regular feed didn’t show any significant difference as in (fig.2).

![Graph showing blood glucose concentration](image)

**Fig.2:** The action of cinnamon (*Cinnamomum Zeylanicum, Breyne*) on blood glucose concentration in alloxan — induced mature male rats with diabetics. The mean values are $\pm S.D.$ ($p < 0.05$).

![Graph showing total glutathione (GSH)](image)

**Fig.3:** The action of cinnamon (*Cinnamomum Zeylanicum, Breyne*) on Glutathione (GSH) in alloxan — induced mature male rats with diabetics. The mean values are $\pm S.D.$ ($p < 0.05$).
Anti-oxidant activity

The results of this study have shown that oxidative stress induces alloxan-induced diabetes, which led to parallel increases in antioxidant enzymes in rats. These effects were observed both during the experiment and with control rats as in (fig.3)(fig.4).

Gene expression analysis:

Table 2 outlines the results of the InsI expression quantification experiment, which demonstrated significantly elevated levels of InsI expression in the tissue obtained from normal control rats as well as Cinnamon-treated group animals. However, In diabetic groups, and not in normal rats, InsI gene expression was increased, while in the group treated with the cinnamon. Insulin gene expression in pancreatic tissue extracted from cinnamon-treated diabetics was much lower than in standardas TABLE 1.

<table>
<thead>
<tr>
<th>NO.</th>
<th>GROUPS</th>
<th>ANALYSIS OF Gene Expression IN INS I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>means</strong></td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>1.003</td>
</tr>
<tr>
<td>2</td>
<td>D</td>
<td>0.009</td>
</tr>
<tr>
<td>3</td>
<td>K</td>
<td>0.655</td>
</tr>
<tr>
<td>4</td>
<td>I</td>
<td>0.843</td>
</tr>
</tbody>
</table>

**The mean values are ±S.D. (p < 0.05).**
Discussion

because the acute ketosis increases the lipid peroxidation activity of diabetes, such as atherosclerosis is more quickly found in diabetics\textsuperscript{21}. The change in the number of the fluidity of the lipid membrane as a result of peroxidation affects enzyme activity, and the number of molecules, as well as on the molecules and activity in the membrane-bound enzymes you know, the mind is capable of a lot more than the body Lipids have been linked to heart disease, cancer, and nerve disorders in people who consume them frequently. schizophrenia and coronary heart disease\textsuperscript{22}. insulin stimulates the production of superoxide, which leads to an increase in reactive oxygen species, which in turn causes an increase in the oxidative stress of the death pathway in diabetics. Lowered levels of malondialdehyde and increased levels of MDA appear to be consistent with the claim. The overall administration of Cinnamon was odd, to say the least. Since we’ve cut back on the grain and the MDA, our system is out of balance. This denotes an increase in the level of oxidation of Cinnamon will experience\textsuperscript{23}. This may be due to the medication given to patients who are insulin-dependent Brewer’s warning: Pay attention to foods that enhance inflammation, as well as disease, because Cinnamon is an excellent source of disease-promoting foods\textsuperscript{24}. Enroflavassing French cheeses result in causes phagocytoblastisisis. This interesting and exciting finding was that the effectiveness increased at higher concentrations of H2O2, or peroxide levels in the presence of catalase and citrate, no substantial increase in oxidation was observed in the solution. diabetic rats exhibited significantly higher M concentrations than those who received insulin or grains to keep their sugar levels constant\textsuperscript{25}. I should have considered whether or get out of this line of work, that questions whether I should continue with my profession above the capability to neutralize free radicals had the same adverse effect on the enzymatic activity as free radicals. Reduced glutathione is needed to keep normal glutathione levels Consider this: As long as you make your album available, we’ll have you onstage at the VIP Lounge for the next five minutes (free stage time)\textsuperscript{26}. Whatever you are doing, your body takes a bit of a beating. Regardless of whether you’re sitting or moving, you experience some strain on your muscles or not\textsuperscript{27}. Some flavonoids are capable of the initiation stage interfering with free radical metabolism because of the presence of the peroxides and inhibiting the microsomal enzyme system There is another reason\textsuperscript{28}. They can scavenge other lipophilic substances They use their radicals or chelates to delay the Fenton reaction. In the experiment described here, we found that the efficacy of the INS I primer was first observed in this way. These have respective solution concentrations of InsI and GapdH at. primer efficiency was under 5% When we need real copy number It is the expression level of target genes of treatment samples as compared to control samples that is important to identify treatments\textsuperscript{29}. make decisions based on incomplete information. Thus, in this study, we used the InsI primers since their effectiveness was discovered to be at a concentration of (Livak and Schmittgen) a watched (began to watch) just as intently as (began to pay attention to) she paid attention (attended just as much too) as lack of insulin production and any deviations from normal insulin secretion are essential to almost all cases of adult-onset diabetes Also known as insulin-dependent or non-insulin diabetes, respectively a case of Type 2 diabetics, who have developed a specific version of the disease before and therefore do not have the proper antibodies, show up as incipient early signs of the more common forms of diabetes (MODY)\textsuperscript{30}. insulin has such a crucial effect on the body’s overall regulation of sugar levels, hyperglycemia can occur if there is even a small glucose imbalance in the diet. As both types of diabetes are caused by problems with the insulin gene, this form of diabetes has always been viewed as a possible, as well. Here, the researchers took special care to determine the temporal pattern of insulin gene initiation and expression (InsI) In mature male rats, the InsI gene expression was about 6 times that of females. The reduction in insulin sensitivity in the K group was while that in the D group was lower, and there were significantly higher blood glucose levels in the K group animals. In contrast, there was 4.3-fold more Ins I upregulated in the Cinnamon-treated diabetic
rats 35 percent of the control group were heterozygous and three percent of the experimental group were mutant for that trait. We also obtained quantitative data of InsI genes, to a small extent increase in the serum level of glucose and decrease in the level of insulin The findings in this study provide compelling evidence that Cinnamon treatment is effective. Whole-grain helps to regenerate beta-cell tissues especially in islands of the pancreas. As a result, it’s likely that Langer’s use of Cinnamon entire elements. This family was named the INS I after cDNA was extracted from a rat pancreas in the early 1990s. These have mostly been studied in terms of regeneration and growth, including the endocrine and exocrine pancreas. According to the same facts, these cells have an important role in beta-cell regeneration, i.e. the cell proliferation and hyperplasia in tandem with the rise in Ins expression. It is currently believed that FUS, MIP, and INS ITP1 (more specifically, the INS, MIP, and INS1) are the main insulin biosynthetic genes present in the FHA tissue, based on literature research conducted in the past few years. Thus, it was demonstrated that whole grain Cinnamon components could lower blood glucose levels by inducing proliferation of p-related cells. Consequent to which, in terms of Insulin-like mRNA expression, the current results are in agreement with) our previous research findings. Cinnamon flour canines enhances glucose regulation and raises the aminotrigin levels in Diabetic rats you should pay attention to them as they’re gnawing at the roots of the plant that grow higher as time goes on The results thus prove, since 2007, established in a study that Cinnamon -containing foods promote weight loss.” With the addition of whole grain, the glycemic and insiemic response to food was greatly reduced, but not to drink treatments. compared to non-treated subjects.

**Conclusion**

Concluded that cinnamon helps, as an anti-hypoglycemic and anti-oxidant in model rats given sufficient evidence of their own being diabetic. Elevating the expression of InsI gene expression levels also assists in the biosynthesis of insulin-secreting capacity in pancreatic β-cells. Additional studies are needed to ascertain the uses of cinnamon and its various components in disease states of compromised or stressful health.

**Compliance with Ethical Standards:**

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Ethical Approval:** Ethical approval for the present research was acquired from the Al-Qadisiyah University.

**Source of Funding**- Self

**References**


The Role Sonographic Imaging Features in the Prediction of Necrotizing Enterocolitis Outcomes in Neonatal Patients

Hayder Neamah Hassan
Research Scholar, Faculty of Medicine, University of Kufa, Iraq

Abstract
Necrotizing enterocolitis (NEC) is a devastating inflammatory disease of the intestinal tract that represents a significant source of morbidity and mortality in preterm infants. The objective of the current study is to find out the correlation between sonographic imaging features and the outcomes of necrotizing enterocolitis in children, and their role as a predictive tool for the consequences resulted from necrotizing enterocolitis. Thirty two children (patients) have been included in this study, patients were divided into three groups according to the follow up outcomes: those that been recovered (Group I), those that needed surgery (Group II), and those that died (Group III). The study was achieved at Al-Zahra Teaching Hospital in Al-Najaf City, in Iraq, during the period between December 2020 to March 2021. The results showed that there is a significant difference (P value <0.05) among the three groups in terms of the following items: fluid-filled dilated bowel loop, decreased peristaltic movement, focal fluid collection, portal gas. The study concluded that the presence of Fluid-filled dilated bowel loop, peristaltic movement, local fluid collection, portal gas can be considered as predictive tool for the poor outcomes of necrotizing enterocolitis.

Keywords: Necrotizing Enterocolitis, Portal Gas, Focal Fluid, Prediction

Introduction
Necrotizing enterocolitis (NEC) is considered a crucial reason of neonatal deaths specially in pre-term infant. It was recorded that about 5.0% to 14.0% of infant patients with weights below than 1500 grams at birth may have (NEC) and approximately 25.0% to 40.0% may die due to this condition, and because of impaired neurodevelopment, such as high risk of cerebral palsy, visual cognition, and impairment psychomotor functions. The diagnosis and treatment of NEC are very important for good outcomes because of the rapid progression of the disease into intestine perforation, shock and inflammation of peritoneum. The majority of premature neonates need long-term hospitalization because of deteriorating factors such as decreased gestational age and low weight birth (BW). Necrotizing enterocolitis is considered the main cause of death because of gastro-intestinal disease in pre-term neonatal individuals, which affected 5.0–12.0% of neonatal patients with a very low BW (below 1500 grams)

The pathophysiology necrotizing enterocolitis is usually considered to be controlled by many factors, important risk factors include decreased gestational age at delivery, decreased birth weight, chorio-amnionitis. Researches about the pathophysiology of necrotizing enterocolitis have further revealed many causative factors such as genetic disorders, intestine immaturity, alterations in micro-vascular tone, and pathologic microbial infections. In spite of there are no data have discovered an obvious genetic pattern correlated strongly with necrotizing enterocolitis.

There are many studies that investigated the importance of sonographic imaging in the diagnosis and prediction of necrotizing enterocolitis outcomes.
researches have investigated the abdomino-sonography (AUS) as an accessory measurement in management of infant patients with necrotizing enterocolitis. Abdominal sonography can enable the accurate evaluation of the thickness and conditions of the intestinal wall on gray-scale images, peristaltic movement on real time images and intestinal wall perfusion. In addition, the abdominal sonography similar can enable assessment of the status of the cavity of the peritoneum for free fluids and focal-fluid collection, in addition to free gas\(^5\).

Neonatal patients who recover after medical or surgical management for necrotizing enterocolitis are high risk for prolonged GI and neuro-developmental consequences. Surgical therapies of necrotizing enterocolitis include re-section of ischaemic intestinal portions; the consequences of these neonates depend on the length of rest portions of intestine and its capability to absorb digested food efficiently. Typically, re-section of ileum might result in Gastrointestinal dysmotility, abnormal mucosal membrane, bacterial opportunistic growth, and deficiency of vitamin B12, resulting in mal-absorption of digested food\(^6\).

The objective of the current study is to find out the correlation between sonographic imaging features and the outcomes of necrotizing enterocolitis in children, and their role as a predictive tool for the consequences resulted from necrotizing enterocolitis.

### Methods

Thirty-two neonates (patients) with NEC have been included in this study, patients were divided into three groups according to the follow up outcomes: those that been recovered (Group I), those that needed surgery (Group II), and those that died (Group III). The study was achieved at Al-Zahra Teaching Hospital in Al-Najaf City, in Iraq, during the period between February 2020 to March 2021. The following data have been collected: ((Age, Gestational Age, Birth Weight, Pneumoperitoneum, Fluid-filled dilated bowel loop, Free peritoneal fluid, Decreased Peristaltic movement, Bowel perfusion, Focal fluid collection, Portal Gas, Bowel wall thickness)). Statistical analysis was done by SPSS program (version 25) including both descriptive (frequency and percentage) and inferential statistics (Chi Square).

### Results

Table (1) is General characteristics and differences between Group I (recovered), Groups II (need surgery) and Group III (died). This table shows that there is a significant difference (P value <0.05) among the three groups in terms of the following items: Fluid-filled dilated bowel loop, decreased Peristaltic movement, Local fluid collection, Portal Gas.

<table>
<thead>
<tr>
<th>Items</th>
<th>Categories</th>
<th>Group I (Total = 17)</th>
<th>Group II (Total = 11)</th>
<th>Group III (Total = 4)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
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<td>5</td>
<td>7</td>
<td>4</td>
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</tr>
<tr>
<td></td>
<td>7-10</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11-14</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Gestational Age / weeks</td>
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<td>3</td>
<td>2</td>
<td>1</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td></td>
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<td></td>
<td>36</td>
<td>4</td>
<td>3</td>
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<tr>
<td></td>
<td>37</td>
<td>3</td>
<td>1</td>
<td>0</td>
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</tr>
</tbody>
</table>
**Table (1) General characteristics and differences between Group I (recovered), Groups II (need surgery) and Group III (died)**

<table>
<thead>
<tr>
<th></th>
<th>Group I (recovered)</th>
<th>Group II (need surgery)</th>
<th>Group III (died)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Weight/Kg</td>
<td>1.0-2.0</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2.1-2.5</td>
<td>8</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2.6-3.0</td>
<td>3</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Pneumoperitoneum</td>
<td>Yes</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>16</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Fluid-filled dilated bowel loop</td>
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<td>0</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>17</td>
<td>3</td>
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</tr>
<tr>
<td>Free peritoneal fluid</td>
<td>Yes</td>
<td>17</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
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<td>Yes</td>
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<tr>
<td></td>
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<td>7</td>
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<td>10</td>
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</tr>
<tr>
<td></td>
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<tr>
<td>Focal fluid collection</td>
<td>Yes</td>
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<td></td>
<td>No</td>
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<td></td>
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<td>Bowel wall thickness</td>
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</table>

**Discussion**

The first indicator included in this study was focal fluid collections (table 1.), which were found in (50%) of those patients who died, indicating that these are significant sonographic findings for predicting a bad outcome. This is expected, because these findings are a direct complication of intestinal perforation. It was reported there was a high correlation between the sonographic findings of free intra-peritoneal gas or focal fluid collection and neonate demise or requirement for surgical therapy. This aids the interpretation that focal fluid collections are predictive of intestinal perforation.

Our Study showed that only one patient with a focal fluid collection recovered after medical therapy, while one other patient needs surgery for treatment.

The another factor that may have contribution to poor outcome is decreased bowel wall perfusion, resulting in the occurrence of echoic peritoneal fluid and the occurrence of free gas and focal fluid collections in those neonates who may have directly bowel perforation but have no prove for free gas on sonographic imaging which is also crucial for identifying which neonate may have necrosis in bowel before perforation, as these neonates may also have a bad outcome.

Necrotizing enterocolitis is considered as an ischaemic process of the intestine that results in necrosis and inflammation, including the progression of intra-mural gas. As necrosis develops, the mucosa, sub-mucosa and muscularis layers can slough, making very thin wall. The most intensively affected loops of intestine may perforate, and this is correlated with a high mortality. Detection of bowel necrosis by sonographic imaging before perforation may strongly affect surgical intervention and permit the surgical staff to manage those neonates with necrosis before the formation of free gases.

One of the sonographic imaging feature that was observed to make bad outcomes in neonates. Prediction of a bad outcome of NEC may result from the following sonographic imaging: Fluid-filled dilated...
bowel, Focal fluid collection, echogenic free-fluid, high intestinal wall echogenicity and high intestinal wall thickness.(11).

The portal venous gas (PVG) observed by X-ray or sonographic imaging is also considered as a typical diagnostic feature of confirmed necrotizing enterocolitis. Some data reported that PVG can be a predictive diagnosis for the onset of NEC, which was first identified by Yue et al., who discovered the occurrence of portal gas before the onset of NEC(12).

**Conclusion**

The study concluded that the presence of Fluid-filled dilated bowel loop, decreased peristaltic movement, local fluid collection, portal gas can be considered as predictive tool for the poor outcomes of Necrotizing enterocolitis.

**Ethical Clearance:** Taken from University of Kufa ethical committee

**Source of Funding:** Self

**Conflict of Interest:** Nil

**References**


Handwriting Change in Breast Cancer Patients

Heba H Rohym¹, Ghada Mustafa Al-Galad², Noha Abdel Rahim Mohamad³, Mohamed Ragab Teleb⁴
¹Lecturer, ²Professor and Head Department, Forensic Medicine and Clinical Toxicology, Fayoum University, Egypt, ³Fraud and Counterfeiting Officer Forensic Medicine Department - Ministry of Justice, Egypt, ⁴Demonstrator Department of Forensic Medicine and Clinical Toxicology, Fayoum University, Egypt

Abstract

Background: Breast cancer is the most common cancer. In the course of cancer progression, there are specific neuromuscular disturbances that directly affect coordination. Handwriting is regarded as a vital constituent when it comes to the tracing of neuromuscular coordination. The Study aimed to study handwriting analysis in female cancer breast patients trying to find simple useful, non-invasive way help in early detection and diagnosis and follow up of those patients

Methods: The study included 160 participants. The study participants were asked to write the same text (two Arabic sentences and her name in Arabic language) and draw lines in another paper, and then all handwriting samples were scanned and examined.

Results: Breast cancer patients age ranged from 30 – 76 years old with a mean ± SD of 50.3 ± 10.5. Patients according to Educational level parameter were 5 groups; Illiteracy eradication program, primary, middle, secondary, graduation and post gradates were 14 (8.75 %), 10 (8.75 %), 16 (10 %), (80) 50 %, 40 (25 %) respectively.

By analysis of handwriting samples: Tremor, wide spaces between words, heavy writing pressure, slop or slant of writing upward slant or down slant and heaviness of Initial and terminal strokes were observed.

After re-observation of pervious Handwriting changes and make a relation between these changes and cancer stages and lines of treatment which received, it was noticed that these changes were in samples of patient received multiple lines of treatment, in patients with metastasis or has another associated tumor as ovarian, bladder cancers or lymphoma.

Conclusion: There is no evidence of significant handwriting Changes in breast cancer samples unless patient received multiple lines of treatment, in patients with metastasis or has another associated tumor as ovarian, bladder cancers or lymphoma.

Keywords: handwriting analysis, cancer breast patients, writing traits

Introduction

Handwriting analysis is the window to the soul. It may be true that it can help to know a lot about someone’s personality from his handwriting. It’s been said that this science can be used to diagnose mental illness (6). Handwriting is a complex act, resulting from a combination of coordinated actions between the eye, brain, and muscles of the arm, hand, and fingers; a complex perceptual-motor task or a neuromuscular task (9).

Some researches named handwriting as” brain writing”. Experiments included having writers perform with their opposite hand, foot and even mouth, which established conclusive similarities indicating that writing, was centrally organized, the role of the writing central in brain is that it is responsible for the mental
image of writing. An example of this is when dictating a phrase that everyone writes according to its mental image of it in the brain. It also gives orders to the hand as a writing tool and thus implements the mental image in the brain. Therefore writing is brain write and the hand is just enforcer\(^5\). The main uses of handwriting and graphology include behavior analysis, forensic evidence and disease analysis. Script reveals the exact personality including emotional status, fears, defenses, honesty and much other individual personality attribute of a person through the strokes and patterns revealed by script\(^7\).

The authenticity of a person’s signature or script in the suicide note is frequently subjected to forensic document examination during investigation in order to verify authorship\(^2,10\).

Breast cancer creates from breast tissue. Signs of breast cancer may incorporate a knot within the breast; alter in breast shape, dimpling of the skin, liquid coming from the areola, a newly-inverted areola, or a ruddy or flaky fix of skin. there may be bone torment, swollen lymph hubs, shortness of breath, or yellow skin\(^13\).

The most common device that specialists utilize to portray the arrange is the TNM framework. Tumor (T)Node (N): the tumor spread to the lymph hubs, In the event that so, where, what measure, and how numerous? Metastasis (M) the cancer spread to other parts of the body. There are 5 stages of breast cancer: organize (zero), which is non-invasive ductal carcinoma in situ (DCIS), and stages I through IV (1 through 4), which are utilized for obtrusive breast cancer. The organize gives a common way of depicting the cancer, so specialists can work together to arrange the most excellent medicines\(^14\).

Early breast cancer detection is very important; helping early proper treatment and decreasing death rate due to cancer breast, and this can be performed through regular self-exam of the breast, regular physical examination and investigation including breast mammography, Contrast-enhanced (CE) digital mammography, Ultrasound, Magnetic resonance imaging (MRI). Each of these screening tools has strengths and weakness\(^13\).

In the medical field, handwriting can be used as an aid in diagnosis and following of diseases like Alzheimer’s disease, Parkinson’s disease, and even cancer as in Kanfer neuromuscular Test’s in cancer patients\(^12\).

Handwriting changes in case of cancers occur in case of metastasis, treatment by chemotherapy, sever pain, psychological disturbance or pressure of cancer on nerves or any condition affect nervous system in cancer patient. If cancer patient has these conditions, handwriting changes appear which help in early detections of cancer and follow up the patient\(^3\).

Handwriting changes as tremors which indicated by an involuntary, rhythmic, and recurrent movement of the pen from side to side. These tremulous strokes are instant changes from the desired direction of the pen lines and are attributed to nervous impulses affecting the muscles indicating loss of control of the pen. There are many different reasons that tremor occurs in handwriting including aging, illness\(^11\).

Pressure refers to the hand’s grasp on the writing instrument, and the amount of pressure used to push the pen across the paper. It varies for different writers from light to heavy\(^3,11\). Slant or slope refers to the direction in which the writing leans. It may in the right or in the left, or it may be vertical, when a writer in disease, there are change the slant, but not in all cases\(^3\).

Materials and Methods

Study design

This study started from September 2019 to September 2020 in forensic medicine and clinical toxicology department-faculty of medicine, aiming to studying handwriting analysis in female cancer breast patients trying to find simple useful, non-invasive way help in early detection and diagnosis and follow up of those patients.
Subjects

The local ethical committee approval was obtained before starting of the study. The aim and benefits of the study were discussed with participants before collection of handwriting samples. And a written consent for participation of the study was taken, and confidentiality of all data was ensured to all participants.

160 Participants of our study are 120 breast cancer patients and 40 breast cancer with other types cancer patients (lymphoma, bladder cancer and ovarian cancer) attending to different Fayoum hospitals

Prior to inclusion, personal and medical history was taken from each patient including age, Educational level, job, number of siblings, her age at first pregnancy, family history for cancer and treatment she received. At the time of testing all patients had stabilized general condition, adult age with at least primary educational level.

We excluded males patients and any patient with disease affecting handwriting i.e. hemodialysis patients, any neurological disease that affect hand movement as parkinsonism, CNS tumors, Thyroid disease including hyperthyroidism and thyroid tumors, Hepatic patients, Illiterate patients and Alcohol abstinence

Materials:

White papers (A4 60 gm), Floscape papers (70 gm), Finegrip s Blue Pen (0.7 mm), Laptop, Scanner (Hp PSC 1410), Digital camera with high resolution, Compact video microscope 2000 (CMV 2000), Leica microscope MRZ 420, Stereoscopic binocular microscope, Hand magnifiers.

Methods

Each patient was asked to write the same text (two Arabic sentences and her name in Arabic language, three times on two separate sheets of paper (one blank and one lined sheets - Floscape paper) and draw lines in another paper according to model they given in the same session. Subjects were asked to write on the blank sheets first then on the lined one, using similar blue pens (0.7 mm).

All samples of handwriting were obtained in the daylight, then the samples of handwriting of each participant (3 sheets of paper) were kept a in a file that was given a serial number from 1 to 160.

Patients were asked to bring a pervious handwriting sample before becoming diseased or receiving any treatment i.e. old signature, to be compared with handwriting samples after becoming diseased or receive treatment

All samples were scanned using Scanner machine (Hp PSC 1410) and their digital images were collected and collaged to gather by photo collage maker- photo editor & photo collage (a computer graphics application (version 1.28.92) which enables combination and editing of multiple samples. All samples (original and scanned versions) were examined; letter by letter, by experts of handwriting in Ministry of Justice-Egypt using hand magnifiers, Compact video microscope 2000 (CMV 2000), Leica microscope MRZ 420, Stereoscopic binocular microscope (giving 3D magnification) Every mark on the script or unusual movement in the trace was noted during the analysis and recorded individually for each participant.

Various writing traits were analyzed including writing speed, pen pressure grades, spacing between words, initial and terminal strokes, slop/slant and tremors

Statistical Analysis

The collected data were organized, tabulated and statistically analyzed using SPSS software statistical computer package version 22 (SPSS Inc, USA). For quantitative data, the mean, standard deviation (SD) and Range were calculated.

Results

According to age; Patient age ranged from 30 – 76 years old with a mean ± SD of 50.3 ± 10.5, as shown in table (1) The studied patients were 4 categories: age (30-40), (40-50), (50-60), above 60 years group.
34 (21.25 %), 50(31.25%), 48 (30 %) and 28(17.5 %) respectively as in Table (2). shows most cases between 40-60 years 98 (61.25%), less in age 30-40 years 34 (21.25%). Patients according to Educational level parameter were 5 groups; Illiteracy eradication program, primary ,middle, secondary, graduation and post gradates with Percentage 14 (8.75 %) , 10 (8.75 %) ,16(10 %),(80) 50 %,40 (25 %) respectively Breast cancer patients educational level, secondary level predominate other level; 50 % of all studied samples. as shown in table (3) Table (4) showed that according to cancer stage, our samples were three groups : group 1 (stage 0,1,2 TNM staging system) group 2 ( stage 3, 4 TNM) group 3 (breast cancer with lymphoma, bladder cancer and ovarian cancer) 80 (50 %), 40(25 %)40 (25 %) respectively. According to line of treatment used with cancer patients included in our study, our sample were grouped into seven group: group 1 (received no treatment), group 2 (underwent breast surgery) , group 3 (received chemotherapy), group 4 (received hormonal therapy), group 5 (underwent breast surgery and received chemotherapy), group 6 (underwent breast surgery and received radiotherapy) group 7 (underwent breast surgery, received chemotherapy, radiotherapy and hormonal therapy) 48 (30%), 32 (20 %), 12 (7.5 %), 4(2.5 %) , 32 (20 %) ,4(2.5 %), 28 (17.5 %) respectively many cases were early detected and haven’t received treatment yet as shown in table (5). By analysis of handwriting samples: Tremor was observed in 20 out of 160 writing samples(12.5%), which showed variations in the form of slight changes in writing impulses up to marked deteriorations to the level of illegibility. Few samples 14 of 160 writing samples(8.75%) showed wide spaces between words. 26of 160 writing samples(16.25 %) showed heavy writing pressure. It was observed that 8 samples(5 %) showed slop or slant of writing upward slant or down slant. By observation of the beginning and end of the word, 14 samples(8.75%)showed heaviness of Initial and terminal strokes. After re-observation of pervious changes and make a relation between these changes and age, cancer stages and lines of treatment which received, it was noticed that these changes were in samples of patient received multiple lines of treatment, in patients with metastasis or has another associated tumor as ovarian, bladder cancers or lymphoma (5 cases had breast cancer with ovarian cancers – 15 cases had breast cancer with bladder cancer – 20 cases have lymphoma).

<table>
<thead>
<tr>
<th>Table (1): Age of studied patient (in years)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Descriptive Statistics</strong></td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Valid (N)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table (2): Frequency and percentage of age categories among the studied patients (n=160)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Table (3): Frequency and percentage of educational level in the studied sample (n=160).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Category</th>
<th>Count</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Educational level</td>
<td>Illiteracy eradication program</td>
<td>14</td>
<td>8.75 %</td>
</tr>
<tr>
<td></td>
<td>Primary schools</td>
<td>10</td>
<td>8.75 %</td>
</tr>
<tr>
<td></td>
<td>Middle schools</td>
<td>16</td>
<td>10 %</td>
</tr>
<tr>
<td></td>
<td>Secondary schools</td>
<td>80</td>
<td>50 %</td>
</tr>
<tr>
<td></td>
<td>Graduation-post graduation</td>
<td>40</td>
<td>25 %</td>
</tr>
</tbody>
</table>

Table (4): Frequency and percentage of cancer stage in the studied samples (n=160).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Category</th>
<th>Count</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Group 1 (stage 0,1,2)</td>
<td>80</td>
<td>50 %</td>
</tr>
<tr>
<td></td>
<td>Group 2(stage 3,4)</td>
<td>40</td>
<td>25 %</td>
</tr>
<tr>
<td></td>
<td>Group 3(breast cancer with lymphoma or bladder cancer or ovarian cancer)</td>
<td>40</td>
<td>25 %</td>
</tr>
</tbody>
</table>

Table (5): Frequency and percentage of different treatment lines used in the studied samples (n=160).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Category</th>
<th>Count</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line of treatment</td>
<td>No treatment</td>
<td>48</td>
<td>30 %</td>
</tr>
<tr>
<td></td>
<td>Surgery</td>
<td>32</td>
<td>20 %</td>
</tr>
<tr>
<td></td>
<td>Chemotherapy</td>
<td>12</td>
<td>7.5%</td>
</tr>
<tr>
<td></td>
<td>Hormonal therapy</td>
<td>4</td>
<td>2.5 %</td>
</tr>
<tr>
<td></td>
<td>Surgery+ chemotherapy</td>
<td>32</td>
<td>20 %</td>
</tr>
<tr>
<td></td>
<td>Surgery+ radiotherapy</td>
<td>4</td>
<td>2.5 %</td>
</tr>
<tr>
<td></td>
<td>Multiple lines</td>
<td>28</td>
<td>17.5 %</td>
</tr>
</tbody>
</table>
Table (6): Writing changes in the studied samples (n=160).

<table>
<thead>
<tr>
<th>Handwriting change</th>
<th>No.of cases</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tremors</td>
<td>20</td>
<td>12.5%</td>
</tr>
<tr>
<td>Spacing between words</td>
<td>14</td>
<td>8.75%</td>
</tr>
<tr>
<td>Heavy pressure</td>
<td>26</td>
<td>16.25%</td>
</tr>
<tr>
<td>Slant</td>
<td>8</td>
<td>5%</td>
</tr>
<tr>
<td>Initial and terminal strokes</td>
<td>14</td>
<td>8.75%</td>
</tr>
</tbody>
</table>

**Discussion**

In Egypt; breast cancer occupied the second rank among different cancers (1).

Breast cancer is typically detected either during screening, before symptoms have developed, or after a woman notices a lump. Most masses seen on a mammogram and most breast lumps turn out to be benign (not cancerous). When cancer is suspected, tissue for microscopic analysis is usually obtained from a needle biopsy (fine-needle or larger core-needle) and less often from a surgical biopsy. Selection of the type of biopsy is based on multiple factors, including the size and location of the mass, as well as patient factors and preferences and resources (4).

Handwriting is fingerprint of the mind; therefore handwriting analysis can serve as x-ray of individual physical state. Handwriting analysis needs to be informed of any factors that could influence the handwriting being examined. The principle factors considered include age, health, substance abuse, and mechanical factors. Health factors include physical and mental health and any medication that may affect handwriting. Mechanical features cover the writing surface, writing instrument, and lighting. The writing position may also affect the writing act (11).

Changing your handwriting means changing life, in addition, what we write is controlled by the conscious mind, but how we write is controlled with sub-conscious mind. Cancer -or tumor- makes pressure on neurons that affects motor nervous system, controller of handwriting, which causes some dramatic changes in the patient’s handwriting. Hence, we worked on early detection of cancer through handwriting (1).

A typical criteria of normal hand writing that is a mature neuromuscular condition with a normal range of coordination. Normal handwriting is manifested in the smooth, continuous flow of movement, both in the descending and ascending strokes (uniform flow of ink throughout the strokes and sharp, continuous delineations to both sides of each stroke) In case of cancer as lymphoma: The strokes have an oval shape; the turns from descending to ascending strokes are narrow, curved, and show continuity of movement throughout. A regular pattern of heavier (wider and darker) descending strokes and lighter ascending strokes (9).

In our study, most distribution of age categories among the studied patients were 4 groups age, (30-40),(40-50),(50-60),above 60 years group 21.25 %,31.25 %,30 %,17.5 % respectively.

In this study; we noticed decrease in incidence rates

Most cases between 40-60years ; This agree with national cancer institute (NCI) and center of disease control (CDC) and prevention that most breast cancers are found in women are 50 years old or older (8).
in patients above 60 years, this agrees with American Cancer Society Surveillance Research 2019 (4).

In this study, Breast cancer patients according to Educational level parameter; Illiteracy eradication program, primary, middle, secondary, graduation and postgraduates with the Percentage 8.75%, 8.75%, 10%, 50%, 25% respectively. Breast cancer patients educational level, secondary level predominate other level; 50% of all studied samples. Most cases are from high level of education (secondary schools and graduates) may be due to patient awareness about the disease and doing self-examination and early asking of medical advice; in contrast of lack of disease awareness and delayed seeking medical advice in less educational level as in Illiteracy eradication program and primary school level this agrees with American Cancer Society Surveillance Research 2019 (4).

In our study; it is noticed that some writing samples showed changes as tremors, wide space between words, upwards and downwards slop and heavy pen pressure but all these changes were in patients of treated with multiple lines of treatment or cases associated with other cancers as lymphoma, ovarian and bladder cancer or metastasis.

**Conclusion**

By general observation and analysis of samples of handwriting from breast cancer patients, there is no evidence of significant difference between breast cancer samples and normal human standards ratio and no common characters in a breast cancer samples (no tremors in stroke, no unusual configurations).

**Recommendations**

- Establish clinical-radiological and graph logical coloration of cancer patient.
- Analysis of handwriting changes in others types of cancers i.e. hepatic cancer – brain tumors.
- Analysis of handwriting changes in Diabetes mellitus.
- Analysis of handwriting changes in thyroid diseases

**Abbreviations**

**TNM:**

Tumor staging system for classifying the extent of spread of cancer

**Ethical Clearance:** Faculty of Medicine Fayoum University Research Ethical Committee Permission number 434.

**Conflict of Interest:** Nil

**Source of Funding:** Self-Funding

**References**


Extraction and High Purification of Nicotine from Iraqi Tobacco Leaf Formanufacturing, Pharmaceutical, and Medicinal Uses

Husam Alaa Hameed Al-Khinuliu¹, Essam Fadel Al-wan Al-Jumaili²

¹Final Year Post Graduate Student, ²Professor, Biotechnology Dept. Genetic Engineering and Biotechnology Institute for Postgraduate Studies. University of Baghdad.Al-Jadriya Campus, 10071 Baghdad, Iraq

Abstract

Background: Nicotine is highly addictive plant derived alkaloid and the most important species in human use today is Nicotianatabacum. There are direct health effects of chronic nicotine exposure. Even in low doses, nicotine causes vasoconstriction and other cardiovascular effects related to catecholamine release and promote angiogenesis, neuroteratogenicity, and possibly some cancers. Methods: A preliminary investigation to analyze the nicotine contained in Iraqi tobacco leaves was carried out using gas chromatography-mass spectrometry (GC-MS). Nicotine is an alkaloid, and alkali methanol and Lipophilic solventsystem methods (LSS) have been extracted and determined by GC-MS from tobacco leaves. Results: The detection limit for nicotine was for non-selective monitoring at the ppm level and for selective detection at the nanogram level. This is a simple method of thin layer chromatography (TLC) and chromatography mass spectrometry (GC-MS) for the tobacco leave analysis of nicotine. The final purity of nicotine is 99%. Conclusion: the methods which used in this study gave very high purity of nicotine after converting the crude nicotine to its esters.

Key words: Nicotine, Iraqi tobacco, Lipophilic solvent, TLC, GC-MS chromatography.

Introduction

Nicotine is the predominant alkaloid in Tobacco plant (Nicotiana tabacum) represent above 90% from total alkaloid. In the recent decade there are increasingly demanded on it for industrial uses as one of the most important component in E-cigarette flavors and nicotine gum. In the pharmaceutical and medicinal fields using as a curative agent against some of psychotic disorders such as Schizophrenia, degenerative disease such Alzheimer. The aqueous two-phase system and solvent reverse extraction by using isopropyl alcohol/(NH₄)₂SO₄. The recovery rate of nicotine was 96.1% with a purity of above 99% when optimal conditions were used [(NH₄)₂SO₄ 25%, pH 9, temperature 35°C, isopropyl alcohol 5 mL]. Marked solubility differences of nicotine in the ionic liquids [C(2)mim][NTf(2)], [C(2)mim][EtOSO(3)], and [C(n)mim]Cl, 6 ≤ n ≤ 10, are observed through the analysis of the corresponding phase diagrams. These show the potential of commonly used ionic liquids to extract and purify this important compound. From a fundamental standpoint, the generally enhanced solubility of nicotine in these ionic liquids as compared to that of aromatic and aliphatic hydrocarbons can be assigned to the presence of the aromatic pyridine ring and the large aliphatic N-methyl-
The molecular genetics of tobacco alkaloids have not only provided plant biologists with insights into the mechanisms underlying the synthesis and accumulation of this important class of plant alkaloids, they have also yielded tools and strategies for modifying the tobacco alkaloid composition in a manner that can result in changing the levels of nicotine within the leaf, or reducing the levels of a potent carcinogenic tobacco-specific nitrosamine (TSNA). The chemical components of nicotine got the general formula C_{10}H_{14}N_{2} with molecular weight 162 Daltons, and the regular name pyridine 3-(1-methyl-2-pyrrolidinyl), maximum absorption in 254nM, its heterocyclic alkaloids have different tow rings (pyridine) and (pyrrolidine) the two nitrogen in pyridine and pyrrolidine rings capable of being ionized :N-pyridine pKa =3.4, N-methylpyrrolidine pKa =8.1. So, the behavior of nicotine is pH dependence, the entire nicotine soluble in lipophilic solvent in pH above 9 and not soluble in hydrophilic solvent, in pH =2 or less its miscible in the water at 25°C or less.Dalton et al.,[10] found that most regarded of nicotine derivatives is its esters that have many uses because it have less harsh test in mouth or burning feel on skin and in I.V injection. Physically nicotine is the oily liquid, colorless or pale yellow solution, turn to brown when exposure to air or light, fish-like odor when warm with density =1.000925 at 25°C and Boiling point (B.P) = 247°C at atmosphere pressure. The aim of this study is to purify the nicotine in high grade and prepare the salt of nicotine that used in industrial, pharmaceutical and medicinal fields.

**Materials and Methods**

The Iraqi tobacco leafs is purchased from local market, dried in the oven on 40°C for 60 min, crushed by electric grinder to fine powder. The powder (500gm) potted into closable glass container, add 500ml of D.W thin NaOH crystals was added till reached the pH to 13.35, heated in water bath to 80°C for 20 min, cooled to 50°C, add ethanol 99% 4:1 ethanol: mixture reheated to 60°C for 120 min let to cool to the 25°C, added chloroform in ratio 1:4, adjusted the pH to 1.0 by H_{2}SO_{4} 0.5 M, stirred for 60 min at 25°C, tow layer are preform, keep the mixture in [-5°C] overnight, separate by separation funnel took the ethanol layer and rewashed again by 1:4 by chloroform under same conditions, took the ethanol layer and adjusted the pH to 13.35 by NaOH solution 0.5 M added chloroform solvent in 1:4 ratio and stirred the mixture 60 min at 60°C in closed container thin separated, discarded the ethanol layer and took the chloroform layer rewashed by ethanol 75% pH 8.5 under the same conditions, took chloroform layer and evaporate the solvent by rotary evaporator. The pale yellow liquid obtained turn to brown yellow very thick slurry after exposure to air, this substance called crude nicotine.

Preparation of nicotine esters (Nicotine bitartarate salt): To prepare nicotine bitartarate mix approximately 1:3 mol/mol crude nicotine: tartaric acid. where: Molecular weight of nicotine is 162.23g/mol, so 8.1g =0.05 mol. Molecular weight of tartaric acid is 150.087 g/mol. so 7.505g= 0.5 mol.

Dissolved 7.505 g ×3=22.51g of tartaric acid in 100 ml cold methanol then added the 8.1g of nicotine and stirred the white crystals was observed the solution was potted in refrigerator at 4°C overnight, collected the crystals by filter paper, washed the crystals by 50 ml methanol then 50 ml chloroform, this crystals represent nicotine bitartarate. Recovered nicotine from nicotine bitartarate with some modification. 20g salt dissolved into 100 ml cold methanol then added 0.5 M NaOH till the pH reached to 13.35 heated the mixture to 65 for10 min, added 100 ml of chloroform adjusted the pH to 13.35 and stirred with heat 30 min at 60°C add a drop of distill water until separated the two layers took chloroform layer and dried, the yellow slurry material represent the pure nicotine.

**Results and Discussion**

The final material was examined by several tests first by preliminary screening by Wagner’s and Mayer’s reagent gave the result positive, Second by TLC 20×5 cm plate was used, system solvent was methanol: ammonia 200:3*, dyeing by iodine vapor, the RF=56.8 this result was very near compared to RF =57 (Figure 1)\textsuperscript{13,15}. Third test by using GC-MS test to identifying the identity of
substance on a specimen, the 99% of area mass was obtained to the nicotine component in specimen, this ratio is represent the purity of nicotine.

The solvent system, pH and temperature and time is a critical points in extraction efficiency of nicotine, chloroform - aqueous system have a good extraction ratio because the highest distribution coefficient between the two solvents, nicotine trend to dissolved in aqueous solvent whenever pH 3 or less and temp 25 or less than, vice versa nicotine dissolved in organic solvent at pH above 9 or higher and temp above 25°C. The purity of nicotine rose from 16.43% to 99% after converting crude nicotine to its esters (nicotine bitartarate)\textsuperscript{16}. Therefore, because of the capacity of nicotine and few substances under the above conditions to make crystal esters. Nevertheless, all compounds (hydrophilic and lipophilic) seeded by wash only leave filter paper to arrest nicotine salts. This technique can be adopted to purify nicotine at least from Iraqi Tobacco, based on the above findings.

![Thin layer chromatography plates of nicotine compound using solvent system](image)

**Figure (1):** Thin layer chromatography plates of nicotine compound using solvent system

(methanol : ammonia (200: 3) . A: pure nicotine B: crude nicotine .

The used of alkali material not only toinccrease pH value in extraction and recovery of nicotine, but return to capacity of this material with heat to break down the forms of nicotine esters (most nicotine contained in esters forms in plane tissue) and release nicotine in free form, the time, alkali concentration and temperatureare so important in nicotine extraction and yield due to nicotine degradation\textsuperscript{17,18} (Figure 2).
Figure 2: Critical points in extraction of nicotine: Alkali concentration, Time and Temperature.

Table 1: GC-MS analysis of crude nicotine.

<table>
<thead>
<tr>
<th>Peak NO.</th>
<th>Retention Time</th>
<th>AREA %</th>
<th>Compound Name</th>
<th>REF</th>
<th>CAS #</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-MS Column Name</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Agilent 19091S-433UI</td>
</tr>
<tr>
<td>1</td>
<td>12.435</td>
<td>16.43%</td>
<td>S- pyridine 3-(1-methyl-2-pyrrolidinyl)</td>
<td>32085</td>
<td>000054-11-5</td>
</tr>
<tr>
<td>2</td>
<td>16.341</td>
<td>4.45%</td>
<td>Dimethyl-3-Butene-1,2-diol, 1-(2-furyl)-2,3 Octacosyl acetate 1-Heptacosanol</td>
<td>46869, 227661, 210517</td>
<td>019757-51-8, 018206-97-8, 002004-39-9</td>
</tr>
<tr>
<td>3</td>
<td>25.130</td>
<td>5.92%</td>
<td>Methoxyacetic acid, 2-tetradecyl ester Tetratetracontane Heptacosane, 1-chloro ethan</td>
<td>132964, 241527, 217413</td>
<td>1000282-04-8, 007098-22-8, 062016-79-9</td>
</tr>
<tr>
<td>4</td>
<td>26.428</td>
<td>15.91%</td>
<td>Tetratetracontane 2-tetradecyl Methoxyacetic acid ester Tritetracontane</td>
<td>241527, 132964, 241174</td>
<td>007098-22-8, 1000282-04-8, 007098-21-7</td>
</tr>
<tr>
<td>5</td>
<td>27.557</td>
<td>14.65%</td>
<td>Octadecane, 1-chloro-ethan Tritetracontane Methoxyacetic acid, 2-tetradecyl ester</td>
<td>134594, 241174, 132964</td>
<td>003386-33-2, 007098-21-7, 1000282-04-8</td>
</tr>
<tr>
<td>6</td>
<td>28.561</td>
<td>11.22%</td>
<td>1-bromo Octadecane Tritetracontane Hexadecane, 1-bromo</td>
<td>170753, 241174, 147852</td>
<td>000112-89-0, 007098-21-7, 000112-82-3</td>
</tr>
<tr>
<td>7</td>
<td>29.483</td>
<td>5.96%</td>
<td>tert-Hexadecanethiol Oxalic acid, dodecyl propyl ester Hexadecane, 1-bromo</td>
<td>109272, 144569, 147852</td>
<td>025360-09-2, 1000309-26-5, 000112-82-3</td>
</tr>
</tbody>
</table>
Nicotine purity rose from 16.43% to 99% after converting the crude nicotine to its esters, According to figure 3 and 4 (nicotine bitartarate). Therefore, because of the capacity of nicotine and few substances under the above conditions to make crystal esters. Nevertheless all substance (hydrophilic & lipophilic) seeded by wash leave only filter paper to arrest crystals. Therefore in order to obtain the high purity of nicotine, the pH –dependent inversion solubility of nicotine from polar solvent is not necessary to obtain 99 percent purity of nicotine.
Figure (4) : Pure nicotine GC-MS assay the purity of nicotine reached.

Table 2 : GC-MS analysis of pure nicotine.

<table>
<thead>
<tr>
<th>GC-MS Column Name</th>
<th>Agilent 19091S-433UI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak NO.</td>
<td>Retention Time</td>
</tr>
<tr>
<td>1</td>
<td>12.326</td>
</tr>
<tr>
<td>2</td>
<td>16.336</td>
</tr>
<tr>
<td>Total</td>
<td>32 Min</td>
</tr>
</tbody>
</table>

#chemical abstracts serves.

**Conclusion**

In this study, we can conclude that the nicotine high purity rose 99% after converting the crude nicotine to its esters and the technique used in this study can be adopted to purify nicotine at least from Iraqi Tobacco.

**Ethical Clearance** : Taken from institutional ethical committee.

**Sources of Funding** : The research was funded by the authors.

**Conflict of Interest** : None

**References**


Association of the TP53 Codon 72 Polymorphisms with PCOS Female Infertility in Karbala City

Inam Joudah Radhi⁴, Abdulmutalb Badr Manhy Alkhaleeli², Hameedah Hadi AbdulWahid³
¹Assist. Lecturer, ²Lecturer, College of Pure Science, Department of Chemistry, University of Kerbala, Iraq,
³Consultant Gynecologist, Kerbala Obstetric and Gynecology Teaching Hospital, Kerbala Health Directorate,
Ministry of Health, Iraq

Abstract

To training the association of polycystic ovaries with p53 and 72 polymorphisms in the population of female infertility in central Karbala and to search for possible interaction with the polymorphism. The Iraqi environment was suffered from acts of profanation by due to wars in Iraq, since 1990 and after 2003, An enormous number of damages and deaths were affected by destructive chemicals and radioactive resources. These events resulted in either cancer or infertility. Infertility is one of the medical, social and psychological burdens in Iraqi society. After 12 months or more of failure to a clinical pregnancy lead to a regular unprotected sexual intercourse.

Keywords: Women sterility, PCOS, polymorphism, Codon p53 and 72.

Introduction

Infertility is defined as a disease of the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [¹].

The disability to become pregnant consequences in an important affecting and profitable tolls, among artificial nervousness and sadness are more public in sterile couples than sterile couples, also Assisted Reproductive Technologies (ART) is accessible toward care for sterility, then these are pricey. The credit of changeable hazard influences for sterility might guide to economical then helpful interferences expected at preclusion [²]. It is accepted that cancers are the result of the mutation accumulation in the genome. Breast cancer has been a significant for P53 role in the formation and progression, it is also known as the genome guard. The results of different studies indicate that the p53 gene, one of the tumor suppressor genes, is involved at least in various cancers. P53 has been known “the guardian of the genome”, which aids in DNA stability by preventing genome mutation. (SNPs) in the p53 gene There are some different single nucleotide polymorphisms that the biological special effects of most of these polymorphisms have been planned and their clinical results are well known. According to the molecular epidemiological studies, p53 gene mutations are estimated to be identifiable in breast cancer cases. The codon 72 is one of the main SNPs through p53 that has been frequently studied in breast cancer. This polymorphism should be 15% worldwide, > 30% in some developing countries, and 17.28% in manufacturing countries. Polymerase chain reaction (RFLP-PCR) will be restricted by several methods such as fragment length and polymorphism. Direct DNA sequencing and amplification-refractory mutation system-PCR(ARMS-PCR) have been used to examine the polymorphism of the p53 gene codon 72. [³].

The 4 exon codon 72 regions are lies by the most significant is rs1042522. This praline-rich region is
primarily responsible for the show of the apoptotic feature of the TP53 gene. It is a functional polymorphism which is found on exon 4 and foundations the replacement of praline with arginine residue at the regulatory portion of the TP53 gene, causing in altered instruction of apoptosis. Different studies have been established by TP53 codon polymorphism in the growth of endometriosis [4].

The p53 is a growing suppressor gene, governing the instruction of cell tumor and the regulation of the cell cycle, which is complicated in cell spreading like the progression of different procedures of cancer and brings apoptosis or huns the cell cycle in according to DNA damage, which make it possible for cells to be damaged or returned before reinitiating of DNA replication [5]. In contrast, germline variants of the p53 gene are not regularly recognized, and many differences found about their importance in different malignant tumors. The p53 codon 72 gene are derived by two variants labelled from the replacement of a single nucleotide which is resulted in the attendance of proline or arginine in the protein product [6]. The improvement of cancer has been described by revisions the arginine homozygote in codon 72 because it is a danger feature, whereas others exposed a raised danger to persons with the proline homozygous genotype. The malignant transformation of some detectives, abnormalities, chromosomal and aberrations has been related toward p53 by ovarian endometriosis then can be associated with did not notice the different appearance of p53 in endometriosis [7]. The aim of this research has been determined by the frequency of p53 codon and 72 polymorphisms in PCOS in Karbala city women sterility and other symptoms of the disease.

Materials and Methods

The study was showed during the period from (2019). A cross-sectional study was designed; 70 persons, 40 patients and 30 healthies, aged from (19) to (40) years, the diagnosis was based on the clinical history, presentation long-established by UTS and numerous surveys of PCOS biomarker.

DNA extraction:

Two ml of blood sample collected in EDTA tube for genomic DNA extraction used for molecular analysis. DNA extraction kit was purchased from Gene aid (G sync DNA Extraction Kit).

P53 genotyping:

For each allele double autonomous polymerase chain reaction (PCR) tests were used. Genomic DNA improved by using PCR primers which notice p53 codon 72 in the proline form (178 bp. (5’GCCAGAGGCTGCTCCCCC-3’; 5’ CGTGCAAGTCACAGACTT-3’) and arginine form (142 bp) (5’ T C C C C C T T G C C G T C C C an A - 3’ ;5’ CTGGTGCAAGGGGCCACGC-3’) [8].

The PCR was obtained in a 20-µL final volume PCR (premix™) kit was used to amplify. It contained 2 µl of extracted DNA (1 µl of each primer and of 10 pmol/µl primers were mix in total volume of 20 µl. Then the mixture was added to lyophilized PCR premix formula PCR amplification was carried out (cleaver, USA) thermal cycler using the following protocol: 5 min at 95°C, 30 s at 95°C, 30 s at 58°C for Arg72 and 60°C for Pro72, and 45 Second at 72°C for 35 cycles, with an additional 5 min at 72°C after the last cycle The PCR products were separated on a two percent TBE agarose gel and stained with ethidium bromide. A DNA ladder marker (BIONEER, South Korea) was used to determine the size of DNA fragment.

Result

(PCOS) Poly Cystic Ovarian Syndrome is the most public endocrine complaint of reproductive-age women. The analysis of PCOS is mostly founded on the resulting three components: TSH, FSH, and BMI. Prominently, the diagnostic criteria and complications related to Poly Cystic Ovaries Syndrome PCOS are age-dependent, showed in table-1.

The allelic frequencies in the patient group were Arg 47% and Pro 33%; however, the genotype (P<0.001) and allele (P=0.01) distribution showed significant differences between cases and controls (‘P’ values < 0.05). There was a significant increase of P/P
genotype frequency in Poly Cystic Ovaries infertility that they were compared with healthies. The allele frequency also followed similar trend indicating that ‘P’ allele might confer risk of developing PCOS and ‘R’ allele provides protection against the disease, showed in table2

Table-1: age and hormones related pcos.

<table>
<thead>
<tr>
<th></th>
<th>CASES AVAR± SD NO(40)</th>
<th>CONTROL AVAR± SD NO(30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27.96±5.5</td>
<td>23.00±5.63</td>
</tr>
<tr>
<td>Tsh</td>
<td>2.02±1.01</td>
<td>3.90±1.04</td>
</tr>
<tr>
<td>Fsh</td>
<td>7.21±5.77</td>
<td>10.00±2.11</td>
</tr>
<tr>
<td>Bmi</td>
<td>26.91±5.16</td>
<td>21.9±4.53</td>
</tr>
</tbody>
</table>

Table-2: Distribution of TP53 Codon 72 Genotypes in PCOS Cases and Controls.

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=40) n%</th>
<th>Control (n=35)n%</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pro allele</td>
<td>33(41.25)</td>
<td>3(4.29)</td>
<td>1rf</td>
<td></td>
</tr>
<tr>
<td>arg allele</td>
<td>47(58.75)</td>
<td>67(95.71)</td>
<td>15.6(4.5-54.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p/p</td>
<td>8(20)</td>
<td>0</td>
<td>1rf</td>
<td></td>
</tr>
<tr>
<td>p/r</td>
<td>17(42.5)</td>
<td>3(8.57)</td>
<td>1.41(0.126-15.78)</td>
<td>0.77</td>
</tr>
<tr>
<td>r/r</td>
<td>15(37.5)</td>
<td>32(91.43)</td>
<td>17.06(1.95-149.1)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Discussion**

P53 which are participated in the instruction of glucose are: fatty acid, amino acid (glutaminolysis) and influences mitochondrial integrity and oxidative phosphorylation, insulin sensitivity, antioxidant response, autophagy and mammalian Target of Rapamycin [10]. The unstable production and apoptosis of granulosa cells could result to unusual folliculogenesis [9].

Additionally, the use of oocytes with lacking tumor suppressor genes role may be prematurely eliminated, resultant in initial weakening of egg replacement and, as a result, principal ovarian deficiency [10].

The previous studies say that P/P genotype is related to an enlarged appearance of genes adaptable cell cycle apprehension, which consequently results with the stop of follicle growth [11].

Other Indian study ends with similar conclusion that P/P genotype of the TP53P/R polymorphism was related with developed danger of PCOS, associated to the P/P genotype, in addition to the occurrence of the allele which was expressively higher in Poly Cystic Ovaries Syndrome (PCOS) female than in healthy controls P/P.
variant of R/P polymorphism of TP53 is higher in PCOS patients [12]. These studies agree with our results but we still need extra studies and research in this field, because there are no local or even regional studies [13].

**Ethical Clearance**: Ethics committee refer that there is no plagiarism and there is no mistakes or wrong results or plagiarism in this work.

**Conflict of Interest**: The authors declare that there is no conflict of interest.

**Funding Source**: None

**References**

Immunological and Biological Manifestation of Rheumatoid Arthritis Patient in Iraq

RusulH Ahmad¹, AyaidK Zgair²

¹Instructor, ²Professor Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

Rheumatoid arthritis is one of the highly incidence autoimmune disease. In present study the immunological and clinical biochemical test was checked in patients with RA in Iraq. Here 70 serum specimens were obtained from patients suffer from RA and 30 serum specimens from healthy control cohorts. Antinuclear antibodies (ANA) level, C-reactive protein level, erythrocyte sedimentation rate (ESR), activity of alanine aminotransferase (ALT), activity of aspartate aminotransferase (AST), concentration of blood urea and serum creatininand diseases activity score (DAS)-28 were evaluated in sera of patients and healthy control cohorts. The results showed that significant elevation in level of ANA, ESR, CRP and DAS-28 in patients group as compared to healthy control group (P<0.05). No significant difference between patients group and healthy control group in terms of activity of ALT, AST, concentration of blood urea and serum creatinine. The person test (r) was done to evaluate the correlation between ESR, ANA, CRP, ALT, AST, blood urea and serum creatinine. The results showed significant relation between levels of ESR, CRP and ANA and activity of disease in terms of DAS-28. The results showed no significant relation between level of CRP, ALT, AST, blood urea and serum creatinine in patient’s sera and DAS-28. It can be concluded from present study that the some laboratory parameters related with activity of RA such as ESR, ANA, CRP and other parameters do not related with activity of RA such as ALT, AST, blood urea and serum creatinine.

Key words: Rheumatoid arthritis, DAS28, CRP, ESR, Elisa, AST, ALT.

Introduction

Autoimmune diseases are characterized by intrinsic immune alterations, which may lead to chronic inflammation in multiple organ systems. The disease itself and many of its therapies are related to an elevated risk of serious infections [1]. Virtually every place in the body, including the endocrine system, connective tissue, gastrointestinal tract, heart, skin, and kidneys, can be affected by autoimmune diseases. The direct outcome of an autoimmune reaction is considered to be at least 15 diseases, although circumstantial evidence includes >80 autoimmune disorders [2]. DAS28 is a widely used indicator of the activity of the disease in RA patients. Our goal was to test the DAS28’s long-term reliability rather than its short-term error calculation. In order to estimate the pure instrument (method) error and situational effects of the DAS28 determination, short-term reliability is typically evaluated, while long-term reliability includes all short-term repetition effects plus the effects of nonsystematic changes in disease behavior under stable therapeutic conditions. In a study by Uhlig and colleagues, the short-term variability of DAS28 scores was assessed in 28 patients who were evaluated at 2 points 5-7 days apart by the same qualified study nurse [3]. DAS28 scores range from 0 to 9.4 and are calculated using tender joints, swollen joints, general health, and a laboratory measure of acute inflammation [4]. DAS28 can be characterized using erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP).
Despite the routine use of disease activity scores in guiding treatment, existing guidelines do not specify how cutoffs for high disease activity differ between DAS28-ESR and DAS28-CRP [5].

Some of the most common bacterial infections are urinary tract infections (UTIs), which affect 150 million people worldwide each year [6]. Patients with symptomatic UTI are often treated with antibiotics, which can lead to long-term alteration of the usual vaginal and gastrointestinal micro-biota and the development of multidrug-resistant micro-organisms [7].

Materials and Methods

**Collection of blood sample**

Patients and controls blood were collected by venipuncture; 5 ml of blood was drawn using disposable syringes. The blood was divided into two aliquots. The first aliquot (3 ml) was placed in gel tubes, then left to stand at room temperature (18-25°C) to clot. Sera were separated by centrifugation for 5 minutes at 3000 round per minute (rpm). The separated serum was distributed into 2 aliquots in Eppendorf tube and kept at -20ºC until being assayed [8]. Another 2 ml was place in anticoagulant tubes and use immediately for checking erythrocyte sedimentation rate (ESR).

**ESR**

ESR should be done within 2 h of the collection of the blood. Two hundred microliter of ESR solution (3.8-gm of Trisodium citrate dissolved in 100 mL of distilled water) was mixed with 1.8 ml of blood the Wintrobe ESR tube was filled with mixture of blood and ESR solution. The tube was mounted in the ESR stand. The results were checked after 1 h.

**Serological test (ANA and CRP)**

The instructions of manufacture companies of production of ANA (AESK, USA) and CRP (Linear, Spain) were followed to evaluate the level of CRP and ANA in sera of patients group and healthy control group.

**ALT and AST**

The instructions of manufacture companies that products ALT and AST were followed to evaluate the activity of liver enzymes (Linear, Spain) in sera of patients group and healthy control group.

**Kidney function tests (blood urea and serum creatinine)**

The instructions of manufacture companies that produced blood urea (Linear, Spain) and S. creatinine (Linear, Spain) were followed to evaluate the concentrations of blood urea and s. creatinine in sera of patients group and healthy control group.

**Statistical Analysis**

All data represented in mean and standard deviation the ANOVA test was used to identify the significant difference between patients and control groups The person test (r) was used to detect the relationship between all parametersthat used in present study with disease activity score (DAS)-28 [8,9].

**Result**

The results of the current study showed that the percentage of women that suffer with RA (85.71 %) is much higher than the percentage of men with RA (14.28 %). The average age of patients with RA was 51.92 ± 7.4 year, therefore, the average age of healthy people used in this study was 48.5 ± 6.1 year which is very close to the average of patients with RA in order to achieve an objective and scientific comparison between the group of patients with RA and control group (Table 1).

The present study showed increase in the level of ESR in patients with RA (44.6 ± 25.1 mm/h) when compared to the level of ESR in the healthy control group (16.1 ± 7.2 mm/h). The change in the level of ESR was significant (P<0.001). Significant elevation (P<0.05) in the level of CRP was seen when comparing that with the level of CRP in blood of a healthy control group. Similar significant results was found in case of ANA (Table 1) The results showed shows an increase in the level of ANA in patients with RA (0.29 ± 0.15)
when compared to the level of ANA in the healthy control group (0.13 ± 0.03). The change in the level of ANA was significant (P<0.05).

Table 1. Clinical tests for patients suffering with RA that treated with biological therapy and healthy control.

<table>
<thead>
<tr>
<th>No</th>
<th>Test</th>
<th>Patients (n: 70)</th>
<th>Control (n: 30)</th>
<th>P value</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
<td>51.92 ± 7.4</td>
<td>48.5 ± 6.1</td>
<td>P&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>10</td>
<td>5</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>% of male</td>
<td>14.285</td>
<td>16.667</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>Female</td>
<td>60</td>
<td>25</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>% of female</td>
<td>85.714</td>
<td>83.333</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>6</td>
<td>ESR</td>
<td>44.6 ± 25.1 mm/h</td>
<td>16.1 ± 7.2 mm/h</td>
<td>P&lt;0.001</td>
<td>Sig. difference</td>
</tr>
<tr>
<td>7</td>
<td>CRP</td>
<td>113.7 mg/l ± 112</td>
<td>38.1 mg/l ± 24</td>
<td>P&lt;0.05</td>
<td>Sig. difference</td>
</tr>
<tr>
<td>8</td>
<td>ANA</td>
<td>0.29 ± 0.15 OD. 520</td>
<td>0.13 ± 0.03 OD. 520</td>
<td>P&lt;0.05</td>
<td>Sig. difference</td>
</tr>
<tr>
<td>9</td>
<td>ALT</td>
<td>22.12 ± 16 U/I</td>
<td>21.5 ± 12 U/I</td>
<td>P&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>10</td>
<td>AST</td>
<td>20.17 ± 10.6 U/I</td>
<td>17.97 ± 6.27 U/I</td>
<td>P&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>11</td>
<td>Blood urea</td>
<td>27.9 ± 8.6 mg/dl</td>
<td>25.5 ± 7.8 mg/dl</td>
<td>P&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>12</td>
<td>S. creatinine</td>
<td>0.75 ± 0.21 mg/dl</td>
<td>0.63 ± 0.24 mg/dl</td>
<td>P&gt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure 1 showed significant relationship (P<0.05) between ESR and DAS-28 in patients group and control group. The results showed significant relationship between CRP and DAS-28 in patient group while no significant difference was observed between CRP and DAS-28 in healthy control group.

Figure 1. Relationship between ESR and level of serum C-reactive protein (CRP) in mg/l and DAS-28 value as an indicator of RA activity in patients group (a) and healthy control group (b). The positive and significant relation was found between ESR and CRP with DAS-28 value in RA subjects (P < 0.05).

Figure 2 showed significant relationship between levels of ANA and DAS in patient group (P<0.05) while no significant difference was observed between ANA and DAS-28 in healthy control group (P>0.05).

Figure 2. Relationship between ANA (Optical density at 520 nm) and DAS-28 value as an indicator of RA.
activity in patients group (a) and healthy control group (b). The positive and significant relation was found between ESR and CRP with DAS-28 value in RA subjects (P < 0.05).

Figure 3 showed no significant relationship between enzyme activity of ALT and AST with DAS-28 in patients and healthy control groups (P>0.05).

![Figure 3](image)

**Figure 3.** Relationship between ALT and AST with DAS-28 value as an indicator of RA activity in patients group (a and c) and healthy control group (b and d). No significant relationship was observed (P > 0.05).

Figure 4 showed no significant relationship between concentration of blood urea and S. creatinine with DAS-28 in patients and healthy control groups (P>0.05).

![Figure 4](image)

**Figure 4.** Relationship between blood urea and s. creatinine with DAS-28 value as an indicator of RA activity in patients group (a and c) and healthy control group (b and d). No significant relationship was observed (P > 0.05).
**Discussion**

Several studies indicated that the DAS-28 was higher in patients as compared with control groups\textsuperscript{[5,6]}. The several previous study showed that the clinical laboratory markers is higher in patients with RA as compared with healthy control\textsuperscript{[8]}. Present study showed significant relationship in patients and control group with RA activity that indication ESR and CRP is not specifically associated with the activity of RA\textsuperscript{[10]}.

Identifying and quantifying inflammatory disease activity in rheumatoid arthritis remains a challenge. Many studies have suggested that a large proportion of patients may have active inflammation. Although various disease activity scores had been validated, most rely to a large degree on biomarkers such as CRP and ESR. Using the DAS28-CRP with threshold values validated for DAS28-ESR may lead to errors in the determination of disease activity and therefore may lead to errors in the management of patients with rheumatoid arthritis. That are concomitant with our study in terms that these markers are not specific for the disease activity because we found the correlation between ESR and DAS28 in patients group and in healthy control group as well.

Antinuclear antibodies (ANA) are one of the most essential serological markers used for the diagnose of rheumatic diseases (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), and idiopathic inflammatory myopathy (IIM). The number of ANA positive group was higher than control group. In the ANA positive category, the female rate was clearly higher. Rheumatoid factor (RF) positivity, as well as anti-cyclic citrullinated peptide (CCP) antibody positivity was far higher in the ANA positive group\textsuperscript{[11]}.

The results of present study were agreed with the results that obtained by Hoblet\textit{ et al.}, (2012). The found no serious adverse events were noted. They showed no significant difference in AST in blood of patients suffering from RA and that was not related with DAS-28 because of the small sample size who received an initial oral dose of either 15 or 25 mg MTX per week did not reach the level of statistical significance\textsuperscript{[12]}.

The present study indicates that no relationship between disease activity and level of AST in sera of investigated cohort. The results of present study were in line with previous study of Kumar\textit{ et al.}, (2015). They showed no significant differences between DAS-28 and level of enzyme activity of AST because the using of specific treatment within the study for seven weeks in rheumatoid arthritis patients that is why normal kidney and liver function tests were obtained\textsuperscript{[13]}. The results of this study were coincided with previous study of Mahmoodet\textit{ et al.} (2018), they found that there is no significant differences between DAS-28 and kidney function (blood urea) for patient that administrate Azilsartan with methotrexate because Azilsartan can improve the effects of methotrexate on clinical scores and certain inflammatory biomarkers in patients with active RA\textsuperscript{[14]}. Current study were coincided with previous study of Guadagninet\textit{ et al.}, (2021), they found that no differences between DAS-28 and serum creatinine levels in patient that suffer from RA. That happened because the effectiveness of the combined MTX and LFN treatment is equivalent to that of LFN alone. For the combination treatment, there was no rise in toxicity\textsuperscript{[15]}.

**Conflict of Interest:** The authors declare that there is no conflict of interest regarding this study.

**Fund:** This project was funded by University of Baghdad, Ministry of Higher Edution and Scientific Resaerch.

**Ethical committee approval:** This work was approval by the ethical committee of Department of Biology, College of Science, University of Baghdad.

**References**


Isolation and Identification of Bacterial Burn Wound Infection in Iraqi Patient

Jenan A. Ghafil¹, May. T. Flieh²

¹Lecture, ²Professor, College of Science, Department of Biology, University of Baghdad, Baghdad, Iraq

Abstract

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality. In current study, 120 samples were collected from 120 patients suffering from contaminated burns. The study was conducted after obtaining ethical approvals from the ethics committee in the Department of Biology, College of Science, University of Baghdad as well after obtaining the patients’ consent. Samples are collected from patients after they have stopped using antibiotics for 48 hours. After the swabs had been cultured on different media, conventional biochemical tests to identify bacterial isolates and antimicrobial sensitivity to the most common antibiotics were performed by vitek 2 compact. The results showed that the highest percentage of bacterial species was Proteus mirabilis (31.1%). The percentage of isolation of P. aeruginosa was 17.78%. The lowest percentage of bacterial isolates that isolated from infected wound was found in case of Staphylococcus aureus, Pseudomonas fluorescens, Acinetobacter haemolyticus, Burkholderia cepacia, Salmonella ser. gallinarum, Sphingomonas paucimobilis, Comamonas testosterone with 2.2% for each isolate.

Key word: Burn, wound, Antibiotic, Comamonas testosterone, Sphingomonas paucimobilis

Introduction

Burns are one of the foremost common and destroying shapes of injury. Patients with effective thermal injury require prompt specialized care in arrange to reduce morbidity and mortality. Information from the National Center for Injury Avoidance and Control within the United States appear that roughly 2 million fires were investigated each year which result in 1.2 million individuals with burn wounds [¹]. Direct to extreme burn wounds requiring hospitalization account for roughly hundred thousands of the cases, and around 5% of patients with burn wound infection were died on each year from burn-related complications [²].

The survival rates for burn patients have made strides significantly within the past few decades due to progresses in advanced therapeutic care in specialized burn centers. Improved outcomes for seriously burned patients have been credited to restorative progresses in liquid revival, wholesome back, pneumonic care; burn wound care, and disease control. As a result, burn-related deaths are depending on the degree of damage, have been split inside the past 40 years [³]. In patients with serious burns over more than 40% of the total body surface area (TBSA), 75% of all deaths were related to sepsis from burn wound disease or other contamination complications and/or inhalation injury [⁴].

Microbes quickly colonize open skin wounds after burn damage. Microorganisms colonizing the burn wound start from the patient’s endogenous skin and gastrointestinal and respiratory vegetation. Microorganisms may too be exchanged to a patient’s skin surface by means of contact with contaminated external environmental surfaces, water, air and the
dirtied hands of health care persons [5]. Quickly taking after damage, gram-positive bacteria organisms from the patient’s endogenous skin vegetation or the outside environment transcendentally colonize the burn wound [6]. Endogenous gram-negative microbes from the patient’s gastrointestinal greenery too quickly colonize the burn wound surface within the to begin with few days after damage [7].

Staphylococcus aureus got to be the vital etiological agent of burn wound infection. After the discover penicillin G within the early 1950s, which come about within the virtual reducing of Streptococcus pyogenes as a cause of contamination in thermally harmed patients [8]. In spite of the fact that S. aureus remains a common cause of early burn wound contamination, Pseudomonas aeruginosa and Proteus mirabilis from the patient’s endogenous gastrointestinal vegetation and/or a natural source is the foremost common cause of burn wound contaminations in numerous centers. The rate of diseases due to less commonly experienced organisms, counting other gram-positive and gram-negative microscopic organisms, fungi, and viruses, has too expanded relentlessly in subsequent decades [9].

Material and Method

Specimen Collection

In current study, 120 samples were collected from 120 patients suffering from contaminated burns. Samples were collected under sterile conditions using sterile swabs. The samples were immediately transported to the laboratory to be implanted in the appropriate media.

The average age of the patients was 42.6 ± 5.8 years. The number of males was 72 and the number of females 48. The study was conducted after obtaining ethical approvals from the ethics committee in the Department of Biology, College of Science, University of Baghdad as well after obtaining the patients’ consent. Samples are collected from patients after they have stopped using antibiotics for 48 hours.

Bacterial isolation

The collected samples were cultured on MacConkey agar, Blood agar under aerobic and sterile conditions. To diagnose the isolated bacteria the select colonies were re-cultured on mannitol salt agar, SS agar, nutrient agar, XLD agar and EMB agar. For further identification of isolated bacteria catalase test, oxidase test and Gram stain were used to identify the pure isolated bacteria [10].

Microscopic Examination

The morphological identification of the isolates as bacilli was confirmed microscopically by performing Gram staining, for which single colony of each isolate was picked up and stained as per the standard protocol and viewed under oil immersion for similar type of cells.

Catalase test

The collected samples were cultured on MacConkey agar, Blood agar under aerobic condition and sterile conditions, use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide then Place a drop of 3% H2O2 in the glass slide the result observed for the evolution of oxygen bubbles [10].

Oxidase test

The collected samples were cultured on MacConkey agar, Blood agar under aerobic condition and sterile conditions, strip of Whatman’s No. 1 filter paper are soaked in a freshly prepared 1% solution of tetramethylp-phenylene-diaminedihydrochloride, After draining for about 30 seconds, the strips are freeze dried and stored in a dark bottle tightly sealed with a screw cap, for use, a strip is removed, laid in a petri dish and moistened with distilled water. The colony to be tested is picked up with a platinum loop and smeared over the moist area. A positive reaction is indicated by an intense deep-purple hue, appearing within 5-10 seconds, a “delayed positive” reaction by colouration in 10-60 seconds, and a negative reaction by absence of colouration or by colouration later than 60 seconds [10].
Identification using the VITEK 2 fluorescent system (ID-GNB card)

The VITEK 2 DensiCheck instrument, fluorescence system (bioMérieux) (ID-GBB card and ID- GNB card) includes 43 non enterobacterial gram-negative taxa and gram positive. Testing was performed according to the instructions of the manufacturer. Briefly, strains were cultured on nutrient agar for 18 to 24 h at 37°C before the isolate was subjected to analysis. A bacterial suspension was adjusted to a McFarland standard of 0.50 to 0.63 in a solution of 0.45% sodium chloride using the VITEK 2 DensiCheck instrument (bioMérieux). The time between preparation of the solution and filling of the card was always less than 1 h. Analysis was done using the identification card for gram-negative and gram positive bacteria (ID-GNB card) and (ID-GBB) containing 41 fluorescent biochemical tests. Cards are automatically read every 15 min. Data were analyzed using the VITEK 2 software version VT2- R03.1 [11].

Antibiotic susceptibility

The standard method of Mazzarioiet al. (2008) was followed to test the susceptibility of identified bacteria to the several antibiotics (Cefotaxime, Ampicillin, amoxicillin/Clavulanic acid, ampicillin/Sulbactam, Piperacillin/ Tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, imipenem, gentamicin, tobramycin, ciprofloxacin, levofloxacin, nitrofurantoin, trimethoprim/sulfamethoxazole, ticarcillin, amikacin). VITEK 2 DensiCheck instrument (bioMérieux) was used to check the supportability of isolated and identified bacteria [12].

Results and Discussion

Isolation and identification of bacterial species

In present study, 120 swabs were collected from infected burns. The samples were collected from 120 patients. The swabs were inoculated onto number of culture media (Blood agar, MacConkey agar, Manitol salt agar, SS agar and XLD agar) for growing and isolating and then for pre-identification. Most of isolates were grown on blood agar with different shape according to the genera of isolates. The suspected staphylococcus isolates were cultured onto mannitol salt agar to identify the staphylococcus species. Some of isolates were grown on MacConkey agar with pale or pink color. The bacteria that grown onto MacConkey agar with pale color were cultured onto SS agar and XLD to identify whether they were Salmonella or Shigella isolates [13]. Total pre-identified species of bacteria was 50 species but when further identification was done by VITIK 2 technology, only 45 species was identified and 5 was specified as unidentified organism. Thus the further study was done only on the 45 species that isolated from infected wound and identified by VITIK 2 technology (Table 1). The results showed that the highest percentage of bacterial species was Proteus mirabilis (31.1 %). The percentage of isolation of P. aeruginosa was 17.78%. The lowest percentage of bacterial isolates that isolated from infected wound was found in case of Staphylococcus aureus, Pseudomonas fluorescens, Acinetobacterhaemolyticus, Burkholderiacepacia, Salmonella ser. gallinarum, Sphingomonaspaucimobilis, Comamonas testosterone with 2.2 % for each isolate .

Previous study of Forsonet al. (2017) mentioned that P. aeruginosa represented the highest percentage of bacterial species that isolated from burn wound, while Church et al. (2006) reported that the highest percentage of bacterial species that isolated from infected burn wound was P. aeruginosa followed by E. coli and the lowest percentage was found in case of Acinetobacterspp and Bacteroides spp. Similar finding was reported by other investigators[16].
Table 1: Number and percentage of bacterial species that isolated from 120 clinical samples.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Proteus mirabilis</td>
<td>14 (Pm1, Pm2, Pm3, Pm4, Pm5, Pm6, Pm7, Pm8, Pm9, Pm10, Pm11, Pm12, Pm13, Pm 14)</td>
<td>31.1</td>
</tr>
<tr>
<td>2  Escherichia coli</td>
<td>10 (Ec1, Ec2, Ec3, Ec4, Ec5, Ec6, Ec7, Ec8, Ec9, Ec10)</td>
<td>22.2</td>
</tr>
<tr>
<td>3  Pseudomonas aeruginosa</td>
<td>8 (Pa1, Pa2, Pa3, Pa4, Pa5, Pa6, Pa7, Pa8)</td>
<td>17.78</td>
</tr>
<tr>
<td>4  Klebsiella pneumoniae</td>
<td>2 (Kp1, Kp2)</td>
<td>4.4</td>
</tr>
<tr>
<td>5  Serratia ficaria</td>
<td>2 (Sf1, Sf2)</td>
<td>4.4</td>
</tr>
<tr>
<td>6  Burkholderia mallei</td>
<td>2 (Bm1, Bm2)</td>
<td>4.4</td>
</tr>
<tr>
<td>7  Staphylococcus aureus</td>
<td>1 (Sa1)</td>
<td>2.2</td>
</tr>
<tr>
<td>8  Pseudomonas fluorescens</td>
<td>1 (Pf1)</td>
<td>2.2</td>
</tr>
<tr>
<td>9  Acinetobacter haemolyticus</td>
<td>1 (Ah1)</td>
<td>2.2</td>
</tr>
<tr>
<td>10 Burkholderiacepacia</td>
<td>1 (Bc1)</td>
<td>2.2</td>
</tr>
<tr>
<td>11 Salmonella ser. Gallinarum</td>
<td>1 (Sg1)</td>
<td>2.2</td>
</tr>
<tr>
<td>12 Sphingomonas paucimobilis</td>
<td>1 (Sp1)</td>
<td>2.2</td>
</tr>
<tr>
<td>13 Comamonas testosterone</td>
<td>1 (Ct1)</td>
<td>2.2</td>
</tr>
</tbody>
</table>

The diversity of bacteria species isolated from infected wounds was one of the features that distinguished the present study. Bacteria rapidly colonize open skin wounds after burn injury. Microorganisms colonizing the burn wound originate from the patient’s endogenous skin and gastrointestinal and respiratory flora [17]. Microorganisms may also be transferred to a patient’s skin surface via contact with contaminated external environmental surfaces, water, fomites, air, and the soiled hands of health care workers [15]. Immediately following injury, gram-positive bacteria from the patient’s endogenous skin flora or the external environment predominantly colonize the burn wound [18]. Endogenous gram-negative bacteria from the patient’s gastrointestinal flora also rapidly colonize the burn wound surface in the first few days after injury [15]. Microorganisms transmitted from the hospital environment tend to be more resistant to antimicrobial agents than those originating from the patient’s normal flora [19].

Previous study, 185 (61.87%) bacteria were isolated from the wounds of burnt patients. Among the culture positive samples, 112 (60.54%) were from female patients and 73 (39.46%) were from male patients. The most commonly isolated organisms were Pseudomonas species (43%). K. pneumoniae and A. baumannii were second and third predominant bacterial pathogen with a prevalence of 28% and 14.83% respectively. Similar finding with P. aeruginosa a predominant isolate followed by K. pneumoniae and A. baumannii in tertiary care hospital in India were also reported [20]. High
prevalence of these pathogens is associated with their ability to flourish well in a moist environment and persistence in hospital environment [20]. In present study, P. aeruginosa was reported as one of domain species that isolated from burn wound infection.

**Antibiotic susceptibility**

The susceptibility of 45 isolates to different antibiotics was done by VITIK 2 DensiCheck instrument. The antibiotics that used were different according to the group of species of bacteria because the routinely antibiotic that used clinically was different according to the clinical cases and species [21] that covered in the study.

The current results showed that the effect of antibiotics varies greatly according to the species of bacterial used and the type of antibiotics. Where, many types of antibiotics were used in present study. Through an overview of the results, it can be confirmed that there are no bacterial species sensitive to all antibiotics used, and no bacterial isolate that resists to all antibiotics . Figure (1) shows that the P. mirabilis gave the highest percentage of resistance to different kinds of antibiotic, followed by the P. aeruginosa. While, the lowest percentage of sensitivity to different kind of antibiotics was shared among S. paucimobilis: C. testosterone and B. mallei. The present study showed that the highest percentage of intermediate response of bacteria to antibiotics was seen in case of S. paucimobilis followed by P.fluorescens.

Nosocomial infection in the burnt patients is major challenge for a clinician. It has been estimated that 75% of all deaths in burnt patients were associated with infections. Prolonged use of antibiotic leads to the development as well as selection of multidrug resistant (MDR) bacteria which results in treatment failure and intensifies the complications. Thus, the information of microbial flora and the current antibiotic susceptibility patterns are important for the clinician treating burn sepsis [21].

![Figure 1](image_url)

**Figure 1:** The percentages of susceptibility of different species of bacteria to different kinds of antibiotics (P.m: P. mirabilis, E.c: E. coli, P.a: P. aeruginosa, K.p: K. pneumonia, S.f: S. ficaria; B.m: B. mallei, S.a: S. aureus; P.f: P. fluorescens, A.h:A. haemolyticus, B.c: B. cepacia, S.g:S. gallinarum, S.p: S. paucimobilis, C.t: C. testosterone.)
Conflict of Interest: The authors declare that there is no conflict of interest regarding this study.

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Evaluation the Genotoxicity of PHB Nanoparticle by Micronucleus Assay

Jenan A. Ghafil¹, May. T. Flieh²
¹Lecture, ²Professor, College of Science, Department of Biology, University of Baghdad, Baghdad, Iraq

Abstract

Determining the toxicity of substances in vivo is one of the most important tests that judge whether or not they are used in the pharmaceutical field. In the present study, the genetic toxicity of treatment doses of Polyhydroxybutyrate (PHB), PHB nanoparticles, Cefotaxime and complex of PHB nanoparticles and cefotaxime was evaluated. The effect of these substances on the number and percentage of white blood cells (WBCs) in mice was also tested (in vivo). Micronucleus assay was used to assess genotoxicity of above materials in vivo, as well as the technique of WBCs chamber was used to estimate the total number of WBCs in mice administrated with above substances. The differential count of WBCs was measured by staining the smears with leishman stain. The present study demonstrated that there were no significant differences (P>0.05) in the number of micronucleus cells in the mice injected with treatment doses of PHB nanoparticles, Cefotaxime and complex of PHB nanoparticles and cefotaxime when compared with mice injected with normal saline. Similar finding was obtained in terms of counting of total WBCs and differential count in mice injected with treatment doses of PHB, PHB nanoparticles, Cefotaxime and complex of PHB nanoparticles and cefotaxime when compared with WBCs total count and differential count in mice injected with normal saline (P>0.05). It can be concluded that there is no toxic effect of treatment doses of PHB, PHB nanoparticles, Cefotaxime and complex of PHB nanoparticles and cefotaxime on mice.

Keyword: Polyhydroxybutyrate, micronucleus assay, Genotoxicity.

Introduction

Polyhydroxyalcanoate (PHB), a polymer belonging to the polyester classes, is a biodegradable and biodegradable plastic of interest. The poly-3-hydroxybiotrate (P3HP) form of PHB is probably a polyhydroxyalconoate type, but other polymers of this class are produced by a variety of organisms: including poly-4-hydroxybiotrate (P4HPHP).Polyhydroxyhexanonate (PHH), polyhydroxyoctanone (PHO) and their copolymers [1].

In the medical field, many drugs are used to treat bacterial infections [2], and this requires determining their toxicity. The same applies to the PHB, as it has many applications in vivo, including anti-bacterial [3], as well as to contribute to limiting the activity of some diseases such as cancer in the human body [4]. To determine the possibility of its use, it is required to determine cytotoxicity of PHB.

There are a number of methods by which it is possible to determine the extent of the body’s response to foreign substances, including by measuring the number and percentage of white blood cells [5]. These are useful for determining the state of the immune and cellular system in case the body is exposed to foreign substances [5].

There are many methods that can be used to determine the genetic toxicity of medicinal materials, the most important and the most common of which is the Micronucleus assay [6]. A micronucleus (MNs) test is a test used in toxicological screening for...
potential genotoxic compounds. The assay is now recognized as one of the most successful and reliable assays for genotoxic carcinogens. This test is based on the formation of number of MN in treated cells \(^7\). MNs are formed during anaphase from chromosomal fragments or whole chromosomes that are left behind when the nucleus divides. Over time, the assay has evolved to include a pretreatment with cytochalasin-B (Cyt-B), a cytokinesis blocking agent that inhibits cell division, thereby giving the cells a binucleated appearance. This enables more accurate scoring and the ability to sieve out the dividing cells from the non-dividing ones, thereby reducing the incidence of false positives\(^7\).

In the absence of a previous study dealing with the effect of PHB nanoparticles on the state of white blood cells in the body as well as the genotoxic effect of this substance, so the genotoxicity of PHB nanoparticles in vivo was evaluated by using the micronucleus assay and the effect of PHB nanoparticles on the status of white blood cells in terms of number of cells and percentage in vivo was evaluated by using animal models (mice).

Material and method

Polyhydroxybutyrate nanoparticles

Polyhydroxybutyrate (PHB) as a powder was purchase from Sigma-Aldrich, USA the PHB was derived from microbial fermentation. The preparation of PHB nanoparticles was prepared by adding 1 gm of PHB to 50 ml of distilled water and pH was adjusted to 4 by HCl (1 N). The mixture was put in ultrasonic path at 4500 kh for 25 second. The pH was readjusted to 10 by NaOH (1N). The mixture was mixed by magnetic stirrer for 2 h at 21 °C. The mixture was incubated at 21 °C for 18 h and then the pH was readjusted to 7 by HCl (1 N). The synthesis of PHB nanoparticles was evaluated by Atomic force microscopy (AFM), Fourier-transform infrared spectroscopy (FTIR), Ultraviolet (UV) spectrophotometer, X-ray powder diffraction (XRD) and Scanning electron microscopy (SEM).

Mice

BALB/c mice 6–8 weeks old, weighing 20–25 gm was procured from central animal house, AL-Nahrain University, Baghdad, Iraq. Animals were kept in clean polypropylene cages and fed on standard antibiotic free diet. The mice that used in current study were male.

Micronucleus assay

The standard method of Sousa et al. (2016)\(^8\) with little medication was followed to evaluate Percentages of micronucleated polychromatic erythrocytes (PCEMNs) in bone marrow of mice after 48 hours of injection subcutaneous with Polyhydroxybutyrate (PHB), PHB nanoparticles and PHB nanoparticles plus cefotaxime (complex). Briefly, four groups of mice were used in this experiment. Each group consisted of three mice. Group A, mice injected under skin with treatment dose of PHB (1 mg). Group B, mice injected under skin with treatment dose of PHB nanoparticles (1 mg). Group C, mice injected under skin with treatment dose of PHB nanoparticles + cefotaxime (500 µg). Group D (Control group), mice injected under skin with normal saline. The mice were sacrificed 48 hours post administration of treatment doses of above materials under skin. All mice were dissected. Bone marrow cells were collected immediately after the sacrifice of animals. Leg bones of mice were collected and homogenized by mortar aseptically. The bone marrows were collected from homogenised bones. In a Falcon tube previously marked with the animal group. Bone marrow material was re-suspended with fetal bovine serum until homogeneous. The suspension was centrifuged for 5 minutes at 1,000 rpm and the supernatant was discarded and the pellet was re-washed three times with fetal bovine serum. At the end of procedure the pellet was re-suspended with 500 µl of fetal bovine serum then the tube was homogenization gently and smears were prepared dripping off 2 drops of suspension on the tip of a slide (previously labelled with the animal’s group) and with the aid of another slide bent at a 45 degree angle to make the smear and the slides were air dried, two slides per animal were made and stained by leishman stain\(^9\).
The analysis was performed in blind field in an increase of 100x (immersion objective) in a short time by the same observer. Micronuclei were measured in 2,000 polychromatic erythrocytes (PCEs) / animal in bone marrow of adult mice.

**White blood cells (WBCs) total count and differential count**

The standard method of Liu et al. (2020) with little modification was followed to count the number of WBCs in peripheral blood that collected by anticoagulant capillary tube (heparin) inserted in the lateral canthus from the retroorbital sinus of mice. The specific WBC pipette and WBCs solution were used. The blood specimen was diluted 1:20 in a WBC pipette with the diluting fluid (WBCs solution) and the cells were counted under low power of the microscope by using a counting chamber. The number of cells in undiluted blood is reported per microliter of whole blood\(^{[10]}\). The standard method of Nurhayati et al. (2019) was followed with little modification to be in line with approach of the present study\(^{[11]}\) to measure the percentages of WBCs in peripheral blood of mice post smear preparation and stained witleishman stain. The WBCs total count and the percentages of different types of WBCs were measured in blood that collected from different mice groups. Group A, mice injected under skin with treatment dose of PHB. Group B, mice injected under skin with treatment dose of PHB nanoparticles. Group C, mice injected under skin with treatment dose of PHB nanoparticles + cefotaxime. Group D (Control group), mice injected under skin with normal saline.

**Statistical Analysis**

All values have been taken as mean value and standard deviation (SD) calculated. The differences were analyzed using Student’s t-test employing Origin version 8.0 software. A value of \(P < 0.05\) was considered to be statistically significant.

**Result**

**Micronucleus assay**

To ensure the presence or absence of the toxic effect of PHB, PHB nanoparticles, PHB nanoparticles plus cefotaxime, the percentage of micronucleated polychromatic erythrocytes (PCEMNs) in the bone marrow of mice was estimated after they were injected with the therapeutic dose of the following substances, PHB (1 mg) and PHB nanoparticles (1 mg) and the complex consisting of a mixture of PHB nanoparticles plus cefotaxime (0.5 mg). The results of number of PCEMNs in bone marrow of upper groups were compared with a number of PCEMNs in bone marrow of control group (mice injected with normal saline).

The results showed that there were no significant differences in number of PCEMNs in bone marrow of treat groups of mice and number of PCEMNs in bone marrow of control group of mice (\(P < 0.05\)) (Fig. 1). This finding proves that no toxic effect of treatment dose of PHB or PHB nanoparticles or PHB nanoparticles plus cefotaxime (complex). The micronucleus test suggested that the PHB, PHB nanoparticles and PHB nanoparticles plus cefotaxime have no biological toxicity in order to not change the incidence of polychromatic erythrocytes. That confirmed the safety of using of these compounds in vivo.
Figure 1 a, Percentages of micronucleated polychromatic erythrocytes (PCEMNs) in bone marrow of mice after 48 hours post injected subcutaneous with normal saline (control), Polyhydroxybutyrate (PHB), PHB nanoparticles and PHB nanoparticles plus cefotaxime (complex). NS, non significant difference from control group. b, micronucleus in PCEMN.

White blood cells (WBCs) total count and differential count

Fig. 2 shows the number of WBCs in blood of different groups of mice post administrating with treatment dose of PHB (1 mg/kg), PHB nanoparticles (1 mg/kg), PHB nanoparticles plus cefotaxime (0.5 mg/kg) and 0.5 mg/kg of cefotaxime. The results showed no significant difference (P<0.05) between any experimental groups with control. This finding proves that the administration of treatment dose of above materials do not effect on the number of WBCs in vivo.
After calculating the total number of leukocytes (WBCs) in peripheral blood of different groups of mice, the percentages of polymorphonuclear cells (neutrophil, eosinophil and basophil) and mononuclear cells (monocytes and lymphocytes) were calculated in the peripheral blood taken from retro-orbital sinus of mice eyes. The percentages of polymorphonuclear cells and mononuclear cells were calculated in blood samples of groups of mice administrated orally with treatment dose of PHB (1 mg/kg), PHB nanoparticles (1 mg/kg), PHB nanoparticles plus cefotaxime (1 mg/kg), and cefotaxime (1 mg/kg). Fig. 3 shows no significant difference (P<0.05) in percentages of all types of leukocytes [polymorphonuclear cells (neutrophil, eosinophil and basophil) and mononuclear cells (monocytes and lymphocytes)] that obtained from all peripheral blood of experimental groups of mice as compared with percentages of all types of leukocytes that obtained from blood of control group (mice administered orally with normal saline).

![Figure 3](image_url)

**Figure 3.** Differential count of leukocytes that collected from retro-orbital sinus of mice eyes and smeared on slides and stained with leishman stain. There is no significant difference in percentages of all types of leukocytes that obtained from different groups of mice that administrated with treatment dose of PHB, PHB nanoparticles and PHB nanoparticles plus cefotaxime (complex) and cefotaxime. The results were compared with corresponding cells in blood samples that collected from control group.

**Discussion**

During almost 40 years of use, the micronucleus assay (MN) has become one of the most popular methods to assess genotoxicity of different chemical and physical factors, including ionizing radiation-induced DNA damage [7]. In a modern world, humans are exposed to different genotoxic agents present in the polluted environment. Hence, tests are needed to determine the level of exposure and health risk. Although many tests classified as “in vivo biomonitoring” are available, a micronucleus test (MN) is one of the best and the most popular [12]. The assay is also widely used to test genotoxicity in vitro (7). Many previous studies used micronucleus assay (MN) to detect the toxicity of some of the materials used in different field such as therapeutic purpose such as drugs or as a food [13].
There are very few studies assessed the genotoxicity of PHB in vitro or in vivo. It was found only one study focused on this issue. de Sousa et al. (2016) checked the toxicity of membranePHB/Norbixin/Ethylene glycol by micronucleus test and they found that there is no toxicity in the natural material of membranePHB/Norbixin/Ethylene glycol and they can be used for biological purposes and these will contribute to future studies on the effects of the membrane on the healing of skin wounds[8]. This finding is matching with what we got in our study about the safety of PHB that is why we also suggest the possibility of using this material as a medicine for treatment in future.

Estimating the number of white blood cells in the blood is one of the indicators of the state of the immune system, since these cells represent the cellular arm of the immune response. Therefore, estimating its levels gives an indication of the body’s response to an external stimulus, as well as a non-specialized picture of the state of the immune system. Estimating the number and percentage of Polymorphonuclear cells such as neutrophil, eosinophils and basophil and mononuclear cells such as lymphocytes and monocytes gives a more specialized picture of the state of the immune response after the occurrence of infection. However, these two indicators represents non-specific indicators through which it is not possible to determine the nature of the response and for which disease factor, even these to indicators will not give accuracy answer to the causative of the disease but they give general picture about the status of immune response. In present study the total number of WBCs and percentages of these cells were evaluated post PHB, PHB nanoparticles and PHB nanoparticles plus cefotaxime administration to mice orally.

Through searching the literature on the effect of PHB (nanoparticles and non nanoparticles) on the total number of leukocytes (WBCs) in laboratory animals, no article was found dealing with this topic, which supports that the present study is the first study that deals with the effect of PHB, PHB nanoparticles, PHB nanoparticles plus cefotaxime on the total number of leukocytes in peripheral blood of laboratory animals (mice). The finding of no effect of PHB in different form on the total numbers of white blood cells in laboratory animals confirms the safety of using these materials in treatment, and this opens the door for new research dealing with the possibility of using these materials in treatments, especially with regard to infectious diseases.

According to our knowledge there is no published literature covered the effect of PHB (nanoparticles and non nanoparticles) on the percentages of leukocytes [polymorphonuclear cells (neutrophil, eosinophil and basophil) and mononuclear cells (monocytes and lymphocytes)] in laboratory animals. That supports the present study as the first study that deals with the effect of PHB, PHB nanoparticles, PHB nanoparticles plus cefotaxime on the differential count of leukocytes in peripheral blood of laboratory animals (mice).

Several previous studies focused on the effect treatment doses of different medicine especially antibiotics on the number and percentages of polymorphonuclear. Shuman et al. (2012) have identified one category of medications that may cause decreased white blood cell/absolute neutrophil counts when combined with clozapine[14]. Their study supported the use of either ciprofloxacin or moxifloxacin as agents that may have less risk of reductions in white blood cell/absolute neutrophil counts than are seen with penicillins, cephalosporins, and other antibiotics that may ultimately require interruption or discontinuation of clozapine therapy. Similar finding was reported by Nguyen et al (2016) they found that using antibiotics increase the maturation of neutrophil and that reflect on the total count of leukocytes as well[15]. Previous study support strongly that the total count of leukocytes and differential count of all types of leukocytes in blood gives a good indication about the safety of the substances such as drugs or food to be administrated by human and animal [16, 17]. That is why; in this study it was focused on the differential count of leukocytes in peripheral blood of mice post administration with PHB to help in identifying the safety of these substances.
The present study proved that oral administrated of with treatment dose of PHB (1 mg/ kg), PHB nanoparticles (1 mg/ kg), PHB nanoparticles plus cefotaxime (1 mg/ kg), and cefotaxime (1 mg/ kg) do not effect on the total count of leukocytes and percentage of all types of leukocytes. That give another evidence about the safety of these substances (PHB, PHB nanoparticles, PHB nanoparticles plus cefotaxime and cefotaxime) to be using as a treatment substance and that attract us to go further in using of these substances in treating the burn mice model prepared in our laboratory.

Conflict of Interest: The authors declare that there is no conflict of interest regarding this study.

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References


Molecular Screening of KI and WU Polyomaviruses among Patients with Chronic Kidney Disease and Urinary Tract Infections

Manar Hussein AL-Aboudy1, Musa Nima AL- Jiafry2, Mahdi Hussein Al-Ammar2
1Research Scholar, Department of Pharmacy/ College of Pharmacy/ The Islamic University;
2Professor, Department of Biology / Faculty of Science / University of Kufa

Abstract

KIPyV and WUPyV were recognized based on commercial standard and PCR techniques. To occurrence of KIPyV and WUPyV DNA among chronic kidney disease (CKD) and UTI patients using VP2 gene for detection of KIPyV and VP1 gene for WUPyV. Molecular assay results of this study revealed the presence of KIPyV in 6 (5.3%) of CKD patients and 3 (2.7%) of UTI patients while the presence of WUPyV was 1 (0.88%) in CKD patients and negative result in UTI patients. WUPyV DNA and KIPyV DNA was not detected in plasma of healthy persons. The distribution of KIPyV and WUPyV according to gender among CKD showed a no significance difference among both sexes in which (P value = 0.27) and (P value = 0.36) respectively while the distribution of KIPyV and WUPyV among urinary tract infections showed a no significance difference among both sexes in which (P value = 0.13) and (P value = 0.79) respectively .The current study survey about both viruses show that female was higher than male in KIPyV in which 2 (3.22%) of KIPyV DNA were detected in male and 4 (7.84%) in female out of 113 (100%) among chronic kidney disease patients while in urinary tract infection, patients revealed a higher percentage rate of KIPyV in female than male in which 0 (0%) of KIPyV DNA were in male and 3 (4.54%) in female out of 111 (100%) while doesn’t detect any isolate of WUPyV in both sexes.

Keywords: KIPyV, Chronic kidney disease, WUPyV, Urinary tract infections.

Introduction

Polyomaviruses are another type of emerging pathogens that generally cause infection to the urinary tract of humans (1). Primary infection of polyomavirus happens in childhood and it persists for the whole life of the persons, especially in the epithelial cells of the kidneys and urinary tract as well as leukocytes in the blood (2). In the secretions of children with acute respiratory symptoms using high-throughput sequencing technologies, two forms of polyomaviruses have recently been identified, one known as Karolinska Institute Polyomavirus (KIPyV) and the other named Washington University polyomavirus (WUPyV) (3).

The site of persistent infection of these two viruses across the lifespan still unclear (4). These two viruses are closely related to each other than to SV40, BK and JC, but, as shown by the amino acid identity and phylogenetic analysis ratio, they vary greatly from each other (5). Seroepidemiological surveys have shown that the initial infection with KIPyV and WUPyV that occurs early in life could be via the respiratory and oral-fecal pathways, similar to BK and JC polyomaviruses (6). A high rate of co-infection with other major respiratory viruses, such as influenza viruses, parainfluenza viruses, adenoviruses, respiratory syncytial viruses, rhinoviruses,
human coronaviruses, human bocaviruses, and human metapneumoviruses is typically associated with the identification of the genome of these two viruses in respiratory tract samples (7).

In the view of the above mentioned introduction, the design of this study to determine the presence of KIPyV and WUPyV in patients suffering from UTI and CKD in AL-Najaf city-Iraq.

**Materials and Method**

A total number of 224 clinical samples were collected from patients who suffer from chronic kidney disease (CKD) and urinary tract infections (UTI) at age range (15-80 years) and 100 healthy persons as controls at age range (25–65) years. All subjects were admitted to one of the biggest Hospital including Al-Sadr Medical City as well as some chief clinical laboratories in Al-Najaf City/Iraq within the time two months started in January 2020. The patients in this study included 107 males and 117 females while healthy controls was 50 males and 50 females. Blood plasma were collected from each participant was used to extract DNA for detection of KIPyV and WUPyV.

The complete genomic DNA of 113 patients with CKD and 111 of patients with UTI was extracted employing a traditional kit of the entire genomic DNA extraction (iNtRoN, Biotech. Inc., Korea), wheressoever, the extraction was performed based on the guidance of manufacture corporation. The nucleic acid was conserved under -20°C state using the deep freezing device, the PCR technique was employed to examine and detect all the genes described in table (1) that suggested by Gozalo-Margüello et al (2015)(8). The process of gel document (Cleaver, United Kindom), demanded to check and distribute the migration of PCR bands applying 1% agarose (iNtRoN, Biotech. Inc., Korea), back dyeing the gel with ethidium bromide at 0.5 μg/ml concentration.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Primer</th>
<th>Sequence (5'-3')</th>
<th>Product size bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIPyV</td>
<td>VP2-F</td>
<td>CGTCATACTTGCCCCAGTTG</td>
<td>378-bp</td>
</tr>
<tr>
<td></td>
<td>VP2-R</td>
<td>CATCTTGGGCGAGGCTTGAA</td>
<td></td>
</tr>
<tr>
<td>WUPyV</td>
<td>VP1-F</td>
<td>GCCGTACACTGTCAGAGGA</td>
<td>546-bp</td>
</tr>
<tr>
<td></td>
<td>VP1-R</td>
<td>TCTGCAGTTATCATGCGGC</td>
<td></td>
</tr>
</tbody>
</table>

**Statistical Analysis**

The SPSS V.24 program was used in statistical analysis of the data. Chi- Square was used to compare and extract duplicates and percentages

**Result and Discussion**

This study revealed that WUPyV and KIPyV can be detected in both plasma samples from UTI and chronic kidney disease patients, but these viruses were not found in plasma of healthy blood donors. A higher prevalence of KIPyV was observed compared with WUPyV (9/10 vs. 1/10).

Detection of KI and WU polyomavirus in plasma of both groups occurred by using sensitive molecular techniques which include conventional PCR as showed in figure (1). To our knowledge, there is limited studies have been published on KIPyV and WUPyV using conventional PCR in UTI and chronic kidney disease...
patients and this considered the first study that conducted locally. Co-infection with other pathogens has been reported in 74% of KIPyV patients, 68% to 79% of WUPyV patients, and 10% of KIPyV and WUPyV co-infections in the absence of other respiratory viruses\(^9\). In this study, we investigated the KIPyV and WUPyV in the absence of respiratory tract infections.

Neither WUPyV DNA nor KIPyV DNA was detected in plasma of healthy persons in the current study as shown in Table (2). This results is similar to Csoma et al., (2011)\(^{10}\) which doesnot detect these virus in healthy subjects. It also in line with recent study conducted by Kamminga et al., (2019)\(^{11}\) which detected only one of WUPyV DNA in healthy blood donors but contrast to Šroller et al., (2015)\(^{12}\) which revealed about 58% of KIPyV in serum of healthy blood donors as well as to Song et al., (2016)\(^{13}\) which reported 12.5% of KIPyV VP1 DNA in blood specimens from healthy individuals. The role of the KIPyV and WUPyV in the pathogenesis of the urinary tract and their effect on kidney function is not well explained. Some investigations have shown the presence of both these viruses in asymptomatic people\(^{(14,15)}\).

**Prevalence of KIPyV and WUPyV in study groups**

The KIPyV DNA was detected in (6 out of 113) or (5.3%) in patients with chronic kidney disease, and (3 out of 111) or (2.7%) in patients with urinary tract infection while in control group was (0 out of 100). There was no significant difference (Chi-square = 3.5 ; P value = 0.16) among study groups (CKD patients, UTI patients and control group) as shown in Table (2).
Table (2). Prevalence of KIPyV and WUPyV in study groups

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Control Group (N= 100)</th>
<th>UTI Patients (N= 111)</th>
<th>CKD Patients (N= 113)</th>
<th>Chi Square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIPyV</td>
<td>Positive 0 (0%)</td>
<td>3 (8.20%)</td>
<td>6 (5.3%)</td>
<td>3.5</td>
<td>0.16 NS</td>
</tr>
<tr>
<td></td>
<td>Negative 100 (100%)</td>
<td>108 (91.8%)</td>
<td>107 (94.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WUPyV</td>
<td>Positive 0 (0%)</td>
<td>0 (0%)</td>
<td>1 (0.8%)</td>
<td>0.77</td>
<td>0.67 NS</td>
</tr>
<tr>
<td></td>
<td>Negative 100 (100%)</td>
<td>111 (100%)</td>
<td>112 (99.2%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The present study detected 6 (5.3%) out of 113 (100%) of KIHPyV DNA in chronic kidney disease patients. These result is similar to Csoma et al., (2011) (10) which detect (7/195) to KIHPyV in plasma of renal transplant patients and to Csoma et al., (2014) (16) which revealed the occurrence of KIHPyV in plasma of renal transplant patients also to Badura et al., (2014) (17) and Hansen-Estruch et al., (2018) (18). On the other hand the present result is incompatible to Porrovecchio et al., (2013) (19) which seen that All plasma samples from transplant patients were negative for KIPyV DNA. The present study revealed about 3 (2.7%) out of 111 (100%) were positive to KIHPyV DNA in plasma of urinary tract infection patients. These result is similar to csoma et al., (2012) (20) which detected KIPyV DNA in two plasma samples from non-pregnant women out of 200 plasma samples and to Csoma et al., (2015) (21) but is unsimilar to Limam et al., (2020) (22) which not detected any KIPyV out of 112 from Tunisian patients.

The WUPyV DNA was detected in 1 (0.88%) out of 112 or (0.88%) in plasma of patients with chronic kidney disease and (0 out of 111) in plasma of patients with urinary tract infection while in control group was (0 out of 100). There was no significant difference for WUPyV detection and distribution (Chi-square = 0.77 ; P value = 0.67) as shown in table (2) . The current study detected 1(0.88%) out of 113 (100%) of WUPyV DNA in plasma of chronic kidney disease patients while dosent detect any isolate of WUPyV in plasma of urinary tract infections patients. The result of both groups is compatible to with Csoma et al., (2011) (10) which detect 0.8 of WUPyV in blood from renal transplant patients as well as to csoma et al., (2012) (20) which dosent detect WUPyV DNA in plasma of pregnant and non-pregnant women as well as to Limam et al., (2020) (22) which not detected WUPyV out of 112 from Tunisian patients but is incompatible to Csoma et al., (2014) which record from 9.1% and 5.3% of WUPyV in plasma samples in addition to Aghamirmohammadli et al., (2020) (4) which revealed 1.5% of WUPyV in children under 5 years of age in Tehran, Iran. The low detection of WUPyV in samples from chronic kidney disease and UTI groups may be related to the seasonal distribution and environmental conditions which are important for WUPyV transmission.

Table (3) show the differences in the detection of KIPyV and WUPyV DNA by conventional polymerase chain reaction (PCR) among CKD patients classified by gender. This table shows the positive KIPyV DNA was detected in (7.84 %) and (3.22 %) for female and
male patients respectively. There was no significant difference (Chi-square = 1.19; P value = 0.27) among male and female according to KIPyV detection and distribution. These result is contrast to prezioso et al., (2019)(23) which detected KIPyV in 12/31 thalassemic patients (6 females/6 males) as well as incompatible to Al-Obaidi et al., (2018)(24) which reported 27 (43.55) in male and 4 (44.44) in female of JCPyV in patients with kidney transplantation.

The same table shows the positive WUPyV DNA was detected in (0%) and (1.61%) for female and male patients respectively. There was no significant difference for WUPyV detection and distribution (Chi-square = 0.83; P value = 0.36). These result is similar to Sharp et al., (2009)(25) which recorded only one of WUPyV from a 41-year-old man but un similar to Neske et al., (2010)(26) which reported 49.4% of WUPyV were in male and 46.3% of KIPyV in female in plasma of German blood donors. The plasma viral loads of KIPyV and WUPyV in both gender with this disease might be detectable and their impact on the kidney remains to be determined.

Table (3). Prevelance of KIPyV and WUPyV in chronic kidney disease patients according to gender.

<table>
<thead>
<tr>
<th></th>
<th>CKD patients</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chi Square</td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td>KIPyV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2 (3.22%)</td>
<td>4 (7.84%)</td>
<td>1.19</td>
</tr>
<tr>
<td>Negative</td>
<td>60 (96.7%)</td>
<td>47 (92.1%)</td>
<td>0.27</td>
</tr>
<tr>
<td>Total</td>
<td>62 (100%)</td>
<td>51 (100%)</td>
<td>NS</td>
</tr>
<tr>
<td>WUPyV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1 (1.61%)</td>
<td>0 (0%)</td>
<td>0.83</td>
</tr>
<tr>
<td>Negative</td>
<td>61 (98.3%)</td>
<td>51 (100%)</td>
<td>0.36</td>
</tr>
<tr>
<td>Total</td>
<td>62 (100%)</td>
<td>51 (100%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table (4) show the differences in the detection of KIPyV and WUPyV DNA by conventional polymerase chain reaction (PCR) among UTI patients classified by gender. This table shows the positive KIPyV DNA was detected in (4.54%) and (0%) for female and male patients respectively while did not detect any isolate of WUPyV in both male and female. There was no significant difference (Chi-square = 2.23; P value = 0.13) among male and female according to KIPyV detection and distribution nor for WUPyV detection and distribution (Chi-square = 0.07; P value = 0.79). These result is similar to Bialasiewicz et al., (2009)(27) which did not detect WUPyV from different biological samples include blood, urine, and CSF. The high rate of KIPyV among female in UTI group may due to the physiology...
of genital tract in female and the incidence of bacterial infection that possibly regard the risk factor for infection with KIPyV.

### Table 4. Prevalence of KIPyV and WUPyV in urinary tract infections patients according to gender

<table>
<thead>
<tr>
<th></th>
<th>UTI patients</th>
<th></th>
<th></th>
<th>Chi Square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIPyV Postive</td>
<td>0 (0%)</td>
<td>3 (4.54%)</td>
<td>2.23</td>
<td>0.13</td>
<td>NS</td>
</tr>
<tr>
<td>KIPyV Negative</td>
<td>45 (100%)</td>
<td>63 (95.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45 (100%)</td>
<td>66 (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WUPyV Postive</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0.07</td>
<td>0.79</td>
<td>NS</td>
</tr>
<tr>
<td>WUPyV Negative</td>
<td>45 (100%)</td>
<td>66 (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45 (100%)</td>
<td>66 (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS: Non-significant

### Conclusions

The study describes the prevalence of these two viruses in patients with CKD and UTI without respiratory symptoms in Iraq, which demonstrates how these two viruses can be applied as part of virological screening when suspected of etiology of the viral urinary tract and indicate that a candidate respiratory pathogen, KIPyV and WUPyV in the urinary tract can also be identified.

**Ethical Clearance**: Taken from University of Kufa ethical committee

**Source of Funding**: Self

**Conflict of Interest**: Nil

**References**


**Effect of Noise on General Health Status of Electrical Generator Workers in Iraq**

Mohammed Fakhri Azeez¹, Fatima Wanas Khudair², Ali A. Al-Fahham³

¹Research Scholar, Faculty of Nursing, University of Kufa, University of Kufa, Iraq, ²Professor, Faculty of Nursing, University of Kufa, Najaf, Iraq, ³Assistant Professor, Basic Sciences Department, Faculty of Nursing, University of Kufa, Iraq

**Abstract**

Electrical generators in Iraq have been a series problem regarding occupational and environmental pollution. The current study aimed to determine the level of general health status among generator workers in Al Diwaniyah City Iraq, and its correlation with noise and demographic data. A descriptive cross-sectional design was conducted in the current study. It was carried out at the private electrical generators in A-Diwaniyah City from the period between the 15th of October 2020 until the 15th of March 2021. A purposive study of 150 generator workers has been selected. General health was determined by General Health questionnaire (GHQ-28) which is comprised of 28 items, and include four aspects: somatic, social, depression and anxiety. Noise intensity level was measured by sound level meter (SLM). The results of the present study revealed that average level of noise intensity inside the workers’ room was (78.12 db); while it was (104.14 db) near the generator. The results also showed that the assessment of most domain (social, depression, and anxiety) was moderate, except for somatic domain which it was good. There was a significant negative correlation between noise and depression and anxiety. It has been concluded that: Most of the generator workers showed a moderate level of general health status; Most of the generators workers had moderate level for anxiety, depression and social aspects; Noise intensities inside the generator rooms exceeds the upper limits accepted for human health.

**Keywords** - Noise, GHQ-28, General Health Status, Generator Workers

**Introduction**

Based on the World Health Organization’s (WHO) definition, health is a state of complete physical, mental, and social well-being and not merely the absence of disease. Also, General Health (GH) as a sub-directory of the health system is the general condition of the body or mind with reference to soundness and vigor. On the other hand, GH is defined as systematic activities and social acts based on the prevention, Science & Art for disease management, increasing expectancy of life and health promotion that lead to welfare (1).

Workers in their own workplaces are exposed to many different hazards and hazardous conditions that can threaten health and life. Although some hazards are less likely to happen in some work spaces than others, it’s important to assess which hazards are most damaging to the organization and its employees (2).

Electric generators while in operation produce gaseous emissions and high sound levels. These pose serious health risk to not only users but to individuals in the surrounding communities. A recent study conducted in Ibadan revealed high generator noise level of between 91.2 and 100.5 dB(A). This noise level is capable of inducing hearing impairment. Noise Induced Hearing Loss (NIHL) can be caused by one time exposure to noise as well as repeated exposure to noise at various levels of loudness over an extended period (3).

Health effects from generator use can range from auditory (hearing) to non-auditory. Noise and carbon monoxide pose serious health risk and due to the
insidious nature of noise on hearing, many individuals may not discover they have hearing difficulty until it has become worse. In addition, due to the strive to achieve financial sustenance, many often neglect their health and work at relatively short distances from generators, and at long hours. A lack of knowledge is identified as one of the barriers to change. Knowledge about occupational hazards (such as noise from electric generator) is suggested to be a predictor of preventive behaviour at work. Effective behavioural change is facilitated by greater knowledge, experience, and personal risk perception (3).

The current study aimed to determine the level of general health status among generator workers in Iraq, it also aimed to determine the noise levels (by a decibel meter) at the generator location and its correlation with the workers general health domains.

**Methods**

A descriptive cross-sectional design was conducted in the current study. It was carried out at the private electrical generators in A-Diwaniyah City in Iraq from the period between the 15th of October 2020 until the 15th of March 2021. A purposive study of 150 generator workers was taken in the current study. The final study instrument consists of three parts:

**Part 1: Demographic Data**

This part is concerned with participants’ socio-demographic data. A demographic data sheet, consists of (10) items, which contain (age, marital status, educational level, monthly income, residency, duration of work at the generator, type of work, smoking status, alcohol intake, BMI).

**Part 2: General Health questionnaire**

The General Health questionnaire (GHQ-28) is comprised of 28 items include somatic symptoms domains (7 items); anxiety domain (7 items); social dysfunction domains (7 items) & depression domain (7 items), the general health questionnaire is a self-administered screening instrument used to measure the psychological distress and general mental health in non-psychiatric persons. The scale was first developed by Goldberger and Hillier (1979).

**Part 3: Noise intensity measurement**

Noise intensity level was measured by sound level meter (SLM), model: UNI-T; UT352, China, with the ranging of 40dB – 160dB.

**Statistical Analysis**

Descriptive statistics presented as mean, standard deviation, frequencies and percentages. Chi-square test was used to compare frequencies. Pearson’s correlation test was used to assess the correlations. Level of significance of ≤ 0.05 was considered as significant difference or correlation.

**Results**

**Table (1) Mean levels of noise (dBA) at the generation locations**

<table>
<thead>
<tr>
<th>Items</th>
<th>Mean</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside the workers’ room</td>
<td>78.12</td>
<td>6.15</td>
<td>0.000</td>
</tr>
<tr>
<td>Near the generator</td>
<td>104.14</td>
<td>2.87</td>
<td></td>
</tr>
</tbody>
</table>

Table (1) show mean levels of noise (db) at the generation locations, it explains that mean level of noise inside the workers’ room was (78.12 db); while mean level of noise near the generator was (104.14 db). There is a high significant difference in the mean level of noise between inside and outside the workers’ room.
Table (2) shows descriptive statistics of overall assessment for GH-28 domains and overall assessment among generator workers.

<table>
<thead>
<tr>
<th>GH-28 Domains</th>
<th>No.</th>
<th>M.S.</th>
<th>S.D.</th>
<th>95% C.I. for Mean</th>
<th>Ass.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L.b.</td>
<td>U.b.</td>
</tr>
<tr>
<td>Somatic Domain</td>
<td>150</td>
<td>3.1</td>
<td>0.87</td>
<td>2.9</td>
<td>3.2</td>
</tr>
<tr>
<td>Social Domain</td>
<td>150</td>
<td>2.96</td>
<td>1.11</td>
<td>2.63</td>
<td>3.45</td>
</tr>
<tr>
<td>Depression Domain</td>
<td>150</td>
<td>2.96</td>
<td>0.94</td>
<td>2.45</td>
<td>3.45</td>
</tr>
<tr>
<td>Anxiety Domain</td>
<td>150</td>
<td>2.92</td>
<td>1.04</td>
<td>2.14</td>
<td>3.84</td>
</tr>
<tr>
<td>Global Mean of Score for GH-28</td>
<td>150</td>
<td>2.98</td>
<td>0.99</td>
<td>2.92</td>
<td>3.1</td>
</tr>
</tbody>
</table>

MS : Mean of Scores; SD : Standard Deviation ; Poor : MS = 1-1.99 ; Moderate : MS = 2-2.99 ; Good : MS ≥ 2 ; L.b. : lower border ; U.b. : Upper border

Table (2) shows descriptive statistics of overall assessment for GH-28 domains and overall assessment among generator workers, it explains that the assessment of most domain (social, depression, and anxiety) was moderate, except for somatic domain which it was good.

Table (3) shows correlation (Pearson Coefficient) between noise inside and outside workers’ room and domains and total assessment of 28-GH.

<table>
<thead>
<tr>
<th></th>
<th>Somatic</th>
<th>Anxiety</th>
<th>Social</th>
<th>Depression</th>
<th>Total Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noise Inside the workers’ room</td>
<td>r = -0.119</td>
<td>r = -0.030</td>
<td>r = -0.075</td>
<td>r = -0.172 *</td>
<td>r = -0.167 *</td>
</tr>
<tr>
<td>Noise Near the generator</td>
<td>r = -0.043</td>
<td>r = -0.096</td>
<td>r = -0.170 *</td>
<td>r = -0.028</td>
<td>r = -0.132</td>
</tr>
</tbody>
</table>

* Significant correlation at p value < 0.05

Table (3) shows correlation (Pearson Coefficient) between noise inside and outside workers’ room and domains and total assessment of 28-GH, it shows that there is a significant negative correlation between depression domain and noise level inside the workers’ room (r = -0.172; P<0.05); it also explain that there is a significant negative correlation between social domain and noise level inside the workers’ room (r = -0.170; P<0.05); a significant negative correlation between social domain and noise level inside the workers’ room (r = -0.170; P<0.05).

Discussion

The current study recorded that the noise level near the generator is higher than that inside the workers’ room, this is a logical result, where the walls and the door were used as isolating agents for the noise that resulting from the operation of the generator.
The average level of noise inside the workers’ room in this study is (78.12 ± 6.15 dBA), this level is higher than the lower limit of acceptable indoor noise level, this result agrees with the study conducted by Alnayli et al., they have evaluated noise pollution in three locations in Al-Diwaniya City, and found that noise pollution was about 21% higher than the accepted levels identified by WHO. It was found that noise has undesirable effects on the cardiovascular system exposure to noise greater than 70 dB(A) results in increases in vasoconstriction, heart rate and blood pressure reported a case of persistent ventricular fibrillation upon arousal from sleep by noise, and several reports have suggested that transitional periods during waking from sleep may provoke cardiac arrhythmias. A study conducted by Mohammad have reported that cardiovascular effects are associated with long-term exposure to noise in the range of 65–70 dB or more, indicating the private electrical generators as the main source of noise pollution in urban areas.

The current study has also revealed that the mean level of noise intensity near the generator was (104.14 ± 2.87 dBA) as in table (1). This results is similar to the result recorded by Al-Naemi and Abdel who found that noise intensity inside the generator room was (95.18 ± 0.78 dBA) which had negative effects on the hearing capabilities of the workers, and resulted in increase in lipid profile markers, and decrease in anti-oxidant indicators, in workers compared to healthy control group. Table (3) shows a negative correlation between the level of noise and the level of general health status in all its dimensions, and this result is identical to what has been scientifically proven that noise has effects on health, a loud and continuous voice causes temporary or permanent loss of hearing, increased blood pressure, distorted heart rate, increased adrenaline, disrupted the proper functioning of the kidney. Damage to the human psyche, a feeling of fear and pressure on the nerves and the ability to focus on thoughts, work and learning. Excessive noise is considered a form of violence because it provokes a sense of helplessness, fatigue, and the desire to use violence against the source of the noise.

According to the International Programme on Chemical Safety (WHO 1999), an adverse effect of noise is defined as a change in the morphology and physiology of an organism that results in impairment of functional capacity, or an impairment of capacity to compensate for additional stress, or increases the susceptibility of an organism to the harmful effects of other environmental influences. This definition includes any temporary or long-term lowering of the physical, psychological or social functioning of humans or human organs. The health significance of noise pollution is given in this chapter under separate headings, according to the specific effects: noise-induced hearing impairment; interference with speech communication; disturbance of rest and sleep; psychophysiological, mental-health and performance effects; effects on residential behaviour and annoyance; as well as interference with intended activities.

It was reported that oxidative stress induced by noise and hazardous effects of toxic gases may have direct effects on cardiovascular dysfunctions, hormonal disturbances, and hearing disabilities that may interfere with both physical and mental health domains. A recent study have investigated deeply the environmental pollution resulting from the combustion of fuel used in electric power generators in Iraq, especially in the summer and you are the national electric power supplied by almost non-existent state where this problem is a local phenomenon that has serious dimensions to human health; they found that many types of gases emitted from burning fuel electric generators operating in the province of Baghdad indicating the amount of environmental pollution, which is more risk to humans with permitted by the World Health Organization.

Conclusions

It has been concluded that: Most of the generator workers showed a moderate level of general health status. Most of the generators workers had moderate level for anxiety, depression and social aspects. Noise intensities inside the generator rooms exceeds the upper limits accepted for human health. The is a correlation between...
noise produced by the generators and the decline in the anxiety and depression aspects of the workers.

**Ethical Clearance**: Taken from University of Kufa ethical committee

**Source of Funding**: Self

**Conflict of Interest**: Nil

**References**


Effect Phenytoin Therapy to Fibroblasts and Angiogenesis of Enterocutaneous Fistula in Wistar Rat

Muhammad Budiman Irpan Bachtir1, Hermawan2, Hardian3, Ignatius Riwanto4
1Master Biomedical Student at Diponegoro University Biomedical Master’s Student, 2Lecture at Departemen of Anatomic Pathology, Faculty of Medicine, Diponegoro University, 3Lecture at Department of Physiology, Faculty of Medicine, Diponegoro University, 4Lecture at Department of Digestive Surgery, Faculty of Medicine, Diponegoro University

Abstract

Background: Comprehensive wound care in enterocutaneous fistula (ECF) is the therapeutic of choice currently, which may increase of closure rate without surgery from 19 to 92%. Phenytoin has been reported to have anticolagenase effect on wound healing is hoped to improve the ECF closure.

Aim: The study was aimed to demonstrate the effect of phenytoin on closure of enterocutaneous fistula seen from the number of fibroblasts and angiogenesis

Methods: This study was “Randomized Controlled trial with post test only group design” on 18 male wistar rats with ECF, that were divided randomly into three groups: group(K) control, P1(topical phenytoin), P2(oral phenytoin). After 7 days of treatment, they were terminated and histopathological examinations were performed to do fibroblast cell counting and the amount of angiogenesis by Hematoxilin Eosin staining.

Results: Mean of the number of fibroblast in groups K, P1 and P2 were 69.50 ± 10.07, 155.50 ± 13.50 and 182.16 ± 11.85 respectively (One way Anova P=0.001) and mean of the number of angiogenesis in groups K, P1, and P2 were 95.66 ± 9.72, 178.66 ± 11.75, and 205,16 ± 9.74 respectively (One way Anova P=0.001). Post Hoc Test LSD showed that the number of fibroblast of group P1 vs K(P=0.001), P2 vs K(P=0.001), P1 vs P2(P=0.004) and the number of angiogenesis of group P1 vs K(P=0.001), P2 vs K(P=0.001), P2 vs P1(P=0.002).

Conclusion: The therapy of topical and oral phenytoin increased the number of fibroblasts and angiogenesis in Wistar rat with ECF. Outcome of oral phenytoin therapy better than topical.

Key words: Enterocutaneous fistulas, phenytoin, fibroblasts, angiogenesis

Introduction

Enterocutaneous fistula (ECF) is an abnormal communication between the intra-abdominal digestive tract and the skin.1,2 The ECF occurs spontaneously on 30% due to malignancy, sepsis, radiation or inflammatory diseases of the intestine, whereas due to postoperative complications of more than 75%.2,3 ECF patients are faced with conditions of increased morbidity and mortality due to it complications. Comprehensive wound care accompanied with parenteral nutrition (TPN) is currently the treatment of choice, with closure of fistulas without surgery increasing from 19% to 92%.5-7

After the ECF occurs, therapy must be immediately carried out with optimal patient stabilization and non-operative therapy which is conventional therapy.1
Prognostic factors that allow spontaneous closure of fistulas are influenced by several factors, namely ECF that occurs due to surgery, transferrin value > 200 mg/dl, fistulasize of < 2 cm, absence of obstructive, inflammatory, and intestinal infections. Presence of components in the form of foreign bodies, radiation, inflammation, infection, inflammatory bowel disease, epithelization of the fistula tract, neoplasms, distal obstructions, and steroids (FRIENDS) are indication for surgical intervention. In conventional therapy of ECF wound care plays an important role in the process of spontaneous closure, so that the administration of therapy to the wound in order to speed up closure is very necessary. The healing of passage in fistulas consists of various processes, including cell migration and the formation of new extracellular matrices, one of which is increased of fibrosis.

Presently, wound care at ECF also has many methods that can be done with moist dressing until the treatment using negative pressure wound therapies (NPWT) or one of which is often known as vacuum assisted closure (VAC). The use of VAC is currently reported to improve the quality of life for patients EFC, which the skin around the EFC wound is protected. The implementation of this method has a high cost and expertise of trained stoma nurses, so that there is still a need for an EFC wound care method that can more easily be developed.

Phenytoin drug has long been known as an anti-seizure drug is currently reported from several studies showing it had a therapeutic effect on wound healing. This has been supported by many studies and studies that reported since decades. Several studies have shown the advantages of therapeutic effect of the administration of phenytoin on healing burns, trauma wounds, venous static ulcers.

The mechanism of phenytoin in wound healing at this time is still uncertain, but in vitro research, it is known that there are several mechanisms that can support wound healing. The mechanism consists stimulation of proliferation of fibroblasts, increasing angiogenesis, increasing the formation of granulation tissue, glucocorticoid antagonists, decreasing collagenase activity, increasing collagen deposits, decreasing exudate in wounds and also finding antibacterial effects. In other studies phenytoin was found to have a mechanism to reduce MMP-1 and 9 which prove the anti-collagenase effect of phenetoin. This mechanism is the basis for the application of the case in ECF, with an increase in the number of fibroblasts and angiogenesis will enable the closure of the fistula canal.

Jaber reported in 2013 a case series of studies conducted with intravenous phenytoin giving a positive effect on the healing of gastrointestinal fistulas. The positive effects of phenytoin shown through systemic stimulation are significant because of the side effects that can occur in long-term administration. Teo, in vitro studies show safety in topical phenytoin administration and therapeutic effect on wound healing. So that research into the effects of phenytoin on ECF healing needs to be further developed by topical administration and oral administration which has not been done at this time.

**Materials and Method**

**Subject**

This research is an experimental study with a “Randomized Controlled Trial with Post Test Only Group Design” design. The study used Wistarrat\textit{RattusNorvegicus} strain which will be divided equally into three random groups, 1 control group and 2 intervention groups. All groups were made to have the presence of ECF by surgical procedures. One group will be a control group treated with ECF wounds treated with moist gauze which will be terminated on the 7th day, while other subjects according to the group division will be treated with topical and oral phenytoin wounds which will be terminated on the 7th day.

Experimental animals are Wistarrat strain\textit{(RattusNorvegicus)}. Inclusion criteria were 8-10 weeks old who performed the procedure for making ECF, body weight ± 150-200 grams after acclimation
for a week in individual cages and no visible anatomical abnormalities. While the exclusion criteria were rat appeared to be sick (inactive movements) during the treatment of ECF. Rats that lost > 10% and died at the time of the study were included in the drop out criteria. In this study, the number of samples used 6 ratsin each group. The sample was 18 rats. Each rat was then labeled number 1-18. The division of groups is done randomly by drawing lots.

The research and data collection were carried out for 3 months from April-June 2019. The topical phenytoin production site was conducted at the Chemistry Laboratory of the Sekolah Tinggi Ilmu Farmasi (STIFAR) Yayasan Farmasi Semarang. Procedures for making enterocutaneous fistula conditions, treatment of rats, tissue retrieval, the process of making preparations and HE staining were carried out at the Laboratory of the BioSains Institute of Brawijaya University, Malang.

The independent variables are:
1. Topical phenytoin
2. Oral phenytoin
3. Without the administration of phenytoin

The dependent variables are:
1. Number of fibroblasts in histopathological preparations
2. Number of total angiogenesis in histopathological preparations

**Experimental design**

Rats were acclimatized in the laboratory for one week in individual cages with periods of 12 hours of light and 12 hours of darkness. Rats were fed and drank ad libitum. Providing food with feeds whose nutritional value composition has been standardized. After acclimatization, the rats were carried out the procedure of making enterocutaneous fistulas.

Animals were anesthetized intraperitoneally with 80 mg/kg ketamine (Pantex Holland, Duizel, Netherlands) and 10 mg/kg xylazine (Pantex Holland) diluted in PBS. The cecum was accessed through a standard 7-mm stab incision on the lower left side of the abdomen, sparing the colon upon exposure, and a 5-mm enterotomy was performed and sutured to the abdominal wall to create an enterocutaneous fistula. To allow spontaneous closure of the fistula, the opening in the cecum wall was secured to the borders of the surgical wound without maturation. At this point, the animals were randomly allocated into one of three groups:

1. Control group (CG)—the enterocutaneous fistula wound that was treated with moist gauze and terminated on the 7th day,
2. Topical phenytoin group (TPG)—the enterocutaneous fistula wound that was treated with 10% phenytoin ointment and terminated on the 7th day
3. Oral phenytoin group (OPG)—the enterocutaneous fistula wound that was treated with oral phenytoin and terminated on the 7th day. Oral phenytoin given with a maximum human dose (300 mg/day) is converted into a rat dose (200gr) through a conversion table. Based on the conversion, the dosage is 0.03 mg/grBB given once a day. Drug administration is made by dissolving phenytoin with aquabidest and given orally with a sonde.

**Statistical Analysis**

After the data is collected, editing, coding, tabulation and entry are carried out. Data analysis includes descriptive analysis and hypothesis testing. In descriptive analysis the dependent variable is presented in the form of a mean table, SD, median and box plot graph. The data in this study were normally distributed after the data normality test with the Saphiro-Wilk test both on the number of fibroblasts and the number of angiogenesis in each treatment group. Data analysis continued with the hypothesis test used was the One Way Anova test followed by a Post-Hoc Test to determine differences between groups.

**Research Ethics**

The animal experiments were performed in
8-10-weekold Wistar rat strain (*Rattus Norvegicus*) in which an enterocutaneous cecal fistula was created. The study was submitted and approved by the Diponegoro University Faculty of Medicine Research Ethics Commission No. 78/EC/H/KPEK/FK-UNDIP/V/2019. Eighteen animals were used in the experiments.

**Results**

The study was conducted on 18 Wistar rats, which were divided into 3 groups; i.e. groups of rats who treated with fistula wound treatment with moist gauze (C), topical phenytoin (P1) and oral phenytoin (P2), each group consisted of 6 rats, and until the end of the study were healthy and not included in the dropout criteria.

**Characteristics of Research Samples**

The average calculation result of 18 rats body weight, on the 7th day of acclimatization where that day is also the first day of the treatment process. This can be seen in table.

**Number of Fibroblasts**

![Image of histopathological picture with hematoxylin-eosin staining](image)

*Figure 1. Histopathological picture of the number of fibroblasts with hematoxylin-eosin staining with 400x magnification. a. Without treatment, b. Topical phenytoin, c. Oral phenytoin; arrows indicate fibroblast cells.*
Table 1. Descriptive tables and normality of body weight data (Grams)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (Grams)</th>
<th>p</th>
<th>Homogenity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Median (min – max)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>180,17 ± 5,629</td>
<td>179 (175 – 190)</td>
<td>0,576</td>
</tr>
<tr>
<td>P1</td>
<td>183,50 ± 5,010</td>
<td>183 (177 – 190)</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>182,17 ± 5,981</td>
<td>181,5 (175 – 192)</td>
<td></td>
</tr>
</tbody>
</table>

Information: * Significant (p < 0,05); ** Homogen (p > 0,05)

From table 1, it can be seen that the rat weight data are normally distributed and homogeneous. The highest rat weight was found in the group with topical administration of phenytoin, which was 182.17 ± 5.981 grams.

**Distribution of data**

Variable data on the number of fibroblasts obtained an average number of 69.50 ± 10.07 in the group without phenytoin (C) therapy, 155.50 ± 13.50 in the group of topical phenytoin (P1) and 182.16 ± 11.85 in the oral phenytoin therapy group (P2). From the results of the normality test obtained normal distribution results (p> 0.05)

**Statistical Test for the Number of Fibroblast**

![Figure 2. Boxplot graph of the number of fibroblasts from each group. C: fistula wound treatment with moist gauze, P1: fistula wound treatment with topical phenytoin, P2: fistula wound treatment with oral phenytoin (P2). There was a statistically significant difference (One Way ANOVA P <0.05). Also found significant differences when compared to each group (Post Hoc test); * significant P <0.05)
From the box above, it shows that there are differences in the number of fibroblasts. The lowest number of fibroblasts was seen in the control group without phenytoin therapy, 69.50 ± 10.07, while the highest number of fibroblasts was seen in the treatment group with oral phenytoin administration, which was 182.16 ± 11.85. Based on the One way ANOVA test, it can be seen that the p value <0.05, which means the difference is significant. In the Post-Hoc test there were differences in the number of fibroblasts from the groups given both topical and oral phenytoin therapy had a significant difference compared to the control group in each group (p <0.05), and there were significant differences in the groups given topical phenytoin compared the group was given oral phenytoin (p <0.05).

**Total angiogenesis**

![Histopathological picture of the amount of angiogenesis by painting hematoxylin-eosin with 400x magnification. a. Without treatment, b. Topical phenytoin, c. Oral phenytoin; arrows indicate angiogenesis.](image)

**Distribution of data**

Variable number of angiogenesis data obtained an average number of 95.66 ± 9.72 in the group without phenytoin (C) treatment, 178.66 ± 11.75 in the topical phenytoin (P1) and 205.16 ± 9.74 groups in the oral phenytoin therapy group (P2). From the results of the normality test obtained normal distribution results (p>0.05).

From the boxplot graph, figure 4 shows that there are differences in the number of angiogenesis. The lowest
number of angiogenesis in the control group without phenytoin therapy was 95.66 ± 9.72, while the highest number of angiogenesis was seen in the treatment group with oral phenytoin administration which was 205.16 ± 9.74. Based on the One way ANOVA test, it can be seen that the p value <0.05, which means the difference is significant. In the Post-Hoc test there were differences in the number of angiogenesis of the groups given both topical and oral phenytoin therapy had significant differences compared to the control group in each group (p <0.05), and there were significant differences in the groups given topical phenytoin compared the group was given oral phenytoin (p <0.05).

**Statistical Test of Total Angiogenesis**

![Boxplot graph of the number of angiogenesis of each group. C: fistula wound treatment with moist gauze, P1: fistula wound treatment with topical phenytoin, P2: fistula wound treatment with oral phenytoin. (P2) There is a statistically significant difference (One Way ANOVA P <0.05), and also a significant difference when compared in each group (Post Hoc test); * significant (P <0.05)](image)

**Discussion**

This study aimed to see the effect of phenytoin administration on the spontaneous closure of ECF. The effect was assessed on the number of fibroblasts and the number of angiogenesis in the healing process or spontaneous closure of ECF in Wistar rats. This study was conducted on Wistar rats that met the inclusion criteria and were treated for 7 days and subsequently assessed in fistula tissue by looking at the number of fibroblasts and angiogenesis through HE staining.

From this study, fistula tissue was taken on the 7th day after each group was treated, which time was the end of the inflammation phase of the wound healing process. In the treatment group using topical and oral phenytoin therapy it was found that there was a significant difference in
the number of fibroblasts with the group giving oral phenytoin having the highest number. The higher number of fibroblasts in the administration of phenytoin therapy indicates the effect of phenytoin therapy on ECF, this is in accordance with the theory that the administration of phenytoin has a stimulating effect on fibroblasts proliferation. Increased fibroblast proliferation in the ECF induction will have an effect positive in spontaneous closure of ECF. One of the factors expected to occur in fistula closure therapy is the presence of fibroblasts to support the wound healing process.

In conventional ECF therapy currently popular using VAC which in its application has a mechanism of reducing proinflammatory cytokine and inducing microdeformation on the wound surface and complex wound healing. Lu, Feng et al show that microdeformation in wound healing is related by induction of fibroblasts. The positive effect of the differences in the number of fibroblasts in this study could be a factor in the process of spontaneous closure of ECF. Intravenous administration of phenytoin to ECF in previous studies also showed good results with reduced output of the fistula.

This study also assessed the amount of angiogenesis in skin tissue, on the 7th day after each group was given treatment and found the highest value for the amount of angiogenesis found in the group giving oral phenytoin therapy. Whereas the treatment group that was not given phenytoin therapy had the lowest amount of angiogenesis. From these results it was found that there were significant differences in the number of angiogenesis between groups not given phenytoin therapy and groups who were given phenytoin therapy either topically or orally.

Angiogenesis is an important factor in the tissue’s ability to repair itself and eliminate debris, providing nutrients and oxygen to the wound layer. The formation of granulation tissue which is a dense network of blood vessels, macrophages, and fibroblasts embedded in loose matrix fibronectin, hyaluronic acid, and collagen depends on the vascularization of the wound tissue and begins to appear in the wound about four days after injury.

The higher number of angiogenesis in the treatment group of phenytoin proved that the effect of phenytoin therapy was most likely through M2 stimulation to increase the expression of growth factors, one of which was VEGF which played a role in increasing the amount of angiogenesis. In the process of spontaneous closure of the fistula angiogenesis is also needed so that collagen deposits and granulation tissue can occur.

The treatment using oral and topical phenytoin therapy also aims to see the difference from topical and systemic phenytoin therapy. Systemic administration of phenytoin is known to have side effects in long-term administration, namely osteomalacia, gingival hyperplasia, and hepatic and renal disorders in rare prevalence. The positive results of the phenytoin treatment group in this study also showed a significant difference between the groups given topical and oral therapy both in the number of fibroblasts and the number of angiogenesis observed, so that the study assumed the selection of phenytoin therapy in enterocutaneous fistulas was superior to oral administration. or systemic. Jaber reported in 2013 a case study conducted with intravenous phenytoin giving a positive effect on the healing of gastrointestinal fistulas, in this study, Jaber chose systemic therapy because healing that was considered more important in fibrosis in the fistulacan be achieved through systemic therapy compared to topical given on the surface of the fistula wound.

Conclusion

This conclusion was followed by the number of fibroblasts and angiogenesis in the administration of topical phenytoin in the ECF of Wistar rats, higher than those not receiving phenytoin therapy. In addition, the number of fibroblasts and angiogenesis in oral phenytoin administration in the Wistar rat ECF were higher than those not receiving phenytoin therapy. It can also be concluded that the number of fibroblasts and angiogenesis in oral phenytoin administration in Wistar rat ECF, is higher than those without topical phenytoin.
The author recommends further research with a longer observation time to see the process of closing the ECF on the administration of phenytoin therapy and research on patients who have criteria for spontaneous closure with VAC and phenytoin therapy both oral and topical as additional therapy.

Acknowledgements: Animal experiments were carried out at the BioSains Institute, Brawijaya University, Malang. The Anatomical Pathology Section of the Diponegoro National Hospital conducted a microscopic evaluation. The Chemistry Laboratory of the Sekolah Tinggi Ilmu Farmasi (STIFAR) Yayasan Farmasi Semarang performs topical phenytoin manufactures.

Source of Fund : Self
Conflict of Interest : Nil
Ethical Clearance : The study was submitted and approved by the Diponegoro University Faculty of Medicine Research Ethics Commission No.78/EC/H / KPEK/FK-UNDIP/V/2019. Eighteen animals were used in the experiments

References
Legal Policy in the Prevention of Narcotics in Indonesia

Muhammad Yunus Idy1, Marif1, Handar Subhandi Bakhtiar2
1Faculty of Law, Universitas Islam Makassar, Indonesia, 2Faculty of Law, Universitas Pembangunan Nasional Veteran Jakarta, Indonesia

Abstract
The purpose of law enforcement against criminal acts of narcotics and psychotropic abuse is to improve the health status and human resources of Indonesia. The new law concerning narcotics has two approaches to parties using narcotics illegally or in other words, abusing narcotics. The first approach is to see the drug users as victims so that they must be rehabilitated, and the second approach is to look at drug users as perpetrators of criminal acts. Criminal acts related to narcotics crimes can generally be divided into 3 (three) types, namely supplying which is usually done by producers or importers. The act of distributing narcotics and other illegal drugs carried out by dealers or sellers and then the users or people who abuse the narcotics. Therefore, the efforts to eradicate narcotics abuse must include all those three types of activities or actions. The efforts to eradicate it must start from the upstream part of the supplier. The policy in combating narcotics abuse is a positive legal policy which is not only merely the implementation of normative juridical laws, but also requires a factual juridical approach that is comprehensive and integral.

Keywords: Legal Policy, Narcotics, Psychotropics.

Introduction
Each country has a legal system that reflects the history and experience of the country’s people in the development of their economic, political, cultural and traditional structures. A good legal system seeks to limit actions that harm the society for the security of the community itself. When people feel insecure, vigilante acts will occur.

The opening of the Constitution of the Republic of Indonesia, the fourth paragraph, states that the state intends to protect the entire Indonesian nation, and the whole of the Indonesian bloodshed. The philosophical juridical statement contains 2 (two) fundamental meanings, namely: one of the principles of the administration of the state (government) which must be oriented towards the goal of protecting all Indonesian people, and the principle of protecting the state (government) based on equality before the law. The relationship between the principle of protection and the principle of equality before the law in the overall activities of state administration leads to the framework of law enforcement.

Law enforcement is closely related to the legal system and legal objectives, explain that discussing law enforcement can begin by examining the issue of what will be upheld. When talking about law enforcement, it is essentially talking about the enforcement of ideas and concepts which are beneath abstract. Law enforcement is an attempt to turn these ideas into reality and the state must intervene to realize abstract laws by holding various bodies for this purpose. The legal system which consists of legal substance, legal structure, and legal culture are always directed towards achieving legal objectives, namely: to achieve legal certainty, benefit, and justice. Radbruch taught that we must use the principle of priority where the first priority is justice,
benefit, and finally the certainty. Law enforcement which is controlled by the legal system will be able to realize legal objectives when positioned on a balance between the formal law enforcement which tends to pursue legal certainty and the substantial law enforcement which seeks to achieve legal benefit and justice.

Law Enforcement is covering the duty and authority to defend the law (handhaving van het recht) against a person or group of people who violate the law or committed an unlawful act or denial of a legal commitment including enforcing the law, namely the act of establishing the law regarding things such as the status of an object or objects. Both in theory and tactics, this traditional understanding is incomplete because of the connotation of law enforcement only by mere repressive actions. Whereas in a broader sense, law enforcement also includes preventive measures even broader than that. Law enforcement in the context of the rule of law/legal state (rechtstaat) has 3 (three) main pillars, namely: (1) the legality of all actions of law enforcement officers including citizens; (2) the guarantees for the recognition, protection, respect and enforcement of human rights, and (3) the implementation of a free, fair and impartial court. The imbalance between the three pillars of law enforcement causes discrimination and arbitrariness, and leads to the destruction of the joints of legal life in the legal state.

The relevance of law enforcement (various legal fields) in the context of a legal state with its main pillars (principle of legality, human rights, the impartial court) requires a good judicial system and is able to accommodate all the sub-systems of the judiciary. One justice system that has direct contact with law enforcement against narcotics crimes is the criminal justice system (hereinafter referred to as SPP). The discussion in the field of criminal law policy (penal policy) will be oriented towards the efforts to criminal law reform in the future, specifically related to legislative policy against criminal acts of narcotics abuse in Indonesia. The Criminal Justice System is essentially a criminal law enforcement process. Therefore it is very closely related to criminal law itself, because criminal law is basically a criminal law enforcement system in abstracto which will be realized in law enforcement in concreto. For this reason, this article will discuss about the concept of government policy in the efforts to combat the criminal acts of the narcotics and psychotropic abuse in Indonesia.

Research Methods

This research is a normative-legal research using a statute and conceptual approaches. Those legal material collected are analysed descriptively related to the problems and prescriptively.

Analysis and Discussion

Today’s law enforcement has a broader meaning, which not only concerns law enforcement, but also includes preventive measures. This is different from the English term of law enforcement; the Indonesian law enforcement is more repressive. While the preventive form is the provision of information and instructions called law compliance, which means compliance or legal arrangement.

Law enforcement is essentially an effort to enforce legal norms, both in the form of orders and prohibitions. The purpose of law enforcement against criminal acts of narcotics and psychotropic abuse is to improve the health status and human resources of Indonesia. Therefore, in order to realize people’s welfare, efforts should be made to improve the field of medicine and health services, among others by seeking the availability of certain types of narcotics which are urgently needed as medicines and preventing and eradicating the danger of abuse and illicit trafficking of narcotics and narcotics precursors. The policy of eradicating crime through the making of criminal laws is an integral part of the public protection policy and is an integral part of social politics. Social politics can be interpreted as all rational efforts to achieve people’s welfare and at the same time protect the community.

Pancasila is the source of all legal sources. Therefore, the values contained in Pancasila should be the basis of law enforcement in combating narcotics and psychotropic
crimes, especially the values of justice, legal certainty and the same legal treatment for everyone as contained in Article 27 paragraph (1) of the 1945 Constitution of Republic of Indonesia regulates that all citizens are equal in law and government and are obliged to uphold the law and government with no exception. Whereas the values of humanity as contained in the second principle of Pancasila are closely related to the purpose of punishment. In this case, the punishment imposed on the perpetrators of narcotics and psychotropic crimes must not exclude the values of humanity, in the sense that the punishment continues to uphold human dignity.

Provisions that give the authority to the National Narcotics Agency to carry out arrests that exceed the arrest period in the Criminal Procedure Code states that the Arrest as referred to in Article 17, can be carried out for a maximum of one day, are contrary to the principles of justice and legal certainty as contained in Article 3 of Law No. 35 of 2009 concerning Narcotics, and the same legal treatment for each person.

One of the authorities possessed by the National Narcotics Agency as an investigator is to make arrests for 3 x 24 hours from the date the arrest letter is received by the investigator and can be extended for a maximum of 3 x 24 hours. This is one form of difference in authority between the National Narcotics Agency and other law enforcement officers, namely the police. The authority to arrest done by the police is based on the Criminal Procedure Code. Based on the provisions of Article 19 paragraph (1) of the Criminal Procedure Code that arrests can be made for a maximum of one day. This difference in authority will lead to differences in treatment of suspects who are allegedly involved in narcotics and psychotropic crimes. Suspects in narcotics and psychotropic crimes can only be arrested by the police for a maximum of 1 day, while suspects arrested by the National Narcotics Agency can be held for up to 6 days.

The difference in treatment between the suspects arrested by the National Narcotics Agency and the police will lead to injustice and legal uncertainty. Therefore, in order to realize justice, legal certainty and equal treatment for every suspect of narcotics crime, efforts need to be made to provide the same basis to the National Narcotics Agency and the police in handling or enforcing the law against narcotics and psychotropic crimes. This effort can be done by formulating a separate regulation that gives the same authority to the police and the National Narcotics Agency, or by revising the narcotics law specifically related to the authority of the two law enforcement agencies.

The regulation concerning the Narcotics and Psychotropic Crime in Indonesia cannot be separated from international policies related to the Criminal Actions of Narcotics and Psychotropic abuse. This international policy is formulated in various kinds of conventions. The UN Conventions on Narcotics and Psychotropics include Single Convention on Narcotic Drugs in 1961 and United Nation Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances in 1988. Single Convention on Narcotic Drugs in 1961 along with protocols that change the convention namely Protocol Amending the Single Convention on Narcotic Drugs in 1961 was accepted and ratified by Indonesia with the enactment of Law No. 8 of 1976. While the United Nation Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances in 1988 has also been accepted and ratified by Indonesia with the enactment of Law No. 7 of 1997.

The United Nations Single Convention on Narcotic Drugs 1961 is an international agreement that prohibits the production and supply of narcotics and illegal drugs except under licenses for certain purposes such as medical care and research. The convention was aimed at renewing the Paris Convention on July 13, 1931. This Convention included a number of synthetic opioid products found in the last 30 years and also to facilitate the incorporation of new types of narcotics into the agreement. The previous agreement only controlled the production and illicit traffic of opium, coca, and derivatives such as morphine, heroin and cocaine. This 1961 Single Convention was a consolidation of the previous agreements that expanded the coverage by
including marijuana and other drugs which effects were similar to certain types of narcotics.

The international conventions mentioned above become a reference for Indonesia in making regulations relating to narcotics and psychotropic crimes. These regulations are formulated in several forms of legislation, the last of which is Law No. 5 of 1997 concerning Psychotropic and Law No. 35 of 2009 concerning Narcotics.

Based on the new narcotics law, the use of narcotics can be seen from two perspectives. The first is the legal use of narcotics which is used in accordance with the applicable legal provisions. The second is the use of narcotics which is illegal or contrary to the law which is referred to as narcotics abuse. Narcotics can be used legally and not against the law, if it is done for medical purposes and the development of science.

The new law concerning narcotics has two approaches to parties using narcotics illegally or in other words, abusing narcotics. The first approach is to see the drug users as victims so that they must be rehabilitated, and the second approach is to look at drug users as perpetrators of criminal acts. Elucidation to Article 54 of Law No.35 of 2009: What is meant by victims of Narcotics abuse is someone who unintentionally uses Narcotics because he was persuaded, tricked, deceived, forced, and / or threatened to use Narcotics.

Criminal acts related to narcotics crimes can generally be divided into 3 (three) types, namely supplying which is usually done by producers or importers. The act of distributing narcotics and other illegal drugs carried out by dealers or sellers and then the users or people who abuse the narcotics. Therefore, the efforts to eradicate narcotics abuse must include all those three types of activities or actions. The efforts to eradicate it must start from the upstream part of the supplier. The supplier of narcotics or psychotropics can be a producer or importer. The efforts to eradicate upstream parts can be carried out in the form of strict supervision so that the illegal production and importation of narcotics can be prevented. If this can be done well, the illegal circulation can also be reduced. Furthermore, if the illegal distribution is reduced or even eliminated, then its illegal use can also be reduced or even eliminated.

The focus of efforts to eradicate narcotics and psychotropics should be directed towards the efforts to prevent the occurrence of illegal distribution, not only solely focusing on efforts to eradicate the abuse. If illegal drug trafficking can be handled properly, the distributors will find it difficult to get narcotics to be traded. If they still can get the drug, the price will be very expensive because the supply of the drug is very limited. Thus, illegal users will also find it difficult to obtain narcotics and other illegal drugs because of the limited supply, and the very high prices. The main objective to be achieved in this regard is the creation of a condition that can make it difficult for dealers and illegal users to easily get narcotics and illegal drugs.

The law enforcement in the effort to eradicate criminal acts of narcotics and psychotropics should be more focused on efforts to prevent the occurrence of illegal circulation. This effort can be realized in the form of strict supervision to the producers and importers. Based on the results of the research conducted in 5 (five) sample regions, it is known that the current effort to eradicate narcotics crimes is more focused on the aspect of abuse, not on the efforts to prevent illegal distribution of the drugs. It shows that the law enforcement efforts are directed more at the illegal users. This is difficult to be expected to reduce the occurrence of criminal acts of narcotics abuse because narcotics and other illegal drugs can still be easily obtained. The main factor that causes the crime of narcotics abuse is because narcotics and other illegal drugs can be obtained easily. If the narcotics and illegal drugs cannot be obtained illegally, the use of illegal drugs can also be reduced or even eliminated. Therefore, the efforts to eradicate narcotics crimes must be carried out comprehensively starting from the upstream part, which is the suppliers, either producers or importers, up to the downstream, which is the users.

The policy in eradicating criminal acts of narcotics abuse is a positive legal policy which in essence is not
merely an implementation of normative juridical laws. In addition to the normative juridical approach, criminal law policy also requires a comprehensive and integral factual juridical approach. The law has provided a sufficient legal umbrella and has regulated the suppliers, importers or producers, and the users, but at the level of implementation, law enforcement efforts still need to be improved. This is more related to the performance of the law enforcement officers themselves.

Based on the description in the previous sections, it can be understood that the fundamental problem faced in eradicating criminal acts of narcotics abuse is the lack of legal factors, namely legislation in anticipating the development of the modus operandi of narcotics crime and because of internal systems and performance law enforcement itself. As stated by Friedman, there are 3 (three) main components in the legal system that are effectively related and affecting each other in the law enforcement efforts. The three components are the factors of law (the substance), the structure, and the legal culture. If the legal factors are weak but the law enforcement officers are consistent and firm and supported by adequate management and facilities, then supported by a conducive community legal culture, the performance of law enforcement will take place effectively. However, if the legal arrangements are good but the law enforcement agencies are not firm and inconsistent; the facilities and management are disproportionate; the society is not conducive, the performance of law enforcement becomes ineffective.

Based on the provisions of Article 4 of Law No. 35 of 2009 concerning Narcotics, it can be understood that the policies related to narcotics and psychotropic crimes are inseparable from the objectives of the law, namely: (a) guarantee the availability of Narcotics for the benefit of health services and/or the development of science and technology; (b) prevent, protect and save the Indonesian people from narcotics abuse; (c) eradicate narcotics and narcotics precursors illicit traffic and; (d) guarantee the regulation of medical and social rehabilitation efforts for abusers and drug addicts. Therefore, the formulation of the offense in the narcotics law covers planting, production, distribution, traffic, circulation to the users, including personal use.

Even the results obtained from criminal acts of narcotics and psychotropic abuse, whether in the form of movable or immovable assets, tangible or intangible assets as well as goods or equipment used to carry out narcotics and narcotics precursors crimes are confiscated for the state. This is in accordance with the provisions of Article 136 of Law No. 35 of 2009 concerning Narcotics.

The policy of eradicating crime by using penal means is a form of repressive action that focuses on prosecution after the crime has occurred, namely the imposition of criminal sanctions. The success of the efforts to eradicate psychotropic crime is largely determined by the existence of good integration and cooperation between law enforcement officials both the police and the National Narcotics Agency. This integration will ultimately lead to harmony and balance of life in the society.

Law enforcers have made serious efforts to eradicate criminal acts of narcotics and psychotropic abuse. There have been many parties who have been arrested and have even been convicted, ranging from illegal users, dealers, importers, to the producers. However, other facts show that the level of abuse of narcotics and psychotropic substances in Indonesia continues to increase from year to year. This shows that law enforcement efforts carried out by the law enforcement officials in eradicating criminal acts of narcotics and psychotropic abuse are still less effective.

**Conclusion**

The policy in eradicating criminal acts of narcotics abuse is a positive legal policy which in essence is not merely the implementation of normative juridical laws. In addition to the normative juridical approach, criminal law policy also requires a comprehensive and integral factual juridical approach. The new narcotics law also has two approaches to the parties using narcotics illegally or in other words, abusing narcotics. The first approach is to see the drug users as victims so that they
must be rehabilitated and the second approach is to see the drug users as perpetrators of criminal acts. Criminal acts related to narcotics crimes can generally be divided into 3 (three) types, namely supplying narcotics and other illegal drugs, which is usually done by producers or importers; distributing the drugs, which is carried out by dealers or sellers; and using the drugs or abusing the drugs. Therefore, efforts to eradicate it must also include all three types of activities or actions.

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Recent Perspectives and Upcoming Directions in Molecular Diagnosis of Malaria: A Systematic Review

Ola S. H. Almusaddar1, Cyuzuzo Callixte2
1Postgraduate Student, Department of Nutrition, Faculty of Public Health, Universitas Airlangga, Indonesia, 2Postgraduate Student, Department of Immunology, Postgraduate School, Universitas Airlangga, Indonesia

Abstract

Background: Malaria remains a global leading cause of morbidity and mortality. The absence of effective vaccines is still the vital hindrance to the management and elimination of malaria. From that standpoint, accurate laboratory diagnosis could be the right hand for disease management. Objective: This review intended to assess the recent perspectives and upcoming directions in molecular diagnosis of malaria. Methods: This review was conducted by using internet searching tools where 35 published papers were retrieved from the credible online publishers and among them, 27 papers that satisfy the inclusion criteria were profoundly reviewed.

Results: Among the 27 articles, 22(81.48 %) papers focus on contribution of PCR based-method in malaria diagnosis, 4(14.8%) report on comparison between polymerase chain reaction(PCR) and other molecular techniques, 7(30%) emphasize on advantages and disadvantages of PCR, 4(14.8%) represent relationship among PCR and LAMP, 3(11%) discuss the most promising molecular diagnostic tools, 5(18%) focus on comparative designs of different PCR methods, whereas 1(3.7%) emphasizes on parasite density and 2(7%) on pigment containing monocyte.

Conclusion: This review conclude that microscopy remains the gold standard method for malaria diagnosis and speciation in limited resource settings but also molecular based-methods provide significant alternatives with superior sensitivity and specificity.

Keywords: Malaria, Molecular Diagnosis, LAMP, PCR, Microscopy.

Introduction

Malaria is one of the major public health problems hampering developmental efforts in most developing countries (1). Malaria is spread by contaminated mosquito with parasitic protozoans (a group of single-celled microorganisms) belonging to the genus Plasmodium (2). Plasmodium genus have five species of parasites that affect vertebrates (humans), these species are Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium knowlesi and Plasmodium ovale (1). The disease is most frequently transmitted by an infected female Anopheles mosquito. After biting, the mosquito introduces the parasites from its saliva into a person’s blood (2).

Malaria symptoms typically develop within 10 days to four weeks following the infection. Common symptoms of malaria include: shaking chills that can range from moderate to severe, high fever, profuse sweating, headache, nausea, vomiting, diarrhea, anemia, muscles pain, convulsions, coma and bloody stools (2).

In terms of diagnosis, for more than 100 years, microscopy has been the gold standard diagnostic tool for malaria (3). The World Health Organization (WHO) suggests that malaria can be diagnosed by standard
microscopy or immunochromatographic lateral flow assays (known as rapid diagnostic tests, RDTs) before treatment (4) . The other diagnostic tests for malaria are, serology, fluorescence microscopy and nucleic acid-based amplification techniques like PCR and isothermal amplification where the mostly known is loop mediated isothermal amplification (LAMP) (5). Rapid diagnostic test and quantitative real-time PCR (qPCR), are techniques that are applied to malaria studies and were used to detect Plasmodium species in 10⁹ whole-blood samples from patients after their return from malaria endemic areas (6). Methods of real-time PCR are particularly designed for large scale screening and they can be used in malaria control and elimination programs (7). Even if WHO recommenced the use of PCR to detect the asymptomatic patients and low parasitemia, PCR requires technical expertise, expensive reagents and infrastructures which make it inaccessible in low- and middle-income countries (5).

Blood smear microscopic examination remains the current gold standard method, because of its availability, low cost and ability for detection of malaria and speciation. PCR assay present an alternative to microscopy which has shown a superior sensitivity and specificity (8). Microscopy is most frequently used, but at low parasitemia (<20 parasites/µl), the technique becomes less sensitive and time consuming (9). Rapid diagnostic tests based on Plasmodium antigen detection do frequently not allow discrimination of species as microscopy does, but also become insensitive at <100 parasites/microliter. Judgment concerning malaria treatment depends on the right identification of species causing the disease. Therefore, there is a need for developing new tools of diagnosing malaria that are sensitive, cost effective and capable of performing large scale diagnosis. Here, we review the most promising molecular diagnostics developed so far and give some viewpoints for the near future (9).

This review is intended to describe the recent and upcoming directions in molecular diagnosis of malaria for the detection of low parasitemia in asymptomatic patients from the perspective of high-throughput and practicability in constrained resource environments for the sake of malaria elimination. This review also emphasizes on the advantages and disadvantages of most promising molecular diagnostics and present recommendations on possible future directions in molecular diagnosis of malaria.

Material and Methods

This review was carried out by using important internet searching tool: PubMed. It is actually consisted by the analysis of specific segment of published papers to a given research topic related to malaria diagnostic tools. In other words, it describes, summarizes, evaluates and report on the retrieved papers. This study context provides an update on what is going on in malaria molecular diagnostics and what has been done so far whether it is beneficial to Malaria patients or not. The published articles were carefully searched through PubMed search board in order to obtain different papers which could be perfectly related to the review topic for reviewing. PubMed have been chosen to be the best searching tool due papers accessibility (regularly uploaded and updated research papers), reliability and specificity to medical domain and contains a collection of most of medicine and biomedical sciences that fulfill medical evidence-based guidelines.

PubMed covers most papers in medicine domain and allows a quick and advanced search. A computer-based papers’ search was done on two different dates (12th and 28th February, 2020) to avoid any search bias that may result; as day after day, in the same database more newly published papers get uploaded. This data was retrieved from searching engine based on different research keywords related to the study title. The keywords used were malaria, malaria diagnosis and molecular diagnosis of malaria. The targeted reviewed papers were those related to malaria, malaria diagnosis and molecular diagnosis of malaria. This research reviewed most of the published papers on current and future direction in molecular diagnosis of malaria. It considered and extracted different published papers examining factors associated with either malaria diagnosis or molecular
diagnosis of malaria. All the current and updated papers that are straightly relevant to the topic were considered and reviewed in this work.

During this study, different inclusion and exclusion criteria were set. For a paper to be included in this study had to be published in English, giving information on development of molecular diagnosis of malaria, and being published in 2000 to 2020. The papers that were published before 2000, published in a different language other than English, published as a book, papers having a title which is not related to our topic or having insufficient information were rejected.

After considering the inclusion and exclusion criteria and other described research considerations, a detailed review strategy can be demonstrated on the following flowchart which shows the reviewed 35 Published papers which yielded 27 papers that were used in this current evaluation.

**Reliability and Validity**

The results of the 27 reviewed studies are reliable because all these papers considered for our review were retrieved from PubMed which hosts reliable data. PubMed database uploads health related papers with standard quality, from highly cited journals. For instance, our retrieved papers were published in different good journals as follows: Malaria Journal (impact factor: 3.109); Journal of infectious disease (impact factor: 5.997); Journal of Clinical Microbiology (impact factor: 3.993); A Peer-reviewed Open Access Journal (impact factor: 3.234); Korean Journal of Parasitology (impact factor: 1.151); American Journal of Tropical Medicine and Hygiene (impact factor: 2.699); Tropical Medicine International Health (impact factor: 2.329); Journal of tropical medicine (impact factor: 0.926); Clinical Microbiology and infection (impact factor: 5.768); Southeast Asian Journal of Tropical Medicine public health (impact factor: 0.719); Pathogen Global Health (impact factor: 1.656). The final reviewed paper’s validity is extremely ensured as well, as their content falls within the broad aim of our review.

**Results**

Research results were obtained by using the identification for analysis where common themes used are PCR based-assay, comparison between PCR and other molecular techniques in malaria diagnosis, advantages and disadvantages of PCR based-method, relationship among PCR based assays, most promising molecular diagnosis of malaria, comparative designs of PCR, pigment containing monocyte based-assay which are presented within the selected twenty-seven articles.

**Theme 1: PCR based-assay**

PCR based-assay in malaria diagnosis was reported within by 22 of the 27 selected studies. PCR will be used in the future to screen samples from clinically suspected foci to increase the proportion of detected malaria cases. PCR products can also be analyzed using gel-electrophoresis. For instance, the samples that were found to be negative by db-PCR-NALFIA but positive for *Plasmodium* in their initial diagnosis were determined to be negative by gel analysis as well (10).

Nested-PCR belongs to PCR based-method and was performed in samples from 30 non-human primates nine samples (30%) of them were found to be positive for *Plasmodium* (11). Different PCR forms have been reported including: two single-PCR reactions which are nested 1PCR and nested 2PCR designed to detect *Plasmodium falciparum* infections, one single PCR to detect *Plasmodium vivax* infections, and one multiplex one-step PCR reaction to detect both parasite species (12).

Nested PCR assay was also successful in detecting mixed infections that are not detected by microscopy (13). The study concluded that nested PCR and real-time PCR may be highly suitable for asymptomatic malaria detection in large numbers of clinical samples from areas of endemicity (14). The mixed infections detected by nested-PCR assay were 6.5, 22 and 23.5 percent in samples collected from Afghanistan, Iran and Pakistan respectively (15). Nested PCR, multiplex and real-time PCR were investigated for their sensitivity and specificity for the overall detection of Plasmodium.
species. They were reported to be 96.6% and was 89.4%, respectively while microscopy is still considered as the gold standard for malaria detection and identification(8). PCR based diagnostic tools are capable of detecting very low parasitemia due to high sensitivity. *Plasmodium falciparum, P. ovale, and P. vivax* which are the species of malaria parasite were detected by a TaqMan-based real-time PCR qualitative assay(16); and Polymerase Chain Reaction (PCR) is a popular nucleic acid-based tool that is used in the diagnosis of various infectious diseases(17).

Molecular methods, including nested polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) and histidine-rich protein 2 (HRP-2) based rapid diagnostic tests (HRP-2-RDTs) were used for the malaria diagnosis (18). LAMP presents a high diagnostic accuracy for parasite detection among both fever patients and asymptomatic individuals(19). The same technique is claimed to be a molecular diagnostic test for malaria which is simple and inexpensive to detect the conserved 18S ribosome RNA gene of *P. falciparum* (20). Malaria LAMP displayed diagnostic accuracy similar to that of nested PCR, by using pan-*Plasmodium* genus primers, LAMP did not differ in performance from nested PCR (P = .3447) (21). The method which was found to be the most efficient for the detection of mixed infections is the PCR-based method because of its high sensitivity and specificity (22). The three molecular assays (real-time multiplex polymerase chain reaction, merozoite surface antigen gene [MSP]-multiplex PCR and the PlasmoNex Multiplex PCR Kit) were reported to be involved in detection of plasmodium species, for the real-time multiplex PCR was more sensitive (81%) in detecting *P. vivax, P. falciparum and P. knowlesi* infections compared with the PlasmoNex Multiplex PCR Kit (62%) and MSP-multiplex PCR (50%) and they displayed 100% specificity for detecting malaria samples (23).

Identification of specific malaria parasite infection through PCR often is followed by DNA sequencing of the 18S rRNA gene for confirmatory purpose(24). For instance, quantitative real-time PCR (qPCR) is commonly used as a confirmatory method for malaria diagnosis; it was performed with high efficiencies of more than 94%. The multiplex qPCR assay detects simultaneously *Plasmodium spp., P. falciparum, P. vivax* and human RNaseP gene(25).

The PET-PCR is a new molecular diagnostic tool with similar performance characteristics as commonly used PCR methods that is less expensive and easy to use (26). The RFLP-dHPLC method detected many more mixed species infections than did microscopy. Their use revealed a high prevalence of sub-microscopic infections. The overall prevalence detected by the RFLP-dHPLC method and microscopy were (68.4% and 30.7%) respectively (27).

**Theme 2: Comparison between PCR and other molecular techniques in malaria diagnosis**

Four out of 27 reviewed studies (14.8%) report the comparison between PCR and other molecular techniques in malaria diagnosis. The sensitivities of the two nested PCR and real-time PCR techniques were higher than that of microscopy examination (sensitivity, 100% versus 26.4%; kappa values, 0.2 to 0.5). PCR-based molecular methods for malaria parasite detection are relatively simple and provide improved sensitivity and various advantages compared to microscopy and RDTs (14).

Malaria LAMP also demonstrated diagnostic sensitivity significantly superior to that of expert microscopy (21). Comparison of nested PCR to HRP-2-RDTs show that the poor sensitivity of HRP-2-RDTs indicates that low parasitemia may not be detected after treatment, whereas the low specificity of HRP-2-RDTs indicates that it cannot be applied for treatment follow-up. However, HRP-2-RDTs have similar sensitivity as microscopy but less specific. The db-PCR-NALFIA is much more sensitive than RDT, especially for the detection of *P. vivax* (17).

**Theme 3: Advantages and disadvantages of PCR based- method**
The advantages and disadvantages of PCR based-method in diagnosis of malaria were reported within 7 out of 27 reviewed papers (30%). PCR-based molecular methods for malaria parasite detection are relatively simple and they are highly specific and capable of reporting highly sensitive test values (13). PCR is known to be highly sensitive and specific as an advantage but it presents some barriers including its high cost, and the amount of infrastructure required in terms of equipment and a sophisticated laboratory setup with stable power and refrigerators for reagent storage (16). PCR based-methods have demonstrated high sensitivity and specificity in detection of plasmodium infection and have the ability to quantify parasitemia when used in a quantitative real-time PCR format. The ease and speed of PCR make it an important tool in malaria elimination programs (27). LAMP is simpler and faster as compared to nested PCR with the advantage of detecting low parasitemia becoming a potential point-of-care test for treatment follow-up. The low sensitivity and the high false positive rate of HRP-2-RDTs is the major limitation of its application for monitoring of the therapeutic response; and PCR tools are too sophisticated and expensive as a disadvantage to apply them in most malaria endemic countries (17). Real-time PCR does not require post-PCR processing; this reduces sample handling and minimizes the risk of contamination (15). PCR can be used for precise parasite quantification through qPCR methods (25). Nested PCR, multiplex and real-time PCR are capable of detecting very low parasitaemia (7).

**Theme 4: Relationship among PCR based assays**

This theme was presented by 4 out of 27 selected studies (14.8%), giving an explanation on relationship among PCR based assays. Nested PCR-2 and real-time PCR displayed similar results with higher sensitivities of 100% reported (13). LAMP has similar sensitivity and specificity to nested PCR. With high PPV and NPV (17). Malaria LAMP had a diagnostic accuracy similar to that of nested PCR among collected positive blood samples, one sample characterized as *P. vivax* monospecies infection by nested PCR was found to be positive for *P. falciparum* (20). Specificity of all three assays (real-time multiplex polymerase chain reaction [PCR], merozoite surface antigen gene [MSP]-multiplex PCR, and the PlasmoNex Multiplex PCR Kit) were tested using gDNAs of all five malaria. And in species of human addition the PlasmoNex Multiplex PCR Kit and real-time multiplex PCR showed similar sensitivity for detecting *P. vivax* (22).

**Theme 5: Most promising molecular diagnosis of malaria**

The findings obtained from 3 out of 27 reviewed papers (11%), reported on the most promising molecular diagnosis techniques for malaria. The malaria LAMP is the most promising molecular diagnosis for malaria because it has advantages over other molecular tests in speed, sensitivity, and minimal need for specialist training (20). The same advantages of LAMP have been reported as of being cheaper, simpler and faster. The 100% sensitivity and specificity of real-time PCR methodology supports its status as the best PCR methodology to detect *P. falciparum* and *P. vivax* (8).

**Theme 6: Comparative designs of PCR**

Comparative designs of PCR were encountered within 5 out of 27 selected studies (18%). Real-time PCR technique is based on primer and probe sequences for the gene encoding the 18S Plasmodium rRNA genes; whereas for the nested PCR-2, the species-specific nucleotide sequences of the 18S rRNA genes of *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* are targeted and for the nested PCR-1 strategy is based on use of primers (13). All PCR reactions can be performed in a 20 μL volume containing 250μM each oligonucleotide primer, 10μL of master mix (Promega) (0.3 units of Taq Polymerase, 200μM each deoxyribonucleotide triphosphates and 1.5 mM MgCl2) and 2μl DNA (11). Nested-PCR assay uses 18S small sub-unit ribosomal RNA (ssrRNA) gene (16). Nested polymerase chain reaction (PCR) primers were designed from the 3R region of the 28SrRNA gene (13). A multiplex qPCR assay was designed to simultaneously detect *Plasmodium* species such as *P. falciparum*, *P. vivax* and human RNaseP gene.
as an endogenous control (25).

**Theme 7: Parasite density per microliter of blood**

Only one reviewed paper out of the twenty-seven, is reporting on parasite density per microliter technique. Parasite density per microliter of blood in a patient is determined using parasite count adjusted by average WBC count (8000/microliter) observed in microscopic fields of the thick film (28).

**Theme 8: Pigment containing monocyte based-assay**

Pigment containing monocyte based-assay was reported within two out of twenty-seven selected studies (7%). Those studies include (29–31). At a discrimination level of one or more atypical pigment-containing monocytes (PCM), negative and positive agreement was found to be 95.6% and 91.6% respectively and many analyses showed that the only significant risk factor for the presence of PCM (odds ratio > 200) was malaria infection (31). Malaria PCM as a distinct cluster in scatter diagrams that is well separated from normal leukocytes. From microscopic inspection of sorted cells and PCM has a different average relative frequency according to the patients: for nonimmune patients is 1.5 x 10^-4 (median), for semi-immune patients 8.8 x 10^-4, and for malaria-negative persons 4.4 x 10^-6 (29).

**Discussion**

All the 27 reviewed research and review articles were selected based on molecular diagnosis of malaria. Some of those studies focus on advantages and disadvantages of PCR based essay in diagnosis of malaria. Currently, diagnosis of malaria by use of microscopy or RDT are not sufficient in specificity or sensitivity to detect low-parasite-density infections. Microscopy and RDTs become relatively less sensitive at parasite densities below 100 parasites/μl (11,29–32).

RDTs are inexpensive, simple to perform, and provide results in 15-20 minutes. Despite high sensitivity and specificity for Plasmodium falciparum infections, RDTs have several limitations that may reduce their utility in low-transmission settings: they do not reliably detect low-density parasitemia (≤200 parasites/μL), many are less sensitive for Plasmodium vivax infections, and their ability to detect Plasmodium ovale and Plasmodium malariae is unknown (33). Microscopic examination of blood smears remains the current gold standard for malaria detection and speciation. However, PCR assays present an alternative option to microscopy which has been shown to have superior sensitivity and specificity (8).

PCR based molecular method when compare to thick blood smear this test displayed a sensitivity ranging from 65% to 81% and specificity was close to 100%; and this method of assay detects all five Plasmodium species (22). The highly sensitive and specificity of PCR makes it an advantageous technique, but the same technique presents some barriers including its high cost, and the amount of infrastructure required in terms of equipment and a sophisticated laboratory setup with stable power and refrigerators for reagent storage (17). In a different study, the prevalence of asymptomatic parasitemia was reported at 17% by microscopy and 47% by PCR (34). The easiness and speed of PCR makes it an important tool in malaria elimination programs and should be improved to detect asymptomatic infections (34). The diagnosis of malaria parasites by one of the PCR based assays, nested PCR, in the present assessment is at medium level with an accuracy of 84.21% (48/57). In comparison of microscopy to nested PCR, microscopy showed the sensitivity and specificity of 85.39% and 100% respectively, whereas the sensitivity and specificity of the nested PCR assay was found to be 99.08% and 100% respectively. Nested PCR assay was also successful in detecting mixed infections that are not detected by microscopy (13).

The PCR-based method was used as the reference standard because of its high sensitivity and specificity over microscopy, particularly in cases with low-level parasitemia. When the three PCR-based methods (nested PCR-1, nested PCR-2 and real-time PCR) were examined for detecting malaria infection in residents of regions of Myanmar where malaria is endemic, two
nested PCR and real-time PCR assays showed that asymptomatic infection was detected in about 1.0% to 9.4% of residents from the surveyed areas; and they showed similar sensitivity in genus-specific tests. However, it was observed that nested PCR-1 showed a lower sensitivity in detecting *Plasmodium spp*, especially *P. falciparum* and *P. malariae*, compared to nested PCR-2 and real-time PCR. The limited detection by nested PCR-1 resulted in 14 samples being positive in a genus-specific assay, while reported negative in a species-specific assay. The results suggest that nested PCR-2 and real-time PCR may be highly suitable for asymptomatic malaria detection in large numbers of clinical samples from areas of endemicity(6,14).

All three PCR methodologies (nested, multiplex and real-time PCR) investigated, were sensitive, specific and capable of detecting very low parasitemia. Although economies of scale can be applied to PCR methods to reduce the time and cost involved in processing each sample, the results from the three molecular speciation techniques (nested PCR, multiplex PCR, and real-time PCR) were used to develop a molecular consensus (two or more identical PCR results) as an alternative gold standard. According to the molecular consensus, 9.6% (13/136) of microscopic diagnoses yielded false negative results. Multiplex PCR failed to detect *P. vivax* in three mixed isolates, and the nested PCR gave a false positive *P. falciparum* result in one case. Although the real-time PCR melting curve analysis was the most expensive method, it was 100% sensitive and specific and least time consuming of the three molecular techniques investigated (8).

The sensitivity and specificity results for the four speciation techniques differed depending upon whether microscopy or molecular consensus was used as the gold standard. Using microscopy as gold standard, the sensitivity of all three molecular techniques for the overall detection of *Plasmodium spp* was 96.6% (86/86+3) and the specificity was 89.4% (42/42+5). However, when Molecular Consensus Was Used As The Gold standard the sensitivity and specificity of microscopy was 94.5% (86/86+5) and 93.3% (42/42+3) respectively(8). By evaluating A TaqMan-based real-time PCR, qualitative assay was performed on 122 whole blood samples from patients who presented with malaria-like symptoms and fever, real-time PCR assay showed a detection limit with analytical sensitivity of 0.7, 4, and 1.5 parasites/μl for *P. falciparum*, *P. vivax*, and *P. ovale* respectively. Real-time PCR can yield results within 2 hours and does not require post-PCR processing, which reduces sample handling and minimizes the risks of contamination(16).

The db-PCR-NALFIA is also used for the detection of Plasmodium species directly from blood samples and is much more sensitive than RDT, especially for the detection of *P. vivax* as well as for *P. falciparum*. There were differences in results between the db-PCR-NALFIA and microscopy and RDT. Although in both field settings there were false-negative db-PCR-NALFIA samples that were determined to be positive by microscopy and/or RDT, there was a greater number of false-positive db-PCR-NALFIA samples that were negative with the other methods. The db-PCR-NALFIA is a relatively easy-to-use method that is robust, sensitive, and specific and could have great potential in locations where malaria is endemic; especially in areas where there is low transmission of malaria, and thus a very sensitive technology is warranted (10).

Microscopy remains the gold standard method for malaria diagnosis because of its simplicity, affordability and the ability to quantify the parasite density. As molecular techniques become much more widely used and acceptable as alternate or as confirmatory assays to microscopy, they must meet and exceed qualities and characteristics that have made microscopy popular. Absolute quantitative multiplex qPCR assay simultaneously detects three *Plasmodium* targets (*P. falciparum* and *P. vivax* parasites) and the human RNaseP as an endogenous control. If qPCR assay is going to replace microscopy as the gold standard diagnostic method, the absolute quantification is
reported as parasites/µl, the same units as those used in microscopy. Currently, qPCR assays use relative standard quantification methods to quantify parasite density in a sample where cultures or clinical samples with known parasite density are used.\(^{(25)}\)

PCR-based molecular methods are good for both their sensitivity and specificity values, but too sophisticated and expensive to be applied in most malaria-endemic countries\(^{(28)}\). LAMP has similar sensitivity and specificity to nested PCR, with high PPV and NPV. LAMP is a cheap, simpler and faster method as compared to nested PCR; with its advantage of detecting low parasitemia, it is becoming a potential point-of-care test for treatment follow-up\(^{(18)}\). In a 128 febrile children study, a complete set of four malaria tests was carried out. Positive results for HRP-2-RDTs, microscopy, nested PCR, and LAMP, were 68(53%), 47(37%), 64(50%), and 65(51%) respectively. When nested PCR was used as a reference standard, only LAMP was comparable and both HRP-2-RDTs and microscopy had moderate sensitivity. HRP-2-RDTs had poor positive predictive value (PPV) and a moderate negative predictive value (NPV) for the treatment follow-up. HRP-2-RDTs have similar sensitivity as microscopy but less specificity. However, as compared to nested PCR, the poor sensitivity of HRP-2 and RDTs indicates that low parasitemia may not be detected after treatment; and the low specificity of HRP-2-RDTs indicates also that it cannot be applied for treatment follow-up\(^{(18)}\).

Malaria LAMP had a diagnostic accuracy similar to that of nested PCR, with a greatly reduced time to result. Malaria LAMP demonstrated diagnostic sensitivity significantly superior to that of expert microscopy. In the analysis, using pan-Plasmodium genus primers, LAMP did not differ in performance from nested PCR (P = 0.3447). LAMP with P. falciparum primers was found to have significantly different diagnostic accuracy than PCR (P = 0.004), with a positive predictive value of 83.5% and a negative predictive value of 99.8%. The current format of pan-Plasmodium genus and P. falciparum–specific LAMP is able to identify all P. falciparum–infected individuals. The costs of LAMP reagents are close to those used for nested PCR, but comparing both techniques in terms of equipment and labor costs, reveals that LAMP would be more affordable for laboratories in malaria-endemic countries. The malaria LAMP test, evaluated for the primary diagnosis of malaria has advantages over other molecular tests in speed, and minimal need for specialist training; and it is a suitable test for diagnosing imported cases of malaria in minimally equipped clinical laboratories\(^{(21)}\).

**Conclusion**

In this review on malaria diagnosis, microscopy remains the current gold standard method due to its availability, low cost and its ability to detect and identify various *Plasmodium* species. PCR assay is a molecular diagnostic method presents an alternative tool to microscopy and has shown a superior sensitivity and specificity. Molecular tests are valuable tools for the confirmation of *Plasmodium* species and in detecting mixed infections in malaria endemic regions. However, most of molecular techniques used in malaria diagnosis present some bottlenecks including the high cost of labor and less access to reagents, compared to the examination of blood smears. Among PCR based-assay methods, real-time PCR is ranked the best due to its high sensitivity and specificity (100%) to detect both *P. falciparum* and *P. vivax*. As a major outcome of our review, the LAMP technique is claimed to be a simple and inexpensive malaria- molecular diagnostic test that detects the conserved 18S ribosome RNA gene of *P. falciparum*. In other studies, the same technique has shown high sensitivity and specificity, not only for *P. falciparum*, but also for *P. vivax*, *P. ovale* and *P. malariae*. LAMP appears to be easy to conduct, sensitive, quick and lower in cost than PCR.

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Synthesis and Characterization of New Oxo-Aztedine, Imidazolidine and Thiazolidine Rings on Creatinine and Evaluation of their Biological Activity

Raad M. Muhiebes, Entesar O. A. Tamimi

1 Research Scholar, Chemistry Department, Faculty of Science, University of Baghdad, Iraq, 2 Professor, Chemistry Department, Faculty of Science, University of Baghdad, Iraq

Abstract

This work included preparation of new imine from the reaction of creatinine with aldehydes (4-nitro benzaldehyde, 4-amino benzaldehyde, cinnamaldehyde) and ketone (acetophenone) in ethanol and the presence of few drops glacial CH₃COOH to obtain Schiff bases (1a, 1b, 1c, 1d). Schiff bases were treated with monochloroacetyl chloride to produce (2a, 2b, 2c, 2d), with glycine to synthesize (3a, 3b, 3c, 3d) and with 2-mercaptoacetic acid to prepare (4a, 4b, 4c, 4d). The prepared compounds had been measured by (FT-IR, and ¹H NMR) spectroscopic techniques. Some of the derivatives were studied activity against antibacterial, antifungal and antioxidant.

Keyword: Schiff bases, β-lactam, Oxo imidazolidine, thiazolidine derivatives.

Introduction

Schiff bases are the compounds containing (–C=N–) as a functional group. They are a condensation products of 1 amines with C=O compounds, these compounds were first reported by Hugo Schiff (1-3). Schiff bases have also been reported to have a broad range of biological activities, like antifungal, antibacterial, antimalarial, anti-inflammatory, antiviral, and antipyretic properties (4). Heterocyclic compounds containing four and five-member rings have given industrial and medical reasons. Azetidinone derivatives are one of these compounds, they represent an important commonly known as β-lactam, due to their antibacterial, antifungal, anti-tubercular, antianthelminic, and enzymatic activity (5). Furthermore, they are found to inhibit cholesterol absorption (6). The imidazoles are an important class of heterocyclic and many naturally occurring imidazoles are known to possess biological activity, anti-fungal, anti-bacterial, anti-anthelmintic, anti-neoplastic, antipyretic and anti-spasmyotic activities (7). On the other hand, thiadiazole, fused heterocyclic ring compounds have many biological activities as antimicrobial activity (8), anti-inflammatory (9).

Materials and Methods

All initial chemicals necessary for Schiff bases preparation were obtained from Fluka, Sigma- Aldrich, Alfa Aesar, Japan and BDH used without further purification. The Stuart melting point apparatus was used to measure melting points. IR Affinity-1 Shimadzu as KBr disc, results are given in cm⁻¹. ¹H NMR BrukerSpectrospin ultrashield magnets 300 MHz instruments, using DMSO-d₆ solvent.

Schiff bases (10) (1a, 1b, 1c, and 1d) preparation procedure

Schiff bases were prepared by the reaction of creatinine (0.113g, 0.001 mol) with variable aldehydes and ketone (0.001 mol), in 30 ml EtOH and few drops of glacial acetic acid. This mixture was refluxed for (10-12hrs). The excess solvent was evaporated and the formed product was recrystallized from absolute C₂H₅OH. Physical properties are listed in Table 1.
Synthesis of azitidine-2-oxo derivatives (11) (2a, 2b, 2c and 2d)

Schiff base (1a,1b,1c, and 1d) solution (0.003mol) in (25 ml) THF was quantitatively poured into the mixture of ClCH$_2$COCl (0.34 g, 0.004 mol) and TEA (0.56 g, 0.004 mol). Solutions was well mixed at (0-5ºC), then the reaction was conducted for (6-8) hrs at room temperature. The mixture then kept for additional 2 days in the sealed containers at RT., after that the mixture was poured in a container with ice to obtain the compound in a solid form. The products were filtered and washed by distilled water then dried and recrystallized. Table 1 show the physical properties of the newly generated compound.

Synthesis of 4-oxo-imidazoline derivatives (12) (3a,3b,3c and 3d)

A mixture of Schiff base (1a,1b,1c and 1d) (0.001 mol), glycine (a-amino acetic acid), (0.07g, 0.001mol) in (25ml) ethanol and few drops DMF was refluxed for (20h.), the excess solvent was evaporated. The solid product was collected and recrystallized from absolute CH$_3$CH$_2$OH. Physical properties are listed in Table 1.

Synthesis of 4-oxo-thiazolidine derivatives (13) (4a, 4b, 4c and 4d)

To a mixture of Schiff bases (1a,1b,1c and 1d) (0.002 mol) and 2-mercaptoacetic acid (0.92g, 0.01 mol) dissolved in DMF (25 ml), anhydrous ZnCl$_2$ (0.21g, 0.0016 mol) was added and refluxed for (18-20 hrs). The reaction mixture was then poured into crushed ice, filtered and washed with water, dried, and recrystallized from absolute ethanol. Physical properties are listed in Table 1.

Biological Activity (14)

Using the culture media called Muller-Hinton for determination of the biological effectiveness of the following material (2a to 4d) on ranges of isolated microorganism (Bacteria and Fungi). After being dissolved, Muller-Hinton was sterilized by using autoclave (121ºC for 15 min.) and poured onto petri-dishes (plate-count). Once Muller-hinton and been left to solidify and to be ready for use, the specific microorganism were used in this study. Then 0.1 mm had been added to the material mentioned in the middle of the plates contaminated previously with bacteria or fungi to determine the biological effects of each material. After then the plates were incubated with a particular incubator (37ºC for 24hr.) the inhibition zones were seen clearly after 24hr, for bacterial and 48hr. for fungi incubation on each plate-count. However, these zones were differed according to material in addition to isolated microorganisms used on each Petri dish. The result of all tested compound is listed in table (3).

Results & Discussion

<table>
<thead>
<tr>
<th>No. Com</th>
<th>m.p.ºC</th>
<th>Color</th>
<th>Yield%</th>
<th>v (C=O) amide</th>
<th>v (C=O) lactam</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>220-222</td>
<td>Off-white</td>
<td>90</td>
<td>1666 1421</td>
<td>1707</td>
</tr>
<tr>
<td>1b</td>
<td>230-232</td>
<td>Brown</td>
<td>88</td>
<td>1622 1419</td>
<td>1689</td>
</tr>
<tr>
<td>1c</td>
<td>182-184</td>
<td>Bile yellow</td>
<td>75</td>
<td>1669 1417</td>
<td>1699</td>
</tr>
<tr>
<td>1d</td>
<td>180-182</td>
<td>White</td>
<td>80</td>
<td>1670 1419</td>
<td>1700</td>
</tr>
</tbody>
</table>

Table 1: Some physical properties and FT-IR spectral data cm$^{-1}$ of synthesized creatinine derivatives (1a-4d)
Characterization of all the derivatives was carried out by FTIR and some of others was done by ¹H-NMR. Scheme (1) showed different organic compounds contained heterocyclic rings from imins derivatives.

| 2a | 178-176 | Green | 70 | - | 1435 | 1651 | 2941 | 2802 | 3060 | 1577 | 1500 | - | 1718 |
| 2b | 204-206 | Brown | 65 | - | 1433 | 1633 | 2976 | 2877 | 3062 | 1558 | 1475 | - | 1732 |
| 2c | 152-154 | Brown | 60 | - | 1417 | 1700 | 2939 | 2802 | 3042 | 1505 | 1475 | 1558 | 1739 |
| 2d | 222-224 | Yellow | 70 | - | 1400 | 1627 | 2978 | 2850 | 3064 | 1558 | 1475 | - | 1710 |
| 3a | 160-162 | Orange | 90 | 1672 | 1415 | 1755 | 1714 | 2918 | 2850 | 3080 | 1598 | 1521 | - | - |
| 3b | 122-124 | Brown | 65 | 1597 | 1411 | 1693 | 1662 | 2931 | 2883 | 3022 | 1597 | 1508 | - | - |
| 3c | 88-90 | Yellow | 75 | 1639 | 1415 | 1668 | 1639 | 2902 | 2800 | 3028 | 1595 | 1550 | 1494 | - |
| 3d | 196-198 | White | 80 | 1670 | 1419 | 1750 | 1689 | 2900 | 2806 | 3024 | 1593 | 1500 | - | - |
| 4a | 170-172 | White | 60 | - | 1413 | 1708 | 1755 | 2931 | 2796 | 3050 | 1577 | 1490 | - | - |
| 4b | 180-182 | Yellow | 55 | - | 1406 | 1700 | 1670 | 2983 | 2816 | 3080 | 1544 | 1490 | - | - |
| 4c | 175-177 | Yellow | 50 | - | 1417 | 1745 | 1680 | 2924 | 2852 | 3045 | 1579 | 1492 | 1430 | 1417 |
| 4d | 186-188 | Off white | 45 | - | 1415 | 1732 | 1701 | 2922 | 2852 | 3035 | 1575 | 1506 | - | - |

Cont... Table 1: Some physical properties and FT-IR spectral data cm⁻¹ of synthesized creatinine derivatives (1a-4d)
The derivative compounds (1a, 1b, 1c, and 1d) prepared from the reaction of (4-nitrobenzaldehyde, 4-amino benzaldehyde, and cinnamaldehyde) and acetophenone with Creatinine in the presence of glacial acetic acid, characterized first by the action of them with 2,4-DNPH to give (-ve) test, the reaction showed disappearance of the C=O group. Table (2) show IR spectral data of these derivatives, it reveals the disappearance of the absorption band $\nu_{NH}$ and its appearance at (1670-1622) cm$^{-1}$ due C=N group.$^{15}$

The heterocyclic compounds that contain azetidine-2-oxo ring (2a, 2b, 2c, and 2d) synthesized from the reaction between chloroacetyl chloride and (1a, 1b, 1c, and 1d) derivatives in triethylamine, the IR showed demies absorption band of azomethane group and appearance bands to the C=O group, NMR for some derivatives show appear the chemical shift to (CH proton) in b-lactam ring and (CH proton) fused with chlorine.$^{16}$ The $^1$H-NMR spectrum for compound (2b) show appear signals $\delta$ 1.2 ppm (s, 3H, C$_2$H$_3$); $\delta$ 3.0 ppm (s, 2H, CH$_2$-NH) and $\delta$ 3.8 ppm (s, 1H, -N-C=O-CH-CI), $\delta$ 4.25 ppm (s, 2H, -NH$_2$-Ar), and $\delta$ 7.2-7.75 ppm (m, 4H, Arm.). $^1$HNMR spectrum for (2c) shows signals listed in Table (2).

4-oxo- imidazolidine derivatives (3a, 3b, 3c, and 3d) prepared from the reaction of glycine with Schiff base (1a, 1b, 1c, and 1d) in absolute ethanol, FTIR spectral data of these derivatives list in Table (2). The

Scheme1: Synthesis of substance heterocyclic on creatinine
\( \text{\(^1\)H-NMR spectrum for compound (3a) \( \delta 2.9 \text{ ppm (s,3H,-} \text{CH}_3 \); \( \delta 3.04 \text{ ppm (d, 2H, Ar-CH-NH-CH}_2 \); \( \delta 3.7 \text{ ppm (s,2H, CH}_3\text{N-CH}_2 \); \( \delta 4.0 \text{ ppm (s, 1H,CH}_2\text{-NH-CH} \); \( \delta 7.72-8.1 \text{ ppm (m, 4H, Arom.) and } \delta 8.25 \text{ ppm (s, 1H, NH-CH}_2 \).} \)

(\(^1\)H-NMR spectrum for (3c and 3d) show signals listed in Table (2).)

4- oxo-thiazolidine derivatives (4a, 4b, 4c, and 4d) was prepared from adding 2-mercaptopic acid to derivatives of Schiff base(1a,1b,1c, and 1d ) with anhydrous ZnCl\(_2\), table (1) showed the IR spectra data for these derivatives, the spectrum of IR show disappearance (\(-N=C=\) ) group and appear the absorption bands at (1732-1708) cm\(^{-1}\) due to carbonyl group, \(^1\)H-NMR used to characterization for some of the derivatives show appear signals due to CH\(_2\) and C=O groups in thiazolidinone ring, the \(^1\)H-NMR spectrum for derivative (4a) appear signals 3.08 ppm (s, 3H, CH\(_2\)NC\(_3\)); \( \delta 3.20 \text{ ppm(s,2H, CH}_2\text{NCH}_3 \)), \( \delta 3.72 \text{ ppm (s,2H, C=O-CH}_2\text{-S); } \delta 4.2 \text{ ppm (s, 1H, N-CH-Ar) and } \delta 2.7 \text{ ppm (d,4H, Arom.) while derivative (4b and 4d) are listed in the table (2).} \)

<table>
<thead>
<tr>
<th>Structure</th>
<th>(^1)H-NMR signals data, ( \delta ) (ppm)</th>
</tr>
</thead>
</table>
| ![Structure 1](image1) | \( \delta 1.2 \text{ ppm (s,3H,CH}_3 \); \( \delta 3.35 \text{ ppm (s, 2H, CH}_2\text{-NH) and } \delta 3.64 \text{ ppm (s,1H,-N-C=O-CH}_2\text{-Cl), } \delta 4.3-4.5 \text{ ppm (d,2H,Ar -CH=CCH}_3\text{aliph.) and } \delta 7.2-7.6 \text{ ppm (m, 5H, Arom.)} \)
| ![Structure 2](image2) | \( \delta 2.94 \text{ ppm (s, 3H,CH}_2\text{-N-CH}_3 \); \( \delta 3.36 \text{ ppm (s, 2H, C=O- NH-CH}_2 \); \( \delta 3.64 \text{ ppm (s,2H, CH}_3\text{-N-CH}_3 \), 6.9 ppm (d,2H,Ar -CH=CCH}_3\text{aliph.) } \delta 7.16-8.0 \text{ ppm (m, 5H, Arom.) and } \delta 8.4 \text{ ppm (s, 1H, NH-CH}_2 \).} \)
| ![Structure 3](image3) | \( \delta 2.93 \text{ ppm(s,3H,N-C(CH}_3\text{)-Ar); } \delta 3.69 \text{ ppm (s, 3H,CH}_2\text{-N-CH}_3 \); \( \delta 2.80 \text{ ppm(s, 2H,-C=O- NH-CH}_2 \), \delta 3.44 \text{ ppm (s,2H, CH}_3\text{-N-CH}_3 \), 7.46-7.60 ppm (m,5H,Arom.)and } \delta 8.0 \text{ ppm (s, 1H, NH-CH}_2 \).} \)
| ![Structure 4](image4) | \( 2.96 \text{ ppm (s, 3H,CH}_2\text{NCH}_3 \); \( \delta 3.45 \text{ ppm(s,2H, CH}_3\text{NCH}_3 \), \( \delta 3.63 \text{ ppm (s,2H, C=O-CH}_2\text{-S); } \delta 4.01 \text{ ppm (s, 1H, N-CH-Ar), } \delta 4.70 \text{ ppm (s, 2H, NH}_2\text{-Ar) and } \delta 7.2-8.4 \text{ ppm (m,4H, Arom.)} \)
| ![Structure 5](image5) | \( 1.8 \text{ ppm (s, 3H,Ar-C-CH}_3 \); 3.15 \text{ ppm (s, 3H,CH}_2\text{NCH}_3 \); \( \delta 3.61 \text{ ppm (s, 2H,-C=O-CH}_2\text{- N-CH}_3 \),\( \delta 3.90 \text{ ppm (s,2H, C=O-CH}_2\text{-S); and } \delta 7.0-8.45 \text{ ppm (m,4H, Arom.)} \).} \)
### Table 3: Antibacterial and antifungal activity of some synthesized derivatives

<table>
<thead>
<tr>
<th>Compound Code</th>
<th>Staphylococcus</th>
<th>E.Coli</th>
<th>Asp.flavous</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>2b</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>2d</td>
<td>++++</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>3a</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>3c</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>4a</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>4b</td>
<td>++</td>
<td>++</td>
<td>---</td>
</tr>
<tr>
<td>4c</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>DMSO</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

**Key to symbols =**

- **Inactive** = (-) inhibition Zone <6mm
- **Slightly active** = (+) = inhibition Zone 6-9mm
- **Moderately active** = (+++) inhibition Zone 9-12mm
- **Highly active** = (++++) inhibition Zone 13-17 mm
- **Very high activity** = (++++) inhibition Zone < 17 mm

**Conclusions**

The prepared compounds were confirmed by using spectroscopic techniques (FT-IR and $^1$HNMR). The biochemical studies revealed that the new aztedine, imidazolidine and thiazolidine caused activator effects on two types of bacteria Staphylococcus aureus and Escherichia coli, and one type of fungi Aspergillus flavous. The organisms Staphylococcus aureus, Escherichia coli and Aspergillus flavous show very high activity toward the compounds (2a, 2b, 2d, 3c, and 4c).

**Ethical Clearance**: Taken from University of Baghdad ethical committee

**Source of Funding**: Self

**Conflict of Interest**: Nil

**References**


Evaluation of Diagnostic Accuracy of Ultrasonography and Low Dose Computed Tomography in Acute Appendicitis

Saradiq Mudhafar Jebur1, MahmoodRadhi Jobayr2, Haider Abdulameer Ghayad3

1Research Scholar, Medical City, Welfare Hospital Teaching , Baghdad, Iraq, 2Professor, Middle Technical University (MTU), College of Health and Medical Technology, Dept. Radiology Technology, Baghdad, Iraq
3Research Scholar, Medical City Complex, X-Ray Institute, Baghdad, Iraq

Abstract

In acute appendicitis, abdominal Ultrasonography (USG) to reach a stable diagnosis and use of Low Dose Computed Tomography (LDCT) in the diagnosis of acute appendicitis is crucial. This study aimed at assessing of the accuracy of imaging modalities (USG and LDCT) in the diagnosis of children and adults with acute appendicitis to minimize the rate of negative appendectomy. A total of 42 patients, children and adults were examined, were included in this research (16 females and 26 males) ages was ranged from 5 to 45 years. All patients were examined by USG and LDCT to diagnosis acute appendicitis. The sensitivity of low dose a LDCT scan was used to diagnose acute appendicitis (96.0%) that means LDCT was able to diagnosed (96.0%) Correctly diagnose acute appendicitis patients. The specificity of LDCT in the diagnosis of acute appendicitis was (50.0%) that mean LDCT was able to diagnosed half of those with diagnosis other than acute appendicitis correctly, But the sensitivity of USG in diagnosis of acute appendicitis was (54.1%) that mean ultrasound was able to diagnosed (54.1%) of patients diagnosed by CT scan as acute appendicitis correctly. The use of low-dose computerized tomography (LDCT) provides significantly higher diagnostic accuracy compared to ultrasound for the detection of acute appendicitis.

Keywords: USG, Computed tomography, acute appendicitis, LDCT.

Introduction

Acute appendicitis remains a significant cause of morbidity in children and adults as the most frequent acute surgical condition; appendectomy is the primary basis of care (1). Delay in making decisions can lead to severe problems such as perforation and development of abscesses, while rushing to surgical is related to a high rate of negative appendectomy (up to 15% - 47%) (2). Appendicitis diagnosis of all ages is difficult, acute appendicitis diagnosis can be tough for the reason that medical symptoms and symptoms correlate with different ailments due to the fact they have got comparable symptoms (3). A hole in efficaciously diagnosing appendicitis can result in a worse prognosis, and misdiagnosis can cause useless operations (3, 4). Therefore, precision of diagnosis appendicitis is important. Although it’s essential to have diagnostic accuracy and physical examination outcomes, imaging is frequently used to get a proper diagnosis. Therefore, established diagnostic protocols propose Ultrasonography (USG) because the number one method of imaging and CT as extra image follow-up in misleading situations (5, 6). Nevertheless, in lots of cases, USG effects of sufferers with suspected appendicitis are deceptive, and this confusion can cause delays in prognosis and care and extra costs. There have been additionally tries to restriction to radiation from a
CT examination due to sensitivity from CT detection to radiation. And to limit the quantity of CT scans which are needless. Low-dose CT (LDCT) has emerged as choice to examine sufferers with abdominal discomfort, as correct diagnostic reliability and reduced to ionizing radiation were proven (7). Indeed, different protocols, like those using Iv contrast material alone (8), orally contrast material alone (9), none oral or Iv contrast material (10, 11), and LDCT (12), have additionally been proven to be responsive and specific to appendicitis, because of worry of reduced precision, but LDCT shows fine effects. We consequently suggest the proportional output in LDCT with the detection of acute appendicitis. We were unable to evaluate low and usual dose CT an order to get accurate dose reduction measurements in both cases (13).

In this work, a systematic study comprehensive has been carried out to assess the accuracy of imaging modalities (USG and LDCT), in the diagnosis of children and adults with acute appendicitis. To demonstrate minimize the incidence of negative appendectomy from the emergency department in referred patients suspected of having an acute appendicitis.

Methods

There were 42 patients in total, children and adults were examined were included in this research project (16 females and 26 males) ages was ranged from 5 to 45 years. The data have been collected from November/20/2020 to March/23/2021. City of Medicine (Welfare hospital teaching and Baghdad Teaching Hospital), A Samsung ultrasound device was used to test all patients with suspected acute appendicitis, and a linear high frequency transducer was scanned on the right iliac fossa region, using “a graded compression technique”. Following a USG review, all patients with clinically suspected acute appendicitis had a CT scan. And Using a Siemens SOMATOM definition, a LDCT scan was performed. Scanner with 64 detectors, (Siemens, Germany 2016) tube voltage: 100 kVp tube current: 87/190 mA slice thickness: 5mm rotation time 0.5s with or without dye for the lower abdomen and pelvic, Most scans are quick enough that patients do not need to be sedated to be scanned.

Sedation may be required in some cases for children who are unable to remain still. Motion causes image blurring and degrades the quality of the examination in the same way that it degrades the quality of images. If contrast agent is used, it will be swallowed and intravenous injection (IV), or, rarely, administered by enema.

On USG the inflamed appendix appears as a blind-ending, tubular structure with bowel signature. When pressure is applied, the inflamed appendix is noncompressible. On transverse images that the appendix will have a “target”-sign configuration consists of the alternating layers of the appendix wall. Although no specific measurement describes an acutely inflamed appendix, free fluid in the abdomen or pelvis, swollen mesenteric lymph nodes, and fluid collections adjacent to the appendix that represent abscesses include secondary signs of appendicitis. Acute appendicitis CT results include a cumulative outer luminal diameter greater than 6 mm”, lack of lumen oral contrast (if oral contrast was administered), appendicolitis, and inflammatory changes in periappendiceal. The related effects include swollen mesenteric lymph nodes, free fluid, and adjacent bowel inflammatory changes. The appearance of free air and the development of abscesses are major indicators that the appendix is possibly perforated.

Result and Discussion:

A total of 42 subjects were included: underwent abdominal US and CT Our patient’s ages range from 5 to 45 years old, and they are children and adults were (16 females and 26 males).
Table 1: The sensitivity, specificity US results in diagnosis of acute appendicitis in comparison to Low Dose CT results.

<table>
<thead>
<tr>
<th>Ultrasound diagnosis</th>
<th>Low Dose CT diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>5</td>
</tr>
</tbody>
</table>

Sensitivity of USG in diagnosis of acute appendicitis was (54.1%) that mean ultrasound was able to diagnosed (54.1%) of patients diagnosed by LDCT scan as acute appendicitis correctly. The specificity of USG in diagnosis of acute appendicitis was (40.0%) that mean ultrasound was able to diagnosed (40.0%) of those with normal appendix by LDCT correctly. Positive predictive value was (87.0%) that mean those diagnosed as acute appendicitis by USG was (87.0%) being acute appendicitis by LDCT and negative predictive value was (10.5%) that mean only (10.5%) of those diagnosed as normal appendix by USG being diagnosed as normal appendix by LDCT and overall accuracy was (52.38%).

Table 2: In contrast to surgical outcome, sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of Low Dose CT results in diagnosing acute appendicitis.

<table>
<thead>
<tr>
<th>Low Dose CT diagnosis</th>
<th>Surgical outcome</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>2</td>
</tr>
</tbody>
</table>

The sensitivity of LDCT scan was used to diagnose acute appendicitis (96.0%) that mean LDCT was able to diagnosed (96.0%) of patients incidence of acute appendicitis correctly. The specificity of LDCT in the diagnosis of acute appendicitis was (50.0%) that mean LDCT was able to diagnose half of those with diagnosis other than acute appendicitis correctly. Positive predictive value was (96.0%) that mean those diagnosed as acute appendicitis by LDCT was (96.0%) being acute appendicitis by surgery and negative predictive value was (50.0%) that mean half of those diagnosed as other diagnosis rather than acute appendicitis by LDCT being diagnosed as other diagnosis by surgery and overall accuracy was (92.59%).

In this study, sensitivity of LDCT scan in diagnosis of acute appendicitis was (96.0%) that mean LDCT was able to diagnosed (96.0%) of patients incidence of acute appendicitis correctly. The specificity of LDCT in the diagnosis of acute appendicitis was (50.0%) that mean LDCT was able to diagnosed half of those with diagnosis other than acute appendicitis correctly, as other diagnosis by surgery and overall accuracy was (92.59%). In the diagnosis of acute appendicitis, CT plays an important role. By offering detailed appendix and peril-appendicular changes information and calling
the gold standard technique for determining whether or not a patient has acute appendicitis. These findings are consistent with Fallon et al., Alshamari et al. and Dae Yong Yi et al. (14-16).

Figure 1: shows distribution of patients according to CT diagnosis including (acute appendicitis and normal appendix). Majority (N=37, 88.1%) of patients diagnosed as acute appendicitis by CT scan. Also, in this study, the sensitivity of US in diagnosis of acute appendicitis was (54.1%) that mean ultrasound was able to diagnosed (54.1%) of patients diagnosed by LDCT scan as acute appendicitis correctly. The specificity of USG in diagnosis of acute appendicitis was (40.0%) that mean ultrasound was able to diagnosed (40.0%) of those with normal appendix by LDCT correctly. As normal appendix by USG being diagnosed as normal appendix by LDCT and overall accuracy was (52.38%). Expert graded compression employees can achieve high diagnostic precision, but the technique is highly reliant on the operator’s abilities, experience, and perseverance. It’s especially useful when examining children with low body mass and women with a predisposition to gynecological disorders (15). Recent publications have highlighted the importance of LDCT in reducing false negative findings during surgery (2).

Conclusion

The results of this study indicate that low-dose CT is more effective than USG diagnosis acute appendicitis in children and adults, decreasing the rate a negative appendectomy in patients admitted to the emergency department with clinical suspicion of acute appendicitis. These findings highlight the importance of Low-dose computed tomography (LDCT) for the patients at acute appendicitis to minimize risk of the patient being subjected to the complications of anesthesia and inflammation at the surgical site. Our results confirm Low-dose computed tomography (LDCT) is found to have a better diagnostic value of acute appendicitis. Also, further support control of time as a way to diagnosis of appendicitis to prevent disease complications and costs associated with delayed diagnosis. In addition, In this study, although statistically significant difference is seen in the diagnostic potential of LDCT, however, might be Ultrasonography useful for detecting early of acute appendicitis because ultrasonography is relatively safe and noninvasive.

Ethical Clearance : Taken from Middle Technical University ethical committee

Source of Funding : Self

Conflict of Interest : Nil
References


The Inhibitory Effect of local *T.bovei* Volatile Oil Against ESBL- *E.coli* and *Klebsiella pneumoniae* Isolated From Patients with Urinary Tract Infections

Sawsan Qahtan Taha Al-Quhli¹, Safaa Abed Latef Al Maeni²

¹Research Scholar, Department of Microbiology, College of Medicine, University of Anbar, Iraq; ²Professor, Department of Biotechnology, College of Science, University of Anbar, Iraq

Abstract

This is the first research in Iraq to look in the *T. bovei* volatile oil’s ability to prevent the development of ESBLs. To find successful treatment-combinations for “ESBL-producing” bacteria’s emergent infections, we are evaluating antibacterial properties of phytochemical substances against bacteria that produce ESBL and investigating the effects of phytochemical substances in the presence of antibiotics. The volatile oil of the studied plant was extracted by steam distillation. Oil compounds were detected after extracting the volatile oil from *Thyme bovei* using a Gas chromatography–mass spectrometry (GC-MS). “REMA” was used to determine the MIC of the volatile oil and antibiotic solutions, with minor modifications. The checkerboard approach was used in 96 well microplates to see whether there was a synergy relationship between the volatile oil and antibiotics. *Tbovei* volatile oil had an inhibitory influence on β - Lactamase, according to the findings. Antimicrobial properties Thymol, p-cymene, and “Linalool” were the key constituents contained in GC-MC of *Thymus* volatile oil, which has already been shown to have antibacterial efficacy. The combination of *T.bovei* volatile oil and antibiotics had a synergistic effect on *E.coli* and *K.pneumoniae* producing ESBL growth in the current study.

Keyword: *T. bovei*, Volatile Oil, ESBL, Urinary Tract Infections

Introduction

Bacterial antimicrobial resistance is a worldwide issue that necessitates production new antibacterial agents. As a result of this issue, scientists are increasingly focusing on natural products in order to develop better treatments for multidrug-resistant microbial strains (¹). Antibiotic-resistant bacteria would be more successful if plant extracts with target sites were used (², ³). As a result, essential oils have been used to treat a variety of infectious diseases due to their high concentration of bioactive compounds with anti-oxidant and antimicrobial properties (⁴).

The most common bacterial infections in humans are urinary tract infections (UTIs). They’re also the most popular source of both population- and hospital-acquired infections (⁵). Many conventional antibiotics, such as third-generation cephalosporin’s, are ineffective against UTIs caused by *Enterobacteriaceae* that produce ESBLs (⁶). The evolution of ESBLs, on the other hand, has given these enzymes a new weapon in their arsenal. As a result, ESBLs producers are becoming more prevalent, and ESBLs producer strains are increasing morbidity, morality, and health-care costs (⁷).

Materials and Methods

Clinical isolates

Clinical *E.coli* and *K.pneumoniae* were collected from urinary tract infection (UTI) patients. Traditional Morphological methods and biochemical analysis, as well as automated systems such as Api 20E and Vitek 2, were used to identify them. They were classified as ESBL-producing *E. coli* and *K. pneumoniae* using
screening test, as well as various phenotypic and genotypic approaches according to Al-Quhli, and Al Maeni (2021) (8, 9).

Collection and preparation of medical plant

In July 2019, the leaves of Thyme bovei were brought from Hadethia city’s local plantations. The leaves were cleaned with tap water to remove any dirt that had adhered to their surfaces, and then washed with purified water once more.

Volatile oil extraction

The volatile oil of the studied plant was extracted by steam distillation using a Clevenger-type apparatus (10). After extracting the volatile oil for Thyme bovei, oil compounds were identified by a GC-MS, in Baghdad; Ministry of Ecology.

Determination of minimum inhibitor concentration (MIC)

The Resazurin Microtitre-plate Assay (REMA) was used for determining the MIC of the volatile oil and antibiotic solutions with slight modifications. In aseptic conditions, to all wells of microtitre-plates, a hundred µl Mueller-Hinton Broth was added, followed by transferring the first row of the 96 well plates with a hundred µl material test (volatile oil). Pipette 100 µl of the substance measure in serially decreasing concentrations from the first row to the other rows “(1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, and 1/256)” was used to conduct serial dilutions. Every well was filled with ten microliters of bacterial suspension containing “1×10⁸ CFU/ml”. They were wrapped loosely in para-film and incubated at “35±2°C for 18-24” hours to ensure that the bacteria did not get dehydrated. Following the incubation period, ten microliters of “resazurin- solution” (Alamar- blue) was added to each well, and after another 24 hours, the plate was re-incubated. to observe color change. The findings were visually examined by looking at the color variations in resazurin, with changes from purple to pink, red, or colorless being considered positive. The MIC value was determined as the lowest concentration that caused no change in resazurin color (11).

Study the synergism between volatile oil and some antibiotics on bacterial growth using checkerboard technique

The checkerboard approach was used in 96 well microplates to see whether there was a synergy relationship between the volatile oil and antibiotics (12). The checkerboard assay was planned so that the two antimicrobials to be examined were serially diluted against each other in a cross fashion on two standard 96-well plates. The first antimicrobial “volatile oil” is serially diluted vertically, while the second antimicrobial “antibiotic” is serially diluted horizontally in this manner. The assay was created in such a way that the MIC calculated from the REMA assay for volatile oils could be used (13). The standard technique for assaying hydrolyzed Beta-lactam antibiotics is similar to Sargent’s description (14).

Results and Discussion

The volatile oil was extracted by steam distillation GC-MC; 100 g of leaves were placed in a Clevenger flask with 500 ml distilled water, and after 3 hours of extraction, 1 ml of T. bovei volatile oil was collected. The major compounds in T. bovei volatile oil were beta-ocimene, 3,7-Dimethyl-1,3,7-octatriene, Sabinene hydrate, Y-Terpinene, Linalool, Thyme camphor, p-cymene, p-thymol, thymol, Carvacrol, Thyme bovei. We also found the existence of other active substances, noting that they were the same active substances found in T. vulgaris in the previous analysis (15).

T. bovei volatile oil had an inhibitory effect on β-Lactamase, according to the findings. In the absence of T. bovei volatile oil, E. coli β-lactamase activity was (0.003640) u/ml, while in the presence of T. bovei volatile oil, E. coli β-lactamase activity was (0.001115) u/ml. k.pneumoniae β-Lactamase activity was (0.004443) U/ml in all isolates without T. bovei volatile oil, while the β-lactamase activity of K.pneumoniae isolates was (0.001918) U/ml in the presence of T. bovei volatile oil. Antimicrobial properties Thymol, p-cymene, and
Linalool were key constituents contained in GC-MC of Thymus volatile oil, which is already known to have antibacterial activity \(^{(16)}\).

REMA is characterized by its simplicity, low cost, speed, performance, and dependability. This is a colorization process focused on the oxidation and reduction of resazurin, which is used to test the sensitivity of medicines, antibiotics, plants, and bacteria. The ability to calculate a small volume of plant extracts separates this approach from other conventional methods. A blue reduction pigment (Resazurin) that is widely used as chemical proof is not harmful to cells in the media. Resazurin is a bacterial growth indicator that can be used without a spectrophotometer to assess bacterial growth in a small amount of solution in microliter-plates \(^{(17)}\). The MIC of *T. bovei* volatile oil against growth of bacteria–producing ESBLs was determined in this study and the findings are shown in the tables (1 and 2). Figure (2) showed MICs values of volatile oil, where blue color represent indicate to inhibited of bacterial growth by volatile oils due to not reduce the resazurin, while the pink and red colors were due to the reduction of Resazurin to resorufin by the bacteria \(^{(18)}\).

**Table (1). The MIC of *T. bovei* volatile oil for the growth of *E. coli*-producing ESBLs isolates.**

<table>
<thead>
<tr>
<th>No. of isolate</th>
<th>Minimum Inhibitory Concentration (MIC) <em>T. bovei</em> volatile oil (Titer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli 1</td>
<td>32</td>
</tr>
<tr>
<td>E. coli 2</td>
<td>128</td>
</tr>
<tr>
<td>E. coli 3</td>
<td>128</td>
</tr>
<tr>
<td>E. coli 4</td>
<td>128</td>
</tr>
<tr>
<td>E. coli 5</td>
<td>8</td>
</tr>
<tr>
<td>E. coli 6</td>
<td>128</td>
</tr>
<tr>
<td>E. coli S</td>
<td>256</td>
</tr>
</tbody>
</table>

**Table (2) The MIC of *T. bovei* volatile oil for the growth of *K. pneumoniae*-producing ESBLs isolates.**

<table>
<thead>
<tr>
<th>No. of isolate</th>
<th>Minimum Inhibitory Concentration (MIC) <em>T. bovei</em> volatile oil (Titer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae 1</td>
<td>8</td>
</tr>
<tr>
<td>K. pneumoniae 2</td>
<td>32</td>
</tr>
<tr>
<td>K. pneumoniae 3</td>
<td>128</td>
</tr>
<tr>
<td>K. pneumoniae 4</td>
<td>32</td>
</tr>
<tr>
<td>K. pneumoniae 5</td>
<td>8</td>
</tr>
<tr>
<td>K. pneumoniae 6</td>
<td>32</td>
</tr>
<tr>
<td>K. pneumoniae S</td>
<td>256</td>
</tr>
</tbody>
</table>
Where in *E. coli*, the isolates 1 and 5 were more resistant than isolates 2, 3, 4 and 6 which its resistance to *T. bovei* volatile oil was less. In *K. pneumoniae*, the isolate 3 was less resistant than the isolates 1,2,4,5 and 6. While the stander isolates of *E. coli* and *K. pneumoniae* were not resistant to *T. bovei* oil. Table (1) and (2) shows that *T. bovei* oil has a good effect on bacterial isolates. The ability of volatile oil in inhibiting the growth were due to phenolic compounds that Disrupt the cytoplasmic membrane, the proton motive force, electron flow, active transport, and cellular coagulation are all disrupted (19).

A present study, showed synergistic result of combining *T. bovei* volatile oil with antibiotics on *E. coli* and *K. pneumoniae* producing ESBL growth tables (3) & (4). A rise in bacterial resistance to antibiotics, as well as a shortage of new antibiotics on the market, necessitated the development of alternative methods to deal with infections caused by drug-resistant bacteria (20). Among the possible solutions suggested are the creations of antibiotic alternatives and the discovery or development of adjuvants (21). Some attempts have been made to improve or restore antimicrobial activity against multidrug-resistant bacteria. When volatile oils are added to antibiotics, the antimicrobial MIC is reduced (22). According to Al Dossary & Al Meani (2019), Since volatile oils are multi-component in nature, compared to many traditional antimicrobials that only have a single target site, they are thought to be more promising in preventing bacterial resistance (23). Antibiotic efficacy can be improved by enhancing antibiotic diffusion through bacterial membranes, and/or inhibiting efflux pumps, which are a common mechanism of resistance; in Gram-negative bacteria (24, 25).

Table (3). The effect of combining *T. bovei* volatile oil with antibiotics on *E. coli* –producing ESBLs growth.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml) By REMA</th>
<th>Combination between Antibiotics &amp; T.bovei</th>
<th>FICI (ΣFIC)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftazidime</td>
<td>62.5</td>
<td>0.78125 (128) titer + 7.8125 µg/ml</td>
<td>0.375</td>
<td>Synergistic</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>125</td>
<td>0.78125 (128) titer + 31.25 µg/ml</td>
<td>0.5</td>
<td>Synergistic</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>125</td>
<td>0.78125 (128) titer + 31.25 µg/ml</td>
<td>0.5</td>
<td>Synergistic</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>62.5</td>
<td>0.78125 (128) titer + 7.8125 µg/ml</td>
<td>0.375</td>
<td>Synergistic</td>
</tr>
<tr>
<td>Cefepime</td>
<td>62.5</td>
<td>1.5625 (64) titer + 7.8125 µg/ml</td>
<td>0.25</td>
<td>Synergistic</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>125</td>
<td>0.78125 (128) titer + 31.25 µg/ml</td>
<td>0.5</td>
<td>Synergistic</td>
</tr>
</tbody>
</table>
Table (4). The effect of combining *T. bovei* volatile oil with antibiotics on *K. pneumoniae*-producing ESBLs growth.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml) By REMA</th>
<th>Combination between Antibiotics &amp; T.bovei</th>
<th>FICI (ΣFIC)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftazidime</td>
<td>62.5</td>
<td>1.5625 (64) titer + 7.8125 µg/ml</td>
<td>0.25</td>
<td>Synergistic</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>62.5</td>
<td>1.5625 (64) titer + 15.625 µg/ml</td>
<td>0.75</td>
<td>Additive</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>125</td>
<td>0.78125 (128) titer + 31.25 µg/ml</td>
<td>0.5</td>
<td>Synergistic</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>125</td>
<td>0.78125 (128) titer + 31.25 µg/ml</td>
<td>0.5</td>
<td>Synergistic</td>
</tr>
<tr>
<td>Cefepime</td>
<td>62.5</td>
<td>1.5625 (64) titer + 7.8125 µg/ml</td>
<td>0.25</td>
<td>Synergistic</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>62.5</td>
<td>0.78125 (128) titer + 7.8125 µg/ml</td>
<td>0.375</td>
<td>Synergistic</td>
</tr>
</tbody>
</table>

Thyme is an important medicine that has been used for centuries and has been known as a rich source of bioactive substances with substantial properties that are anti-oxidant and anti-inflammatory, potentially useful in the treatment and prevention of pathological circumstances, but its popularity as an antibacterial molecule that prompted us to choose it (26).

**Conclusion**

In conclusion, our findings revealed that *T. bovei* volatile oil has an inhibitory effect on the growth of “*E.coli* “and “*Klebsiella pneumoniae*” - generating ESBLs, in addition inhibition activity against β-Lactamase enzyme. When combined with antibiotics including “Ceftazidime, Cefpodoxime, Cefotaxime, Ceftriaxone, Cefepime, and Aztreonam”, *T. bovei* volatile oil has a synergistic effect. As well as, *T. bovei* essential oil had strong biological activities and was a potential source of various natural compounds. It’s a powerful inhibitor of *E.coli* and *K.pneumoniae*-producing ESBLs isolated from UTI patients at Al-Anbar Hospital, and it’s led to the development of new medicinal plant-based therapeutics.

**Ethical Clearance** : Taken from Al-Anbar University ethical committee

**Source of Funding** : Self

**Conflict of Interest** : Nil

**References**


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Evaluation of Oral Manifestations of Patients with Parkinson’s Disease—An Observational Study

T. Gopalakrishnan¹, K.M.K. Mastan², P.E. Chandra Mouli³, Naga Leela Guntuku⁴, Priyadharshini A⁵, G. Florence Sangeetha⁶

¹Reader, Department of Oral Pathology And Microbiology, Sri Venkateswara Dental College And Hospital, Thalambur, Chennai. Tamil Nadu, ²Professor and Head, Department of Oral Pathology And Microbiology, Sree Balaji Dental College and Hospital, Chennai, ³Professor and Head, Department of Oral Medicine And Radiology, Sri Venkateswara Dental College And Hospital, Thalambur, Chennai. Tamil Nadu, ⁴Senior Lecturer, Department of Oral Medicine And Radiology, Sri Venkateswara Dental College and Hospital, Thalambur, Chennai. Tamil Nadu, ⁵Senior Lecturer, Department of Oral Medicine And Radiology, Chettinad Dental College And Research Institute, Chennai. Tamil Nadu, ⁶Post Graduate Student, Department of Oral Pathology And Microbiology, Sree Balaji Dental College And Hospital, Chennai

Abstract

Background: Parkinson’s disease is the second most common neurodegenerative disorder which affects around four million people globally. Many of the signs of Parkinson’s disease are found in the head and neck. The typical “masklike” facial appearance with infrequent blinking and lack of expression is caused by bradykinesis. Abnormalities in oral behaviour, such as purposeless chewing, grinding, and sucking movements, are also well recognized in patients with Parkinson’s disease.

Aim and Objectives: The aim of this study is to assess the overall oral health status of the patients with Parkinson’s disease and to analyze the impact of Parkinson’s disease on the oral cavity of the individuals surviving with the disease.

Materials and Methods: The study sample included 50 Parkinson’s disease patients. The patients of all age groups and both genders were included.

Results: Out of fifty patients, majority of the patients were above 60 yrs. 6% of the patients reported with positive family history of Parkinson’s disease. 14% of the patients had gross facial asymmetry, 68% of the patients showed dryness of mouth, 8% of the patients suffered loss of taste, 14% of the patients showed hyper salivation, 54% of the patients showed gingivitis, and 76% of the patients were found having periodontitis.

Conclusion: Treatment for oral and dental problems of Parkinson’s disease affected patients is mandatory. Treatment can be done by supplementing artificial saliva for dryness of mouth, dental fillings for dental caries, periodic scaling for periodontal problems, oral rehabilitation measures and regular dental check up.

Key Words: Parkinson’s Diseases, Oral Manifestations, Facial Asymmetry, Loss Of Taste.

Introduction

Parkinson’s disease is the second most common neurodegenerative disorder after Alzheimer disease which affects around four million people globally. It is a progressive neurological condition which is characterised by both motor and non-motor symptoms such as rigidity, tremors, bradykinesis, impaired postural reflexes, cognitive impairment, and bradyphrenia.

In 1817 James Parkinson first described this disease on his book ‘‘An essay on the shaking palsy’’¹, Charcot also recognised non-tremulous forms of Parkinson disease and exactly pointed out that slowness of
movement should be distinguished from weakness or ‘lessened muscular power’, a term originally used by Parkinson. These clinical manifestations of Parkinson’s disease are caused by a selective degeneration of dopamine-producing neurons in the substantia nigra in the brain stem and the consequent dopamine shortage in the striatum.

There are four cardinal clinical features of Parkinson’s disease - Tremor at rest, Rigidity, Akinesia (or bradykinesia) and Postural instability.

Many of the signs of Parkinson’s disease are found in the head and neck. The typical “masklike” facial appearance with infrequent blinking and lack of expression is caused by bradykinesia. The muscle rigidity also causes difficulty in swallowing, resulting in drooling. Abnormalities in oral behavior, such as purposeless chewing, grinding, and sucking movements, are also well recognized in patients with Parkinson’s disease.

Due to dysphagia and an altered gag reflex, special precautions must be taken to avoid the aspiration of water or materials used during dental procedures. In patients who suffer with hypersialorrhea, maintaining a dry field in certain procedures can be difficult. Xerostomia, on the other hand, is a common side effect of antiparkinsonism medications; the consequent root caries and recurrent decay must be diligently treated. Patients also often have difficulty maintaining their dentition because of their physical disability.

Since the oral aspect of this peculiar disease is less explored, the present study aims at understanding the dental risk among the patients of Parkinson’s disease.

Materials and Methods

The present random sampling study was conducted in Chennai. The patients were comprised of 50 individuals suffering from Parkinson’s disease. Out of 50 patients, 41 were males and 9 were females. All the patients were of varied socioeconomic status and different age group. Patients with other systemic diseases were excluded from this study.

After obtaining a written consent from every participant, a detailed case history was recorded, followed by which intraoral examination was conducted for the study group to observe the oral manifestations.

Results

Among 50 Parkinson’s disease patients 41 patients were males and 9 patients were females of which 12 patients were between 30 - 49 years, 13 patients were between 50 - 59 years and 25 patients were above 60 years of age. Among the oral manifestations dental caries was in 42 patients(84%) , periodontitis was in 38 (76%), Dryness of mouth was in 34 patients(68%), Gingivitis was found in 27 people(54%), Attrition was diagnosed in 14 patients (28%), Hyper salivation, Cervical abrasion and Difficulty in speech was seen in 7 patients (14%), Loss of taste sensation was seen in 4 patients (8%), facial asymmetry and Family history of Parkinson’s disease was seen among 3 patients(6%). Overall, oral manifestations of both hard and soft tissues of the oral cavity was found in Parkinson’s disease patients. (TABLE-1 AND CHART 1)
### TABLE 1- ALL THE ORAL FINDINGS

<table>
<thead>
<tr>
<th>Manifestations</th>
<th>Present</th>
<th>Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Family history</td>
<td>3</td>
<td>6</td>
<td>47</td>
</tr>
<tr>
<td>Dryness of mouth</td>
<td>34</td>
<td>68</td>
<td>16</td>
</tr>
<tr>
<td>Loss of taste</td>
<td>4</td>
<td>8</td>
<td>46</td>
</tr>
<tr>
<td>Hyper salivation</td>
<td>7</td>
<td>14</td>
<td>43</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>27</td>
<td>54</td>
<td>23</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>38</td>
<td>76</td>
<td>12</td>
</tr>
<tr>
<td>Dental caries</td>
<td>42</td>
<td>84</td>
<td>8</td>
</tr>
<tr>
<td>Attrition</td>
<td>14</td>
<td>28</td>
<td>36</td>
</tr>
<tr>
<td>Cervical abrasion</td>
<td>7</td>
<td>14</td>
<td>43</td>
</tr>
<tr>
<td>Difficulty in speech</td>
<td>7</td>
<td>14</td>
<td>43</td>
</tr>
<tr>
<td>Mild facial asymmetry</td>
<td>3</td>
<td>6</td>
<td>47</td>
</tr>
</tbody>
</table>

**GRAPH 1 – ORAL FINDINGS**
Discussion

Age Range

In our study we examined fifty patients of Parkinson’s disease, out of which forty one were males and nine were females with male predominance of 82%, which was similar to the study by Stephen K. Van Den Eeden et al., among five hundred and eighty eight Parkinson’s disease patients, out of which 91% were men. He had also concluded that incidence of Parkinson’s disease varies by race/ethnicity.

In our study of fifty patients, nine were females and they were above 65 years, whereas most of the males were above 55 years. The age distribution between the gender was similar to the study by Charlotte AHaaxmaet al.

WeerasakMuangpaisan et al., conducted a study to find the Worldwide Prevalence and Incidence of Parkinson’s Disease, in his study a literature search was conducted on Medline and EMBASE for studies worldwide investigating the prevalence and incidence of Parkinson’s disease and included publication between 1965 and January 2010. He concluded that Parkinson’s disease is common in the elderly, which was correlating with our study of fifty Parkinson’s disease patients out of which thirty eight were more than fifty years of age.

Family History

In our study, three patients (6%) reported with the family history of Parkinson’s disease that was analogous to the study by Jaan M Autere et al., on two hundred and sixty eight patients, among Finnish population where, ten per cent of them reported an affected first degree relative.

It is noted from the above table that the p value is greater than 0.05 for the demographic variable of Sex and hence the result is not significant at 5% level.

Taste sensation was altered among 8% of our study population howeverSienkiewicz-jarosz H et al., concluded that Parkinson’s disease is not associated with any alterations in pleasant or unpleasant taste stimuli, which is controversial to our study.
Kalf J. G.et al\(^9\) systematically reviewed the prevalence of drooling of saliva and hyper salivation in Parkinson’s diseased patients. Analysis of the data showed that drooling occurred with a frequency of 22–26%. In our study 14\% of the patients were presented with hyper salivation and drooling of saliva which proves that it is a common finding in Parkinson’s disease patients.

Periodontal disease and dental caries take up the highest rank in the oral diseases among Parkinson’s disease patients with 78\% and 84\% respectively. Marco Cicciu et al.,\(^11\) also noted that the frequency of untreated caries, periodontal diseases, and missing teeth of the Parkinson’s disease patients was significantly higher, when he evaluated their Periodontal Health and Caries Prevalence.

Speech impairment remains a challenging issue among the Parkinson’s disease patients where 14\% of our patients presented with difficulty in speech. Aileen K. Ho et al.,\(^12\) had noted that there was leading deficit in the Voice. The study by Hartelius L. et al.,\(^13\) also suggested that approximately out of four hundred and sixty patients with Parkinson’s disease 70\% of the patients had experienced impairment of speech.

Seidler. A et al.,\(^14\) in a quest to identify the possible etiologic relevance to Parkinson’s disease, they identified various factors leading to the Parkinson’s disease, which includes farming activity, pesticide exposures, and animal contacts, toxic exposures such as wood preservatives, heavy metals, solvents and head trauma. Likewise Stewart D. O. et al.,\(^15\) confessed that there exists a relationship between head injury and Parkinson’s disease. In our study, there were history of head injury but it was only up to 2\% out of 50 Parkinson’s disease patients.

Therapeutic management of Parkinson’s disease include Levodopa which has its own side effect of causing decreased salivation leading to dryness of mouth with burning sensation, and our study revealed the same with 30\% of the patients having the manifestation. A study by Melisa Proulx et al.,\(^16\) and Clifford T.J.et al.,\(^17\) concluded that salivary secretion is influenced by levodopa, and Burning sensation in the mouth was 5 times greater than that of the general population.

**Conclusion**

Mastication and orofacial functions are impaired in moderate to advanced Parkinson’s disease and becomes more marked with progression of the disease. One of the frequently observed features of the disease is hyper salivation, usually termed as sialorrhea is probably because of difficulties in swallowing and the loss of facial muscle tone than any actual increase in salivary flow. The condition is progressive and leads to restrictions in mobility and muscular rigidity. This can lead to problems in maintaining oral health.

Treatment for oral and dental problems of Parkinson’s disease affected patients is mandatory. Treatment can be done by supplementing artificial saliva for dryness of mouth, dental fillings for dental caries, periodic scaling for periodontal problems, oral rehabilitation measures and regular dental check up. The seriousness of the dental problems of these patients should be taken into consideration and depending upon the need, multidisciplinary approach upon referral may also be necessary.

**Conflict Of Interest:** Conflict of interest declared none.

**Ethical Clearance:** Taken from ethical committee of BHARATH INSTITUTE OF HIGHER EDUCATION AND RESEARCH, CHENNAI.

**Source of Funding:** Self

**References**


Correlation between Superoxide Dismutase 1 and 2 Polymorphisms in Asthma Patients

Walaa Najah Majid1, Layla Mohsen Mehdi2
1Research Scholar, Biology Department, Faculty of Science/University of Thi-Qar, Iraq,
2Professor, Biology Department, Faculty of Science, University of ThiQar, Iraq

Abstract

This study was designed to investigate the effect of polymorphisms of Superoxide dismutase 1 and 2 genes and how they contribute to the risk of developing asthma. A total 120 were involved in the present study and divided into four groups and each group included 30 samples. First group consisted 30 asthma male patients with waterpipe smoking and second group was also 30 asthma male patients with no smoking, third group was control (no disease) with waterpipe smoking and the fourth group was control group (no disease and without waterpipe smoking). Blood samples were collected from Muthanna hospital and private laboratories from the period mid-August to the mid-November 2020. To study the polymorphism phenomenon of SOD1 and SOD2 genes were assessed using RFLP-polymerase chain reaction (RFLP-PCR) technique. This study confirmed that the phenomenon of polymorphism of the SOD1 gene was not associated with the group of patients infected with asthma compared to control group. This study conducted that the phenomenon of polymorphism of the SOD2 gene is not associated with the group of patients infected with asthma compared to control group in presence and absence of waterpipe smoking.

Keywords: Asthma, Waterpipe Smoking, Oxidative stress, SOD1, SOD2

Introduction

Asthma is a multifaceted inflammatory airway disease with a variety of clinical and molecular phenotypes. In asthma genetic, immunologic, and environmental factors all combine to cause chronic inflammation in the airways. Cigarette smoking is a significant factor in asthma. Asthmatic smokers have a pronounced deficiency in asthma control, a faster deterioration in lung function, increased airflow obstruction, and a worsening of disease severity, according to the data. Normally, oxidative products found in environmental contaminants are inhaled by humans. However, the inflammatory condition of asthmatic patients’ airways can promote oxidative stress by increasing levels of reactive oxygen and nitrogen species (ROS and RNS). This may lead to the inflammatory response’s maintenance and development, as well as disease exacerbation. The NADPH oxidase pathway produces anion superoxide (O2•-) in activated inflammatory cells. The activity of superoxide dismutase enzymes (SODs), catalase (CAT), and glutathione peroxidase (GPX) neutralizes the (O2•-) .

Furthermore, the enzymes that produce nitric oxide (NO) are known as nitric oxide synthase (NOS), another common free radical that forms RNS quickly in the presence of ROS. Peroxidation of membrane lipids, depletion of nicotinamide nucleotides, increased intracellular Ca2+, cytoskeleton breakdown, and DNA damage have all been linked to an excess of ROS and RNS. Tobacco smoke is a significant exogenous cause of oxidative stress in asthma, leading to the maintenance and development of the inflammatory response as well as disease chronicity. Cigarette smoke contains a variety of oxidant compounds that can cause direct and indirect oxidative harm. The current study aims to study polymorphism phenomenon of the SOD1 35 A / C gene and the SOD2 Ala-9Val gene (C / T) of Asthma patients by using (RFLP-PCR) technology to investigate SOD1...
and SOD2 polymorphisms and risk of asthma.

**Materials and Methods**

Samples were collected from blood from asthma patients. As well as the control group from the asthma center of Al-Muthanna hospitals. As the average age of the patient was (15±35) years, collected 120 samples, were examined. A biochemical test was performed on (60) samples had been measured between superoxide dismutase 1 and 2 Polymorphisms in Asthma Patients by immunological method, by using RFLP-PCR reader (Huma Korea origin). Before using the samples, they were placed at room temperature, as well as the reagents. All fluids were used with great care to prevent any errors as the checks were done step by step. All examinations were carried out by the apparatus of the College of Science, The-Qar University. Used RFLP-PCR kit as follows from the global company (measured between superoxide dismutase 1 and 2 Polymorphisms) INtRON Korea.

**Results**

A total 120 were involved in the present study and divided into four groups and each group included 30 samples. First group was consisted 30 asthma male patients with waterpipe smoking and second group was also 30 asthma male patients with no smoking, third group was control (no disease) with waterpipe smoking and the fourth group was control group (no disease and without waterpipe smoking). When genotypes of SOD1 35 AA, AC and CC were compared between groups (Table 1), the distribution rate was considered insignificant. There was no statistical difference for genotype and allele frequency between the groups (p = 0.76). Similarly, genotypes of SOD2 Ala9Val CC, CT and TT were compared between groups (Table 1), the distribution rate was considered insignificant. There was no statistical difference for genotype and allele frequency between the groups (p = 0.83, 0.81, 0.7) respectively.

**Table (1): The genotype and allele distribution in asthma patient and control groups for the SOD1 35 A/C SOD2 Ala-9Val (C/T) polymorphism**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>Patient (n)</th>
<th>Control (n)</th>
<th>Total</th>
<th>P value</th>
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<td>C</td>
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Table (2): The genotype and allele distribution in case and control groups for the SOD1 35 A/C SOD2 Ala-9Val (C/T) polymorphism and risk of asthma

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<td>Non-Smokers</td>
<td>Smokers</td>
<td>Non-Smokers</td>
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<td>0.659</td>
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Table (3): The genotype and allele distribution in the patient and control groups for the SOD1 35 A/C SOD2 Ala-9Val (C/T) polymorphism and their relationship with waterpipe smoking

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<th>Genotype</th>
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<th>Control (n)</th>
<th>Total</th>
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<td>P value</td>
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<td>P value</td>
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<tr>
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<td>P value</td>
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<td>1.091</td>
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**Discussion**

The SOD enzymes play a critical role in protection a cell against free radicalsgenerated by glutathione peroxidase and catalase. SOD enzymes comprise redox metals in the centers of their catalytic zones that transform superoxide radicals to hydrogen peroxide and oxygen\(^{(12)}\). The current study was conducted to find out the association between the SOD1 and SOD2 genetic polymorphism and the incidence of asthma. This study found that the was no relationship between the SOD1 and SOD2 genes and the severity of asthma in terms of the SOD1 35 A/C, CC genotyping frequency and SOD2 Ala-9Val (C/T), the TT genotype frequency in the patient group was statistically no significantly than the control group implying that there is no link between genetic polymorphisms in the SOD1 and SOD2 genes and the occurrence of asthma this corresponds to a study\(^{(13)}\). They discovered that these two genes are unlikely to function as major players in asthma on their own, based on their results. Along with genes involved in oxidative degradation, variants that interact with these genes have yet to be identified, and SOD genes may become significant in inflammatory airway diseases where oxidative stress is common.

The current study was conducted to find out the link between the SOD1 and SOD2 between smokers and non-smoking patients. There was no statistical difference for genotype and allele frequency between the groups (p = 1) between asthma patients with waterpipe smoking and control. At the same way, when the patient smoker’s cohort and control group were compared for the presence of the SOD2 Ala9Val (C/T) polymorphism, the distribution of the genotypes (p = 0.996). The findings of the present study may explain why smoking isn’t considered a risk factor for developing asthma in the samples examined, and the findings of this study were consistent with those of \(^{(14)}\), interpretation of the results might be that another unknown polymorphism, which is in linkage disequilibrium with the SOD1gene and SOD2gene polymorphism and contributesususceptibility to asthma. Although the results have shown a statistically significant enrichment of specific SNPs in SOD genes across the asthma population, however, it is possible that the presence of these polymorphisms increases the probability or enhances the development of asthma through other unknown genetic mutations (such as polymorphic genes coding for enzymes involved in the metabolism of foreign chemicals (xenobiotics) as well as external factors (including pesticides, organic solvents, and metals including iron, copper, and manganese)\(^{(15)}\).

**Conclusions**

Incapabilityto obtain enough patent sample to accurately measure SOD1 and 2 polymorphisms and measuring the enzyme activities of SOD1 and SOD2 genes and this is the ne of limitations of the current study. This may be usefulto highlight the effects of polymorphisms studied, and correlate the genotyping data with different factors

**Ethical Clearance** : Taken from University of Thi-Qar ethical committee

**Source of Funding** : Self

**Conflict of Interest** : Nil

**References**


Mak JC, Leung HC, Ho SP, Ko FW, Cheung AH, Ip MS, Chan-Yeung MM. Polymorphisms in manganese superoxide dismutase and catalase genes: functional study in Hong Kong Chinese asthma patients. Clinical & Experimental Allergy .2006;36.4: 440-447.

The Biological Activity of Alcoholic Extracts of *Cordia myxa* Plant Against *Klebsiella*. Isolated from Infected Patients

Bassam M. Shamkhy¹, Yass K. Abbas²

¹Research Scholar, Department of Biology, Faculty of Education for Pure Sciences, University of Thi-Qar, Iraq, ²Professor, Faculty of Education for Pure Sciences, University of Thi-Qar, Iraq

**Abstract**

Plants have been used as drugs by humans since thousands of years ago. The study aimed to investigate the effectiveness of alcoholic extracts of the fruits, leaves and seeds of *Cordia myxa* plant against pathogenic microorganisms (i.e. Klebsiella). This was done in the laboratories of the Department of Life Sciences / College of Education in Nasiriya southern of Iraq. The results showed that the alcohol extract of the leaf was significantly superior to that of the fruit and seed extract. The results also showed that the growth inhibition and the effectiveness against *Klebsiella* bacteria was at the average concentration of 300mg / mL 32.66 ± 2.08, representing the highest inhibitory concentration. It was concluded that leaves of the *Cordia myxa* is one of the best parts of the plant that have inhibitory effects on Klebsiella bacteria compared to the fruit and seed.

**Keywords:** alcoholic extracts, inhibition, *Cordia myxa*, Klebsiella, infections

**Introduction**

Medicinal plants are the Nature’s gift to human beings to help them pursue a disease-free healthy life. Plants have been used as drugs by humans since thousands of years ago. As a result of accumulated experience from the past generations, today, all the world’s cultures have an extensive knowledge of herbal medicine. Two thirds of the new chemicals identified yearly were extracted from higher plants. 75% of the world’s population used plants for therapy and prevention. In the US, where chemical synthesis dominates the pharmaceutical industry, 25% of the pharmaceuticals are based on plant-derived chemicals(1). The emergence of herbal medicine goes back to about 6000 years with the discovery of a tomb in the cave in northern Iraq in the year (1960). The analyzes conducted on the soil surrounding the skeleton resulted in the presence of pollen grains for eight plants, seven of which are medicinal, which are still used all over the world (2). *Cordia.myxa L* belongs to the family Ehretiaceae, which contains more than 300 genus and that are distributed in tropical, subtropical and warmer regions around the world. It is a medium-sized deciduous tree, the circumference of the whole bearing tree of the trunk is 75.5 cm, with pruned tree branches, soft wood, light gray, no heartwood(3). *Cordia.myxa* Tree is a perennial evergreen tree, medium in size if it reaches a height AD 5-7 and lived to 60 years (4). The fruit is almond with a stone core and is yellow in color and sweetish when ripe. The seed is oval in shape Several chemicals have been identified. Leaves are simple in shape, green in color (5).

The genus Cordia (containing more than 200 species) is one of the largest genera of this family. The fruits are used to make pickles and have many common antibacterial and anti-worm uses (6). It is also widely used in the treatment of diseases of the urinary tract and thoracic tract (7). A botanical study conducted in northeastern Ethiopia reported that *Cordia africana* was traditionally used to treat liver disease, dysentery, stomach pain, and
diarrhea\(^{(8)}\). The dried leaf powder is used to treat malaria and its associated symptoms by traditional healers\(^{(9)}\). Klebsiella species are routinely found in the human nose, mouth, and gastrointestinal tract as normal flora; however, they can also behave as opportunistic human pathogens, it can lead to a wide range of disease states, notably pneumonia, urinary tract infections, sepsis, meningitis, diarrhea, peritonitis and soft tissue infections it may be treated by leaves of *Cordia leucocephala*, popularly known as “Maria Brita”, are used as an infusion to treat dysmenorrhea\(^{(10,14)}\). The genus Cordia belong to the Kingdom : Plantae; Subkingdom: Tracheobionta; Superdivision: Spermatophyta; Division: Magnoliophyta; Class: Magnoliopsida; Subclass: Asteridae; Order: Lamiales, Family: Eretiaceae, Genus: *Cordia* L., Species: *myxa* L\(^{(1)}\).

### Materials and Method

The parts of the *Cordia myxa* tree collected all of the fruits, leaves and seeds from some scattered areas in the city of Nasiriyah, where they were cleaned and washed well with running water to get rid of impurities and dried, and left in the shade to dry, exposed to the air, after which they were ground by an electric grinder and placed in opaque bottles. The alcoholic extract (ethanol) extract was prepared according to the method of Harborne\(^{(11)}\). In this study, *Klebsiella* bacteria were used that were previously diagnosed from patient samples and were taken from the Microbiology Laboratory in the Department of Life Sciences at the College of Education for Pure Sciences at. Dhi Qar University, in order to evaluate the effectiveness of the and alcoholic extract towards the pathological bacterial isolate used during the study, the agar well diffusion method was used as it was mentioned in Hammer\(^{(12)}\). Muller Huntington agar medium was prepared and the bacterial isolates used were activated on this medium and then placed in the incubator at a temperature of 37 °C for a period of 24 hours after which a bacterial suspension was prepared from the developing colonies at a concentration (6 x 10\(^6\)) Using the physiological solution, and comparing the number of bacteria to the previously prepared McFarland. Take 100 ml of the bacterial suspension by means of a sterile micropipette and pour it over the culture medium, then spread the suspension with a glass diffuser spreader sterilized with alcohol and flame, then leave the dishes for one hour to dry the suspension. Three pits with a diameter of 7 mm were made in each dish using a sterile metal cork borer, then 100 micrometers of each concentration of the extract were placed in each hole using a small volume pipette micropipette, as one dish contains three concentrations (100 micrograms / ml and 200 micrograms / ml. And 300 μg. The dishes were incubated in the incubator at 37 °C for 24 hours, after which a transparent corona was observed around each hole, which represented the diameter of the inhibition zone. The diameter of the inhibition zone was measured using a ruler by taking the average of two perpendicular diameters measured in mm. were measured.

### Results and Discussion

*Klebsiella* organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, sepsis, meningitis, diarrhea, peritonitis and soft tissue infections. *Klebsiella* species have also been implicated in the pathogenesis of ankylosing spondylitis and other spondyloarthropathies\(^{(14)}\). The majority of human *Klebsiella* infections are caused by *K. pneumoniae*, followed by *K. oxytoca*. Infections are more common in the very young, very old, and those with other underlying diseases, such as cancer, and most infections involve contamination of an invasive medical device. It was noted through Table (1) that all the concentrations of the ethanol solvent used were effective and with all the concentrations used, and the concentration of 300 mg was one of the best alcohol concentrations used, and through the statistical analysis of the fruit, it was noticed that there were no significant differences between the average concentration of 100 mg / mL of 25.66 ± 1.52\(^b\) and the average The average concentration of 200mg / mL was 26.66 ± 1.52\(^b\), but there was a significant difference in the concentration of 300mg / mL 29.66 ± 0.57\(^a\), as a significant difference was found between it and the concentration of 100mg / mL and the concentration of 200 mg / mL in the paper, through the statistical analysis, it was noticed that
there are significant differences between the average mean concentration of 100mg / mL 24.33 ± 1.15c, the average concentration 200mg / mL 28.33 ± 1.52b, and the average concentration rate 300mg / mL 32.66 ± 2.08a. As for the seeds, it was observed that there are significant differences between the average mean concentration 100mg / mL 23.55 ± 1.50c, the average concentration rate 200mg / mL 27.55 ± 1.66b, and the average concentration 300mg / mL 31.11 ± 1.76a. and the results of the current study are consistent with the results of the study he did(13). The inhibitory action of the alcoholic extract of the Cordia myxa plant may be attributed to the presence of alkaloids and phenols, in addition to triple terpenes, saponins, glycosides, tannins, flavonoids and carotenoids. These substances possess positive and negative bacterial activity as they cause inhibition of the formation of the cell wall of the microorganism or inhibition of the synthesis of essential proteins in it, and the formation of complexes with the cell wall that impede the regularity of permeability, and inhibition of some enzymes that have an important metabolic role in growth and reproduction, and the rupture of cell membranes or change their function. These reasons made the extract an antibiotic and antibacterial(15).

Table (1) the effect of the alcohol solvent of the Cordia myxa plant extract against Klebsiella bacteria that cause infections in human

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<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>100mg</td>
<td>24.55 ± 1.50c</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>200mg</td>
<td>27.55 ± 1.66b</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>300mg</td>
<td>31.11 ± 1.76a</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>27.74 ± 3.15</td>
<td>27</td>
</tr>
<tr>
<td>L.S.D</td>
<td></td>
<td>2.05</td>
<td></td>
</tr>
</tbody>
</table>
Figure (1) inhibitory effects of the alcoholic extract for the (A) leaves, (B) fruits, (C) seeds of the *Cordia myxa* plant, against *Klebsiella* bacteria that cause infections in human.

**Conclusion**

The possibility of adopting the *Cordia myxa* as an influential medicinal plant, as an important and essential source of effective compounds and nutrients, and this confirms the importance in terms of nutritional and therapeutic. The results of the study showed that the *Cordia myxa* contains high concentrations of total phenols that act as antioxidants and have proven their efficiency in scavenging free radicals, in addition to resistance to dangerous diseases related to free radicals. The leaf of the *Cordia myxa* is one of the best parts of the plant used in the current study in terms of the inhibitory effectiveness of bacteria (*Klebsiella*) compared to the fruit and seed used in the study.

**Ethical Clearance** : Taken from University of Thi-Qar ethical committee

**Source of Funding** : Self

**Conflict of Interest** : Nil

**References**


(3) Gupta, R., and Gupta, G. D. A review on plant Cordia obliqua Willd.(Clammy cherry).


Comparison of Akt Expression in the Cerebrum and Cerebellum of Newborn *Mus musculus* Exposed to Physical Stress and Psychological Stress During Pregnancy

Herlina Puji Angesti¹, Hermanto Tri Joewono², Widjiati³

¹Posgraduate student of Reproductive Health Science, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia, ²Obyn MFM Consultant, Department of Obstetry and Gynecology, Faculty of Medicine Universitas Airlangga/RSU Dr. Soetomo, Surabaya, Indonesia, ³Prof. Department of Embriology, Faculty of Veterinary Medicine Universitas Airlangga, Surabaya, Indonesia

Abstract

**Introduction:** Prenatal stress prevalence is almost half of the population of pregnant women worldwide. Stress will stimulate glucocorticoids which can disrupt the PI3K-Akt cascade. PI3K-Akt deficiency will cause impaired fetal brain growth and development.

**Objective:** To compare the Akt expression in the cerebrum and cerebellum of newborn *Mus musculus* exposed to physical stress, psychological stress, the combination of psychological and physical stress, and without stress exposure.

**Method:** This study was experimental laboratory research. Twenty-four female mice were used as samples and divided into four groups: physical stress exposure group, psychological stress exposure group, the combination of psychological and physical stress exposure group, and control group. Akt expression was tested by immunohistochemistry (IHC) staining. Statistical analysis used the One Way Anova and Kruskal Wallis test.

**Results:** There were significant differences in cerebrum Akt expression (p = 0.008) and cerebellum Akt expression (p = 0.047) between the control and stress exposure groups.

**Conclusion:** There were statistically significant decreases in mean Akt expression in the cerebrum and cerebellum of newborn *Mus musculus* exposed to stress during pregnancy.

**Keywords:** Stress, Akt, cerebrum, cerebellum, and *Mus musculus*.

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Introduction

The brain is an organ that controls all body functions. Intelligence, creativity, emotions, and memory are some of the many things regulated in the brain. The brain is a stress adaptation center that triggers a behavioral and physiological response to stress. Stress increases hormone secretion on the hypothalamus-pituitary-adrenal (HPA) axis. After acute experience stress, the HPA hormone axis quickly returns to pre-stress levels, but chronic stress triggers a sustained response that affects mental and physical distress.
Depression and anxiety occurred in pregnancy, with estimated prevalence of 12% for depression and 15.2% for anxiety. Mild to moderate stress has been reported in half of the population of healthy pregnant women worldwide\(^1\). Prenatal stress increased glucocorticoids (cortisol) that can damage the brain\(^12\).

High concentrations of glucocorticoids (GC) combined with glucocorticoid receptors (GR) will inhibit neurotrophic factor (NTF) signaling expressed by many tissues in the brain, especially in the cerebrum and cerebellum. NTF is known to increase cell survival by activating Akt/protein kinase B signaling\(^4\). Phosphatidylinositide 3 Kinase (PI3K)-Akt cascade dysfunction has been recognized as a cause of neurodevelopmental and neuropsychiatric diseases, such as autism, epilepsy, brain injury, and developing brain malformations\(^17\).

**Objectives**

To compare the Akt expression in the cerebrum and cerebellum of newborn *Mus musculus* exposed to physical stress, psychological stress, the combination of psychological and physical stress exposure, and without stress exposure.

**Materials and Methods**

This research is a study laboratory experiment with post-test only with a control group design. Samples were 24 female mice (*Mus musculus*), 2-2.5 months old, weighing 20-25 grams. The research was conducted in Faculty of Veterinary Medicine Universitas Airlangga, from January to March 2021.

After adaptation for a week, female mice were divided into four groups, which are: the physical stress exposure group (K1), the psychological stress exposure group (K2), the combination of psychological and physical stress exposure group (K3), and control group (K4). Below are some explanations:

1. K1 is the group with forced swimming for 5 minutes every day in a box measuring 50 cm x 30 cm x 25 cm with a water height of 18 cm. Water temperature ranging from 24°C-28°C and room temperature of 20°C-25°C.

2. K2 is the group with noise exposure with sound intensity 90 dB through TrueRTA software and measured by the real-time sound analyzer (TES 1358) given for 1 hour per day.

3. K3 is the combination of K2 and K1. Both types of treatment are given on the same day with noise exposure for 1 hour. 5 minutes after that, forced swimming for 5 minutes.

Stress exposure to K1, K2, and K3 is given simultaneously at 09.00. Stress exposure is given starting from day 6-15 of pregnancy.

At the end of the 16th day of the experiment, mice were anesthetized with ketamine (Ketamine Hydrochloride Pfizer®, New Jersey, USA) and acepromazine (Castran®, Venray, Netherlands). Delivery by sectio caesarea (SC). The newborn mice were sacrificed through neck decapitation, and the brain organs were dissected.

Akt expression was tested by immunohistochemistry (IHC) and analyzed using the Remmele semi-quantitative scoring system. This Immuno Reactive Score (IRS) is a multiplication between immunoreactive cells (A) and the color intensity score of immunoreactive cells (B).

The Shapiro Wilk test was used to determine the data’s normality and the Levene test to determine its homogeneity. If the data’s distribution and homogeneity were normal (p> 0.05), then the One Way Anova test was used, followed by Post Hoc LSD (Least Significant Difference). If the data distribution is not normal, then the Kruskal-Wallis test is used, followed by the Mann-Whitney U test to determine the difference between the two groups with abnormal data distribution. Meanwhile, to determine the difference between the two groups with normal distribution, it is necessary to use the T-test analysis. Data analysis using IBM SPSS Statistics version 26.00 (New York, USA).
Results

The research sample consisted of 24 female mice based on inclusion criteria and randomized into four groups.

The results showed the mean difference between K1, K2, K3, and K4, as shown in Table 1. Cerebrum Akt expression was higher in the control group (K4) than the stress exposure group (K1, K2, and K3).

Table 1. Akt expression in the cerebrum of newborn mice in each group.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>8,300 ± 0,726</td>
</tr>
<tr>
<td>K2</td>
<td>6,616 ± 1,487</td>
</tr>
<tr>
<td>K3</td>
<td>5,017 ± 1,038</td>
</tr>
<tr>
<td>K4</td>
<td>9,617 ± 3,468</td>
</tr>
</tbody>
</table>

Statistical analysis used the Kruskal-Wallis test with the result p-value = 0.008. That means there was at least one significant difference between the two groups. The data were normally distributed, so the comparison between the two groups was analyzed using the T-test. T-Test showed significant differences between K1 and K2, K1 and K3, and K3 and K4.

Figure 1. Akt expression (red arrow). Cerebrum Akt expression in the control group (K4) was strongest among the other groups (immunohistochemistry, 400x magnification, Nikon H600L microscope from Nikon Instruments Inc. ™, New York, USA).
Table 2. Akt expression in the cerebellum of newborn mice in each group.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K1</td>
</tr>
<tr>
<td>Akt expression</td>
<td>9,100 ± 2,767</td>
</tr>
</tbody>
</table>

The results showed the mean difference between K1, K2, K3, and K4, as shown in Table 2. The decrease in Akt expression occurred in the stress exposure group. This Akt expression decrease was in line with the increased stress exposure. Statistical analysis used the One Way Anova test with p-value = 0.047, which means significant differences between groups. Post Hoc LSD test (Least Significant Difference) shows significant differences between K1 and K3 and K3 and K4.

Figure 2. Akt expression (red arrow). Cerebellum Akt expression in the control group (K4) was strongest among the other groups (immunohistochemistry, 400x magnification, Nikon H600L microscope from Nikon Instruments Inc.™, New York, USA).

Discussion

The results showed that stress exposure during pregnancy decrease Akt expression in the cerebrum and cerebellum newborn mice. Stress during pregnancy causes an increase in placental GC². The fetal HPA axis is very sensitive to excess GC levels that can alter the regulation of HPA function¹⁶. High GC levels can suppress neurogenesis which endangers cell survival, causing an imbalance in several neurotransmitter systems¹⁰.

The interaction of GC and active GR in adrenocortical cells causes the neurotrophic factor (NTF) signaling pathway to be suppressed. Several proteins have been
classified as NTF: BDNF (Brain-Derived Neurotrophic Factor), IGF-1 (Insulin-Like Growth Factor-1), and GDNF (Glial Cell Line Derived Neurotrophic Factor)⁴. The BDNF system is essential for neural survival and synaptic plasticity in the CNS¹³. BDNF has broad expression in the brain¹⁸. IGF-1 is an important growth factor in CNS (Central Nervous System) development. In early brain development, IGF-1 has an essential role as an autocrine, proliferative, and prosurvival factor⁵. GDNF activates the PI3K / Akt signaling cascades⁴.

NTF plays a role in determining PI3K-Akt regulations. PI3K-Akt signaling activity triggers the catalytic lipid domain of PIP2 to become PIP3⁴. Inactive Akt binds to PIP3 in the plasma membrane, allowing PDK1(Phosphoinositide-Dependent Protein Kinase 1) to access phosphorylate T308 and mTORC2 (Mammalian Target of Rapamycin-2) to phosphorylate S473 as part of the activation of Akt⁷.

The Akt signaling pathway has important biological effects on cells, such as increased survival, inhibition of aging, and physiological activity¹⁹. Akt is a growth factor-induced cell survival mediator and has been shown to suppress apoptotic death in several cell types⁹.

The Akt pathways are negatively affected by glucocorticoid exposure¹¹. PI3K deficiency causes a significant reduction in brain size during embryogenesis³. Many experiments have shown that glucocorticoids can inhibit IGF-1 expression in many tissues and cells⁶. It shows the importance of Akt in brain growth and development³.

The results showed that Akt expression in the cerebrum and cerebellum was significantly higher in the control group than in the stress exposure group. Akt expression in the cerebrum and cerebellum of newborn mice decreased significantly in line with the stress exposure.

**Conclusion**

This study concluded that physical and psychological stress exposure during pregnancy could decrease the expression of Akt in the cerebrum and cerebellum of newborn mice.

**Conflict of Interest:** The authors state that there is no conflict of interest associated with this research.

**Source of Funding:** The authors have not received specific grants from any funding agency in the public, commercial, or not-for-profit sector.

**Ethical Clearance:** This study was approved by the Ethical Committee Faculty of Veterinary Medicine Universitas Airlangga with Number: 2.KE.002.01.2021.

**References**

7. Hemmings BA, Restuccia DF. PI3K-PKB/


Clustering of Provinces in Indonesia based on Maternal Health Indicators

Herti Maryani1, Lusi Kristiana2, Astridya Paramita2, Pramita Andarwati3, Nailul Izza3
1Senior Researcher, 2Junior Researcher, 3Assistant Researcher, Functional Unit of Health Technology Innovation, National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia, Surabaya, Indonesia

Abstract

Indonesian health issues that deserved top priority was maternal and child health because it determines the quality of the human resources of future generations. The objective of this research is to analyze the clustering of provinces in Indonesia based on maternal health indicators. This cross-sectional study was conducted in 34 provinces using secondary data from the Basic Health Research and Statistics Indonesia. Analysis of provincial clustering used FUZZY C-MEANS. Analysis produces six clusters. Cluster 2 has a high mean value of maternal health indicators that exceeded the Indonesian target, consisting of the provinces of DKI Jakarta, DI Yogyakarta, and East Nusa Tenggara. Cluster 2 was formed by the value equation variable Age of first mating, Ownership of maternal and child health monitoring book, Vitamin A Provision, K4, postpartum visits, Iron supplementation tablets consumption and Childbirth delivery in health facilities. Indicators of ownership of the maternal and child health monitoring book for pregnant women had met Indonesia target in all clusters. Meanwhile, the active participation of family planning program indicator was still below the Indonesia target in all clusters.

Keywords: maternal health, indicator, cluster, fuzzy-c-means.

Background

The Maternal Mortality Rate (MMR) and Infant Mortality Rate (IMR), the reduction in the prevalence of stunting, as well as the control of infectious diseases and non-communicable diseases are priority health programs in the Indonesian for 2015-2019 (1).

In developing countries, including Indonesia, maternal mortality is still a major problem. The Indonesia maternal mortality rate in 2018/2019 is 305 per 1000 live births, meaning there are 38 mothers die every day due to illness/ complications related to pregnancy and childbirth. Compared to this, there are 830 mothers die every day in the world because of the same thing. The maternal mortality rate in Indonesia is second highest in ASEAN after Laos. Third place and so are the Philippines, Myanmar, Cambodia, Vietnam and Malaysia (2).

Labor complication is one of major maternal death cause in Indonesia. Based on 2018 Basic Health Research (Riskesdas), the proportion of childbirth complications in women aged 10-54 years in Indonesia was 23.2 percent (3). Based on 2018 National Social Economic Survey (Susenas), it was stated that women in Indonesia who had health complaints the last one month were 45.24 percent (4).

Indonesia’s health problems that need top priority is Maternal and Child Health (MCH) because it greatly determines the quality of human resources (HR) in future generations. The high maternal and child mortality rates reflect MCH services that urgently need improvement,
both in terms of range and quality of services (5).

So far, research conducted related to maternal health was still rarely done. Existing research on maternal mortality and related factors. Research conducted by Nurrizka and Wahyono (2018) on Maternal Mortality Disparities in Indonesia stated that there was still a disparity in maternal mortality that involved an intermediate factor between districts/cities in Indonesia, with the highest risk of maternal mortality occurred in Eastern Indonesia. This study used secondary data from Statistics Indonesia and the Ministry of Health as many as 8 variables, including health variables and demographic variables in 2013 (6).

Research conducted by Mukti and Wibowo (2017) on the Mapping of Maternal and Children Health Areas in East Java Province in 2014 used secondary data on 2014 maternal and children health programs in the East Java Provincial Health Office. This study applied a non-hierarchical cluster analysis method or K-Means Cluster. Eight variables were analyzed, ranging from maternal health service coverage, complications of labor and neonatal health service (5).

Based on the description above, research that has been done related to maternal health area mapping was still limited in one particular province, none of which has been done at the national level. Researcher is interested in analyzing the clustering of provinces in Indonesia based on maternal health indicators and using more health variables.

Cluster analysis produces information that differentiate cluster having maternal health indicators that perform beyond Indonesia’s target and which do not. Indonesian government can employ this cluster analysis to arrange more precise monitoring and evaluation of health program, by prioritizing programs according to the needs of each region.

Methods

This research is cross sectional study that applies Fuzzy-C Means analysis which was conducted in 34 provinces. The research uses data from three sources, namely the National Health Research (Riskesdas) 2018 (3), Welfare Statistics in Indonesia 2018 and Profile of Maternal and Child Health 2018 (4,7).

Riskesdas is a community-based health research that its indicators represent both national level and district/city level. Conducted once every five years, Riskesdas considered having appropriate interval to assess the development of public health status, risk factors, and the progress of health development efforts.

The Statistics Indonesia (BPS) collects data on demographic, education, health, fertility and family planning, housing, information and communication technology, crime, and social protection. These data are presented at the national and provincial levels thus allowing comparisons between regions.

FUZZY C-MEANS was applied to analyze the data. Research conducted by Maheswari (2018), Rahayu (2018), and Wang (2018) shows that cluster analysis using factor analysis results can improve clustering results by providing shorter time data processing. Factor analysis is considered for this study due to its stable clustering characteristics (8–10).

The steps of the analysis are:

1. Perform data adequacy test and correlation test.
2. Perform factor analysis.
3. Perform grouping using FUZZY C-MEANS.
4. Determine the optimal number of clusters using Pseudo F-Statistics.
5. Compare the performance of the method using ICD rate.

The highest Pseudo F indicates optimum number of groups created from data available, meaning the group is highly homogeneous while the intergroup diversity is highly heterogeneous (11). The selection of the best clustering method can be done by choosing the smallest icd rate. The value of icd rate describes the disperse level in the cluster (12).
Results and Discussion

Before clusters is formed, factor analysis is carried out to change the independent variables that correlate with other variables into new variable that is not interrelated.

This new variable is called the principal component (13). The first step in conducting a cluster analysis is to check the adequacy of the data using the Kaiser-Meyer-Olkin (KMO) value. In this analysis the KMO value is 0.707 (> 0.5), indicating that the amount of data used is sufficient to be calculated.

The next step to do is a Barlett test to find out the correlation between variables. This test yields $p$-value = 0.000 (< $\alpha = 0.05$), resulting in a decision to reject $H_0$. It is concluded that there is a correlation between maternal health indicator variables in Indonesia.

Determination of the optimal clusters number is based on the highest Pseudo F-Statistics value. This shows that two optimal clusters are formed. On the other hand, FUZZY C-MEANS method with factor analysis produces the highest Pseudo F-Statistics value of 12.537, which forms six optimal clusters (Table 1).

<table>
<thead>
<tr>
<th>Number of Clusters</th>
<th>With Factor Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7.547577</td>
</tr>
<tr>
<td>3</td>
<td>8.742597</td>
</tr>
<tr>
<td>4</td>
<td>12.30961</td>
</tr>
<tr>
<td>5</td>
<td>11.39988</td>
</tr>
<tr>
<td>6</td>
<td>12.5317</td>
</tr>
</tbody>
</table>

Table 2. Clusters of factor analysis using the FUZZY C-MEANS method

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Member of Clusters</th>
<th>Number of Members</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>South Sumatra, Bengkulu, Bangka Belitung Islands, Central Kalimantan,</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Central Sulawesi, Southeast Sulawesi</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>DKI Jakarta, Yogyakarta, East Nusa Tenggara</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Jambi, Lampung, West Java, Central Java, East Java Timur, Banten, West Nusa</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Tenggara, West Kalimantan, South Kalimantan, East Kalimantan</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Maluku, North Maluku, Papua</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>North Sumatra, West Sumatra, Riau, Riau Islands, Bali</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Aceh, North Kalimantan, North Sulawesi, South Sulawesi, Gorontalo, West Sulawesi,</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>West Papua</td>
<td></td>
</tr>
</tbody>
</table>
Cluster Interpretation

FUZZY C-MEANS method with factor analysis is a good method for grouping provinces based on maternal health indicators. Interpretation of results uses the mean value (centroid) of each variable in each cluster. The mean values of all clusters are shown in Table 3.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>Cluster 4</th>
<th>Cluster 5</th>
<th>Cluster 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1 = First age of mating **</td>
<td>63.02</td>
<td>80.18</td>
<td>61.84</td>
<td>73.99</td>
<td>77.83</td>
<td>68.71</td>
</tr>
<tr>
<td>X2 = Normal birth weight newborn **</td>
<td>81.75</td>
<td>80.45</td>
<td>82.76</td>
<td>53.78</td>
<td>86.65</td>
<td>78.92</td>
</tr>
<tr>
<td>X3 = Ownership of maternal and child health monitoring book *</td>
<td>69.93</td>
<td>79.90</td>
<td>79.77</td>
<td>62.47</td>
<td>65.08</td>
<td>74.56</td>
</tr>
<tr>
<td>X4 = Vitamin A provision *</td>
<td>53.98</td>
<td>63.30</td>
<td>59.71</td>
<td>50.83</td>
<td>49.48</td>
<td>61.00</td>
</tr>
<tr>
<td>X5 = Mothers having health insurance **</td>
<td>67.41</td>
<td>77.41</td>
<td>61.36</td>
<td>69.26</td>
<td>63.31</td>
<td>80.69</td>
</tr>
<tr>
<td>X6 = K1 *</td>
<td>77.93</td>
<td>86.30</td>
<td>86.86</td>
<td>71.13</td>
<td>87.26</td>
<td>75.93</td>
</tr>
<tr>
<td>X7 = K4 *</td>
<td>62.72</td>
<td>79.57</td>
<td>74.94</td>
<td>49.10</td>
<td>72.76</td>
<td>58.84</td>
</tr>
<tr>
<td>X8 = Postpartum visits *</td>
<td>58.84</td>
<td>52.40</td>
<td>40.28</td>
<td>24.30</td>
<td>31.08</td>
<td>29.94</td>
</tr>
<tr>
<td>X9 = Iron supplementation tablets consumption *</td>
<td>23.10</td>
<td>57.00</td>
<td>39.03</td>
<td>23.57</td>
<td>37.68</td>
<td>18.26</td>
</tr>
<tr>
<td>X10 = Childbirth delivery in health facilities **</td>
<td>65.20</td>
<td>90.89</td>
<td>81.63</td>
<td>42.72</td>
<td>85.63</td>
<td>79.11</td>
</tr>
<tr>
<td>X11 = Birth attendant by health professional **</td>
<td>90.92</td>
<td>93.94</td>
<td>93.37</td>
<td>67.05</td>
<td>96.51</td>
<td>92.56</td>
</tr>
<tr>
<td>X12 = Active participant of family planning program **</td>
<td>58.81</td>
<td>47.22</td>
<td>58.43</td>
<td>37.11</td>
<td>49.14</td>
<td>48.71</td>
</tr>
<tr>
<td>Average</td>
<td>64.47</td>
<td>74.05</td>
<td>68.33</td>
<td>52.11</td>
<td>66.87</td>
<td>63.94</td>
</tr>
</tbody>
</table>

Source: *Ministry of Health (Riskesdas) 2018; **Statistics Indonesia (BPS) 2018
Table 3 shows that cluster 2 has the highest average and cluster 4 has the lowest. Cluster 1 has the highest average percentage of active participants of family planning program. The variable with the highest percentage is delivery with the help of health professional, and the variable with the lowest percentage is iron supplementation tablet consumption. Cluster 2 has seven variables with the highest average compared to other clusters which are age of first mating, having maternal and child health monitoring book, vitamin A provision, K4, postpartum visits, iron supplementation tablets consumption, and childbirth delivery at health facilities. The normal birth weight of newborn and active participants of family planning program variables in this cluster show inferior performance compared to other five clusters.

Cluster 3 has two variables with the lowest percentage compared to other clusters, which are age of first mating and national insurance health participant. Other variables in this cluster have a good average percentage. Cluster 4 have the lowest average percentage of maternal health indicators among other clusters. Cluster 4 has eight variables with the lowest average percentage of normal birth weight newborn, having maternal and child health monitoring book, K1, K4, postpartum visits, childbirth delivery at health facility, giving birth with the help of health professional, and active participant of family planning program. The indicator of maternal health with the highest mean in cluster 4 is the age of first mating. Whereas the lowest is iron supplementation tablets consumption.

Cluster 5 has two variables with the highest average percentage than other clusters, that are normal birth weight infant and giving birth with the help of health professional. The provision of vitamin A in this cluster is the variable with the lowest average percentage among all clusters. Indicators of maternal health with the highest mean in cluster 5 is giving birth with the help of health professional, the lowest is postpartum visits. Cluster 6 has the variable with the highest and lowest average percentage of the other clusters, which is national health insurance participation of mother and the iron supplementation tablets consumption, respectively. The variable of iron supplementation tablet consumption is indicator with the lowest mean value in cluster 6. While indicator with the highest mean value is childbirth delivery with the help of health professional.

Provinces with a low average indicator of maternal health are cluster 4 consisting of Maluku, North Maluku and Papua. Provinces with the highest average maternal health indicators are cluster 2, which consists of DKI Jakarta, Yogyakarta and East Nusa Tenggara.

The results shows that the childbirth delivery assisted by health professionals variable is the maternal health indicator with highest mean, almost 100 percent (90.96%). According to Nurhapipa and Seprina’s research in Center of Public Health (Puskesmas) XIII Koto Kampar I Riau (2015), the factors of affordability, family support and attitudes determined the mothers’ choice of childbirth delivery attendants type. When mothers had limited access to health facilities, then the chances of mothers choosing traditional childbirth delivery attendants (“dukun”) were fifteen times greater than those who had better access. While lack of family support gave four times greater chance to choose traditional childbirth delivery attendants (14).

The results of Zahtamal, Tuti Restuastuti and Fifia Chandra’s study (2011) showed that 81.1 percent of health service providers contributed to good maternal health practices, although it was statistically insignificant. Better maternal health practices were more likely influenced by the ease of accessing health services. Substandard maternal health practices were experienced 2.5 times greater by respondents with limited health services access (15).

The Number of pregnant mothers receiving iron supplementation tablets (ITD) are still lower (31.96%) than expected number, which was 98% according to The Indonesian National Strategic Plan 2015-2019. Dewantoro and Muniroh’s (2017) mentioned that limited supply of iron supplementation tablets in Center of Public Health led to low number of iron supplementation tablets utilization. Each Center of Public Health had a
distinct amount of funds spent to meet the needs of the iron supplementation tablets depending on the size of the capitation funds. Thus contributed to pregnant mothers’ compliance on consuming iron supplementation tablets (16).

An interesting finding to be analysed is the low average value on iron supplementation tablets consumption in all clusters. Further finding reveals that the ownership pregnancy monitoring book are quite high in all clusters. This finding is quite contradictory to the fact that maternal and child health monitoring book should be a communications, information and education media on healthy pregnancy issue including information on the importance of iron supplementation (17). The use of maternal and child health monitoring books are still limited to record medical history of pregnant women and have not been as an educational medium for pregnant women yet, as Sulistingsih reported (18). According to Kalsum and Yeni, one of the factors that affecting optimum utilization of the maternal and child health monitoring book were number of parity and the active role of health workers (19).

The provincial grouping results presented by images 1 shows cluster 4 (Maluku, North Maluku, and Papua Province) has the lowest mean value of maternal health indicators among others. This finding is consistent with Rahmah Hida N and Tri Yunis MW’s (2018) study result on the Maternal Death Disparity in Indonesia, which stated the high maternal death toll found in the district/city located in Eastern Indonesia. The provinces with a low maternal health indicator had higher risk on maternal mortality (6). The result of our research is consistent with Rivan D and Robert K’s study finding (2017) on the modeling of the Maternal Mortality Rate in Indonesia using GWPR. The study showed that the eastern Indonesia region had the smallest number of K4 visits (maternal visit on third semester) in comparison to other districts, leading to lack of sufficient health observation of pregnant women in the area, followed by increasing risk of maternal mortality (20).

The results of this research is also consistent with Syafrina and Sumertajaya’s study finding (2019) on Regency and City Clustering in Indonesia and also Fuzzy K Radala’s study finding that state most of the regencies and cities on Java Island are situated in cluster 2, which is upper middle class of Indonesian Human Development Index (HDI). The lower middle-class Indonesian HDI category is mostly occupied by Eastern Indonesia regencies and cities, whilst West Papua Province has the lowest Indonesian HDI. In contrast, some parts of Special Region of Jakarta, West Sumatra, and North Sumatra managed to reach high Indonesian HDI (21).

Family planning programme participation rate is one of the indicators of maternal health. The highest of active family planning programme participants (58.81%) is in cluster 1 which consist of six provinces. Based on the 2017 Indonesian Demographic and Health Survey (SDKI) 2017, active participation rate of family planning programme, both traditional and modern method, of the majority of the provinces in the cluster 1 (except Southeast Sulawesi 35.2%) are beyond Indonesia’s National average (46%). However, this is still below the national’s target set in 2019 which is 66%. Family planning programme is not entirely successful as measured through Contraceptive Prevalence Rate (CPR). The family planning program’s achievement in 2017 reached 63 percent, which was increased by 1.1 percent compared to 61.9 percent in 2012 (22).

The Indonesian Demographic and Health Survey showed that the proportion of unmet need was increasing from 9.1% in 2007 and 11.4% in 2012. These also depict shortage on contraception supply contributes to The family planning program’s underperformed condition. This condition should be important because it causes unexpected pregnancy to trigger abortion that escalates the risk of maternal mortality. Nurul Huda F., Ratno Widoyo and Fauziah Elytha (2016) found that lacking of husband’s support in participating birth control, raising the risk of unmet need to be 2.2 times (23).
Cluster 2 has the highest average value on all 7 maternal health indicators, amongst all clusters. This also indicates provinces in cluster 2 perform better in implementing health programs amongst all provinces in Indonesia. Cluster 2 consists of three provinces which have similarities on value of the variables. The average value of childbirth delivery at a health service facility (90.89%) on clusters 2 has exceeded the national target of 2018 (82%) \(^{(24)}\). Indonesian Demographic and Health Survey 2017 mentioned that most provinces in the clusters 2 (except East Nusa Tenggara) performed beyond national target, which was 79.4%, on childbirth delivery at health service facilities program. Similarly, healthcare staff-attended childbirth delivery average values in cluster 2 (except East Nusa Tenggara) exceeded the national average of 90.9\% \(^{(22)}\).

The visit 1 (K1) or minimum once visit to health care facilities during first trimester of pregnancy is one of the maternal health indicators that reaches the highest average value (86.30%) in cluster 2, which is also exceeds the national target of 2018 (85%) \(^{(24)}\). Indonesian Demographic and Health Survey 2017 showed that provinces in cluster 2 (except East Nusa Tenggara) performed better than the national average of K1, which was 97.5%. Likewise, average value for visit 4 (K4) or minimum four times visit to health care facilities during pregnancy (once in the first trimester, once in second semester and twice in third trimester of pregnancy) in cluster 2, which was 79.57%, performed beyond the national target 2018 (78%) \(^{(24)}\). According to Indonesian Demographic and Health Survey 2017, most provinces in cluster 2 (except East Nusa Tenggara) implemented K4 above the national target 2017 (77.4\%) \(^{(22)}\).

Analysis results show the mean value of the KMS ownership in cluster 2 is higher than other clusters, surpassed the targeted achievement rate by General Directorate of Family Health, 50% \(^{(25)}\). The analysis indicated that the average value of the postpartum women received vitamin A in cluster 2 is higher than the other clusters. Despite this, the results of the Nutritional Status Monitoring (PSG) (2017) nationally is still high at 53.5\% \(^{(26)}\).

Analysis results show that clusters 3 had satisfying mean value of most maternal health indicators, but still have the lowest average on indicator of the first mating age and mother having National Health Insurance. Cluster 3 consists of 10 provinces dominated by provinces situated in West Indonesian region. Over the past three years, many of the pregnant women in Indonesia’s western region have rarely utilize the NHI scheme as they prefer pay for medical service. On the other hand, most women in Central and Eastern Indonesia area had higher utilization rate of NHI \(^{(27)}\). Desra and Idris’s study (2019) on the mass-level Determinant in West Sumatra province indicated that the mother’s age had a significant influence on infant mortality in West Sumatra. The older the mother’s age, the higher risk for infant mortality. Furthermore, first mating age also plays significant role in infant mortality rate issue \(^{(28)}\).

Cluster 4 has the lowest average value of eight maternal health indicators. Normal birth weight leads to optimum growth and healthy generation. The low percentage of normal birth weight infants increase the risk of infant mortality and health disorders. Indonesian Demographic and Health Survey (SDKI) 2017 showed provinces in cluster 4 (Maluku 67\%, North Maluku 68.3\%, Papua 52.4\%) had much lower normal birth weight infants number than national average (87.5\%). The ownership of toddlers growth chart and milestones card (KMS) in cluster 4 is the lowest amongst others. Through this KMS, children’s growth are monitored every months. According to SDKI 2017, Papua, which is grouped in Cluster 4, had the lowest average in ownership of KMS (67.2\%) among all provinces in Indonesia \(^{(22)}\).

The childbirth delivery assisted by health professionals variable has the highest average amongst all variables. The results of Alhidayati and Asmutiliyanti (2016) study showed that the coverage of childbirth delivery assisted by health professionals in Hulu-Riau Center of Public Health (Puskesmas) was 79.86\%, while the rest 20.14\% assisted by non health professionals.
The percentage of iron supplementation tablet consumption is the lowest amongst all variables in cluster 5, also the lowest compared to all provinces in Indonesia. Contrary to Nutritional Status Monitoring (PSG) 2017 results, provinces grouped in cluster 6 which got sufficient amount of iron supplementation tablets was only 31.3%. While the rest 16.5% did not get any iron supplementation tablets and the other 52.2% only got less than 90 iron supplementation tablets. This clustering analysis can be employed by the central government to conduct more targeted monitoring and evaluation, by providing special programs with the requirements of each cluster. Out of the 12 variables analyzed, the iron supplementation program needs even greater effort to increase the participation level of pregnant women across the clusters. The enactment of maternal iron supplementation means improving the maternal mortality rate due to bleeding as well as reducing the neonatal mortality rate.

**Conclusion**

The Indonesian provincial group based on Maternal Health Indicators using FUZZY C-MEANS Clustering produces six clusters, which is found with high, medium and low in average value of maternal health indicators. Indicator for ownership of pregnancy monitoring book has met national targets in all clusters. Indicator for active family planning participation is still below the national target on all clusters. Cluster 2 which consist of the Special Region of Jakarta, Special Region of Yogyakarta and East Nusa Tenggara is a cluster with high average maternal health indicators and perform beyond the national target. Meanwhile, the clusters of the Maluku, North Maluku and Papua provinces are clusters with low average maternal health indicators and many indicators under the national target. Other clusters have maternal health indicators with medium and varied average values.

**Additional Informations**

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**References**


Steroidogenesis Mechanism, Disruption Factor, Gene Function, and Role in Male Fertility: A Mini Review

I Gede Widhiantara¹,², Anak Agung Ayu Putri Permatasari¹, I Wayan Rosiana¹, Putu Angga Wiradana¹, Bagus Komang Satriyasa³

¹Study program of Biology, Faculty of Health, Science, and Technology, Universitas Dhyana Pura, Jalan Raya Padangluwih, Dalung, North Kuta, Badung, Bali (80361) Indonesia, ²Doctoral student of the Medical Science Study Program, Faculty of Medicine, Udayana University, Jalan PB. Sudirman, Denpasar, Bali (80234) Indonesia, ³Department of Pharmacology, Faculty of Medicine, Udayana University, Bali (80234), Indonesia

Abstract

Male fertility can be defined as the ability of the male reproductive system to impregnate a woman, while infertility is the condition of a married couple who have been married for one year or more and have had sexual intercourse regularly or without using contraception but do not have a pregnancy or offspring. About 10% of married couples experience infertility. The main organ of male reproduction is the testes because in the testes the process of forming spermatozoa and the hormone testosterone occurs. The hormone testosterone plays a direct role in the continuity of spermatogenesis. Testosterone is produced through a series of steroidogenesis mechanisms in testicular Leydig cells. Several factors influence the course of steroidogenesis such as Leydig cells, steroidogenesis proteins, related genes to the influence of free radicals. These factors are closely related to diet and lifestyle. This study is important to understand in efforts to prevent infertility in men.

Keywords: Male fertility, Steroidogenesis, protein StAR, steroidogenesis disorder, reproduction, animal trials.

Introduction

The health of the reproductive and sexual systems is closely related to mental, physical attitudes, and the social relations of each individual. The incidence of sexual dysfunction (SD) is estimated to be able to affect the sexual activity of men aged over 40 years by 52% and has the same potential for fertility in men aged under 30 years. Feldman et al.¹ A decrease in reproductive function in men can be indicated by a decrease in the quality and number of spermatozoa which can be used as biomarkers for male reproductive health.² Disease and lifestyle such as cardiovascular disease, obesity, depression, anxiety, and smoking are some examples of “classic” risk factors that correlate with reproductive and sexual system problems.³

Spermatogenesis is a biochemical process in the body that is regulated and acted on by endocrine hormones and several other related regulatory factors such as Luteinizing Hormone (LH), Folliclestimulating hormone (FSH), and testosterone and growth hormone in men. Spermatogenesis is facilitated by the presence of other hormones, but only the hormone testosterone plays a very important role in maintaining and maintaining the stability of the spermatogenesis process. Testosterone can be produced by changing cholesterol through a steroidogenesis process initiated by the StAR protein (Steroidogenic Acute Regulatory Protein). StAR protein works under the stimulation of Luteinizing Hormone (LH) in mobilizing and transporting cholesterol to the inner membrane of the mitochondria of Leydig cells.⁴ Research shows that there are several roles played by the
StAR protein as a biomarker of steroidogenesis activity, one of which is that the inhibition of this protein is capable of causing disruption of the hypothalamus-pituitary-testis axis which subsequently impacts LH secretion\(^5\,^6\). Apart from stimulating the performance of Leydig Cells for Steroidogenesis, LH together with androgens also play an important role in the proliferation and differentiation of Leydig cells\(^7\).

Currently, disease monitoring using biomarker and molecular approaches has been widely practiced in the field of health research and clinical practice. One of the uses of biomarkers that can be done in monitoring sexual and reproductive health is through understanding the pattern of steroidogenesis. The steroidogenesis process is regulated at various levels, especially at the level of transcription of genes encoding steroidogenic enzymes as well as several co-factors to post-translational processes associated with Steroidogenesis. Understanding steroidogenesis factors can also be facilitated by identifying genetic lesions/defects that can disrupt this process. An understanding of steroidogenesis is very important to be able to determine the occurrence of sexual differentiation, reproduction, fertility, hypertension, obesity, and physiological homeostasis\(^8\).

In this review, we will provide information and some important findings regarding the mechanism, disorders factors, gene function, and the role of steroidogenesis in male fertility. Several examples of research on Steroidogenesis disorders will also be represented in this review.

**Methods**

This study is based on the results of scientific research related to Steroidogenesis published from various local and international scientific sources, thesis, and dissertation. The internet is also used for data collection that has been published in various scientific journals\(^9\).

**Testis and Leydig Cells**

The testes are the reproductive organs in male and male animals. Men have two testes that are wrapped with a scrotum. In mammals, the testes are located outside the body, are connected by the spermatic tube, and are located inside the scrotum. This is consistent with the fact that the spermatogenesis process in mammals is more efficient at temperatures lower than body temperature (<37°C). The testes are covered by a fibrous layer called the Tunica Albuginea. In the spermatic tube, there is a cremaster muscle which when contracted will lift the testicles closer to the body. When the temperature of the testicles will be lowered, the cremaster muscles will relax and the testes will move away from the body. This phenomenon is known as the cremaster reflex\(^10\,^11\).

The testes have two main functions, namely, where spermatogenesis and steroidogenesis occur. Spermatogenesis occurs in a structure called the seminiferous tubule. These tubules are grooved in lobules where all the ducts then leave the testis and enter the epididymis. Androgen production by Leydig cells contained in the interstitial space. The hormones testosterone and spermatozoa are the two main products of the testes. The seminiferous tubule is the site of the Spermatogenesis process and Leydig cells which have a role in producing the hormone testosterone are located in the cavity between the seminiferous tubules. Leydig cells can be a single number or in groups. Apart from Leydig cells, there are also cells in the interstitial space such as macrophages, mast cells, fibroblasts, nerves, and endothelium cells. Leydig cells are surrounded by fibroblasts, macrophages, and binding tissue\(^12\).

**Biomarker Related to Steroidogenesis**

The regulation carried out by steroid hormones includes various processes both in development and physiology from the fetal phase to the adult phase. All components of steroid hormones are synthesized from cholesterol and have a structure known as Cyclopentanophenanthrene. This structure was discovered in the 1930s and became a precursor to the understanding of steroidogenesis\(^13\).

Substantially, studies have focused more on the performance of steroid hormones than on how they occur. This could be because steroids are a widely used drug and
something that steroid hormone disruption only occurs in people with rare genetic lesions. Advances in science and technology, especially in the health sector, have succeeded in providing the latest information regarding steroidogenic enzymes and the role of their genes used in the diagnosis of certain diseases such as hypertension and polycystic ovary syndrome through this approach. A list of genes in humans that can be used as biomarkers in studying steroidogenic function is presented in Table 1 below.

**Table 1. List of human genes coding for enzymes and steroidogenic and their roles that potential can be used as a reference for biomarkers**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Gene</th>
<th>Gene size (kb)</th>
<th>Function and References</th>
</tr>
</thead>
<tbody>
<tr>
<td>StAR</td>
<td>STAR</td>
<td>8</td>
<td>Steroidogenesis, cholesterol transports to cytochrome P450scc in the inner mitochondrial membrane(14)</td>
</tr>
<tr>
<td>P450scc</td>
<td>CYP11A1</td>
<td>30</td>
<td>Catalysis of the synthesis of cholesterol, sex hormones, and other steroid hormones such as estrogen, testosterone, aldosterone, and cortisone(15). Loss of enzyme activity will cause hermaphroditism, decreased estradiol, decreased testosterone which leads to male fertility.</td>
</tr>
<tr>
<td>P450c11β</td>
<td>CYP11B1</td>
<td>9.5</td>
<td>Neurosteroid biosynthesis expressed in the brain(16)</td>
</tr>
<tr>
<td>P450c11AS</td>
<td>CYP11B2</td>
<td>9.5</td>
<td>Gene expression occurs only in the adrenal zona glomerulosa and has an important role in adrenal steroidogenesis(17).</td>
</tr>
<tr>
<td>P450c17</td>
<td>CYP17A1</td>
<td>6.6</td>
<td>Androgensynthesis regulator and the only enzyme that has the capacity to convert the C21 precursor to the androgen precursor, 17-ketosteroid(18).</td>
</tr>
<tr>
<td>P450c21</td>
<td>CYP21A2</td>
<td>3.4</td>
<td>Synthesis of cortisol, as well as a decrease in this enzyme, causes congenital adrenal hyperplasia(19).</td>
</tr>
<tr>
<td>P450aro</td>
<td>CYP19A1</td>
<td>130</td>
<td>Regulates the calcium-binding protein, calbindin which has the potential to determine sexually dimorphic brain structures(20).</td>
</tr>
<tr>
<td>3β HSD1</td>
<td>HSD3B1</td>
<td>8</td>
<td>Synthesis of potent intratumoral androgens from extragonadal precursors(21).</td>
</tr>
<tr>
<td>3β HSD2</td>
<td>HSD3B2</td>
<td>8</td>
<td>Adrenal and gonadal steroid biosynthesis and deficiency/mutation of these genes and enzymes will cause a rare disease of congenital adrenal hyperplasia(22).</td>
</tr>
<tr>
<td>11β HSD2</td>
<td>HSD11B2</td>
<td>6.2</td>
<td>Blood pressure regulation activates 11-hydroxy steroids in the kidneys so as to protect non-selective mineralo-corticoid (MR) receptors from occupation by glucocorticoids(23).</td>
</tr>
</tbody>
</table>
Table 1. List of humangenescoding for enzymes and steroidogenic and theirrolesthatpotentialcan be used as a reference for biomarkers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 β HSD1</td>
<td>HSD11B1 Regulation of conversion from inactive cortisone to active cortisol. Thus, this enzyme is considered an effective marker for the treatment of diabetes(24).</td>
<td>7</td>
</tr>
<tr>
<td>17 β HSD1</td>
<td>HSD17B1 Synthesis of estradiol and currently known inactivation of dihydrotestosterone (DHT), which exhibits dual function in breast cancer cell proliferation(25).</td>
<td>3.3</td>
</tr>
<tr>
<td>17 β HSD2</td>
<td>HSD17B2 Estradiol estrogen metabolism(26)</td>
<td>63</td>
</tr>
<tr>
<td>17 β HSD3</td>
<td>HSD17B3 Markers are used in endocrine evaluation in prepubertal patients by measuring levels of androstenedione and testosterone(27)</td>
<td>67</td>
</tr>
<tr>
<td>17 β HSD6</td>
<td>HSD17B6 Regulation of retinoid homeostasis in the eye(28)</td>
<td>24.5</td>
</tr>
<tr>
<td></td>
<td>(RoDH)</td>
<td></td>
</tr>
<tr>
<td>AKR1C1</td>
<td>AKR1C1 Accelerate the progesterone metabolism to 20α-hydroxyprogesterone in cervical fibroblasts. The increase in this gene can impact the possibility of premature birth(29)</td>
<td>14.3</td>
</tr>
<tr>
<td>AKR1C2</td>
<td>AKR1C2 Progesterone receptors(30)</td>
<td>13.8</td>
</tr>
<tr>
<td>AKR1C3</td>
<td>AKR1C3 Producing intratumoral testosterone and 17β-estradiol by reducing androgen and estrogen precursors(31)</td>
<td>13.0</td>
</tr>
<tr>
<td>AKR1C4</td>
<td>AKR1C4 Specific functions related to the liver can also be associated with hypomanics in men(32–34)</td>
<td>22.1</td>
</tr>
<tr>
<td>5α-Reductase 1</td>
<td>SRD5A1 Trans-activation of androgen receptors and inhibitors in the treatment of benign prostate disease(35)</td>
<td>36</td>
</tr>
<tr>
<td>5α-Reductase 2</td>
<td>SRD5A2 Testosterone production and deficiency of this enzyme have an impact on male fertility(36)</td>
<td>56</td>
</tr>
<tr>
<td>SULT2A1</td>
<td>SULT2A1 Contributes to the metabolic activation of procarcinogens and is widely expressed in the liver, small intestine, and adrenal cortex(37)</td>
<td>17</td>
</tr>
<tr>
<td>PAPSS2</td>
<td>PAPSS2 Contributes physiologically to androgen activation(38)</td>
<td>85</td>
</tr>
<tr>
<td>P450-oxidoreductase</td>
<td>POR Steroid hormone metabolism and deficiency of this enzyme can lead to impaired sexual development(39)</td>
<td>69</td>
</tr>
<tr>
<td>Ferredoxin</td>
<td>FDX1 The main regulator of mitochondrial steroidogenesis attempted in the zebrafish interrenal glands(40)</td>
<td>35</td>
</tr>
</tbody>
</table>
**Cont...** Table 1. List of humangenescoding for enzymes and steroidogenic and theirrolesthatpotentialcan be used as a reference for biomarkers

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Gene</th>
<th>Size</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferredoxinreductase</td>
<td>FDXR</td>
<td>11</td>
<td>Steroidbiogenesis(41)</td>
</tr>
<tr>
<td>Cytochrome b5</td>
<td>CYB5A</td>
<td>32</td>
<td>Steroidogenesis regulates and has a number of functions in clinical conditions(42)</td>
</tr>
<tr>
<td>H6PDH</td>
<td>H6PD</td>
<td>36.5</td>
<td>Glucocorticoidsynthesis and possibly other roles in electrontransfer for the steroidogenic enzyme P450(43)</td>
</tr>
</tbody>
</table>

Note: Enzyme, Gene, and Gene Size sourced from Miller and Auchus et al.(13)

**SEVERAL FACTORS INHIBITOR OF STEROIDOGENESIS PROCESS**

Several factors can cause Steroidogenesis disorders in men, including:

a. Decrease in the Number and Function of Leydig Cells

The incidence of infertility in men is a fundamental problem from the initial failure of the spermatogenesis process(44,45). Genetic factors are only a small part of several factors causing the decrease in the number of Leydig cells(46–48). One indicator that can be used as a basic reference regarding the potential for male infertility is lower serum testosterone levels(49). In addition, the decline in the function of Leydig cells in increasing age is also the reason for the incidence of infertility in men(50) but until now, this is still being debated. This is evidenced by the results of research from Petersen et al(51) who conducted experiments using male subjects of various ages and paid attention to the total number of Sertoli and Leydig cells in their testes. These results indicated that there is a significant decrease in the number of Sertolicells as men age. It is interesting that there was no decrease in Leydig cells with a unilateral mean number of $99 \times 10^6$ (range: $47 \times 10^6$ to $245 \times 10^6$, coefficient of variation (CV) = 0.48).

Food has been reported to reduce the number of Leydig cells in several studies. As reported by Dantas et al(52) that food contaminated by synthetic herbicides such as Ametryn can cause reproductive performance disorders (decreased Leydig cells, lipid peroxidation, Superoxide dismutase, catalase) and animal life (male Wistar rats). Consumption of foods high in fat can also cause a decrease in the number and diameter of Leydig cells in male Wistar rats(53).

b. Adrenal steroidogenesis defects

The incidence of defective adrenal steroidogenesis can cause impaired sexual development. This incident not only affects the disruption of sexual development but is also capable of causing mild to severe disturbances in the synthesis of glucocorticoids and mineralocorticoids. Therefore, an examination of glucocorticoids and mineralocorticoids can be done to find out information about steroidogenesis defects in patients.

c. StAR protein

**Steroidogenic acute regulatory protein** (StAR) is a mitochondrial protein that has a molecular weight of 30 kDa in the adrenal and gonads and plays an important role in facilitating the rapid movement of cholesterol from the outside to the inner mitochondrial membrane(54). On the other hand, StAR protein has a characteristic role in regulating steroid biosynthesis in steroidogenic tissues(55).
The role of StAR in regulating steroidogenesis has been demonstrated in patients suffering from congenital lipoid adrenal hyperplasia (lipoid CAH), an autosomal recessive disorder that causes impaired adrenal and gonadal biosynthesis due to mutations of the StAR gene\(^{56–58}\). Recent research has shown that hormone-sensitive lipase (HSL), a neutral cholesteryl ester hydrolase (NCEH), plays an important role in regulating the expression of the StAR gene in adrenal and gonadal cells\(^{59}\).

The results of other studies confirm that StAR protein plays an important role in the steroidogenesis process. According to the results of research by Walsh et al.\(^{60}\) using environmental pollutants, organochlorine insecticide lindane and organophosphate insecticide Dimethoate on MA-10 cells directly inhibits the expression of StAR protein which correlates with steroidogenesis in Leydig cells during consumption of vegetable and fruit products contaminated with insecticides.

d. Free radicals by cytochrome P450

Free radical production and lipid peroxidation are potential initial mediators in the physiological processes of the testes and their disruption. Increased levels of Reactive Oxygen Species (ROS) are seen in 80% of infertility men. Certain levels of free radicals are necessary for normal sperm function, but in excess, they can have a detrimental effect on the steroidogenesis process. Oxidative stress can occur when an imbalance arises between the process of free radical formation and antioxidant levels in the male reproductive system, especially in the spermatogenesis process.

**DEVELOPMENT OF STEROIDOGENESIS RESEARCH IN ANIMAL STUDIES**

Currently, molecular mechanisms have led to the use of biomarkers in understanding the function of steroidogenesis in the testes. Several growth factors such as fibroblasts 9 (FGF9) are also reported to be able to be used as an indicator of early gonadal development and testicular steroidogenesis function during the process of sexual maturity\(^ {61}\). The role of the steroidogenesis gene can be identified from histone H3K9 trimethylation (H3K9me3) which was studied in vivo in rat testes exposed to arsenic for a long time. The function of steroidogenic genes such as Lhr, Star, P450scc, Hsd3b, Cyp17b, and Arom decrease after arsenic exposure, but increased histone H3K9me3 methyltransferase. These results indicate that arsenic exposure is able to suppress steroidogenic gene expression by activating the H3K9me3 status in the process of inhibiting steroidogenesis in rat testes\(^ {62}\). The following are some of the results of research reports on the effects of several toxic substances on steroidogenesis in the testes (Table 2).

<table>
<thead>
<tr>
<th>No</th>
<th>Animal subject</th>
<th>Material induction (dose &amp; duration)</th>
<th>Results and References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male guineapig</td>
<td>Exposure to aluminum (300 mg AlCl3 / L) and fluoride (150 mg NaF / L) orally for 13 weeks</td>
<td>Fluoride exposure can induce a decrease in testosterone and sperm count as well as downregulation of Steroidogenesis genes such as StAR and P450scc. Al is only able to attenuate the toxicity effects of F to a certain time(^ {63})</td>
</tr>
<tr>
<td>2</td>
<td>Male mice</td>
<td>Copper sulfate pentahydrate (CuSO4.5H2O, 200 mg / kg, p.o) was given for 90 days. And three groups were given treatment with Tribulus terrestris extract (TTE) (10 mg / kg, p.o); Enalapril (30 mg / kg, p.o), and Losartan (10 mg / kg, p.o).</td>
<td>TTE and Enalapril can protect against damage to testicular steroidogenesis caused by excessive exposure to Cu, so they can be developed as prophylactic drugs of choice in the face of hypertension and testicular dysfunction(^ {64})</td>
</tr>
</tbody>
</table>
Cont... Table 2. Effects of several toxic, drug, and chemical exposures on testicular steroidogenesis in several animal

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3-month-old rams (pre-puberty)</td>
<td>Eight rams were used as control and eight others were given linseed oil (LO) (4% dry matter of total feed) for 81 days.</td>
<td>LO administration was able to increase the development of the testes (seminiferous tubules and the number of Sertoli cells) during the peri-puberty period with the expression of expressions related to steroidogenesis in the testes of rams (65)</td>
</tr>
<tr>
<td>4</td>
<td>Male golden hamster</td>
<td>Male hamsters were exposed to different photoperiod conditions, namely critical (CP; 12.5L: 11.5D); short days- (SD; 8L: 16D) and Long days- (LD; 16L: 8D) for 10 weeks</td>
<td>Photoperiod by regulating circulation and local melatonin levels and expression of the MT1R gene in the testes can enhance the steroidogenesis process to improve the reproductive status of male golden hamsters (66)</td>
</tr>
<tr>
<td>5</td>
<td>Male mice</td>
<td>Bisphenol A (BPA) exposure was given orally at a dose of 0.005; 0.5; 50; and 500 µg / kg body weight / day for 45 days.</td>
<td>BPA is able to interfere with insulin signaling and glucose transport processes in the testes of rats, resulting in impaired testicular function (67)</td>
</tr>
<tr>
<td>6</td>
<td>Male Wistar rat</td>
<td>Administration of tert-butylhydroquinone (tBHQ) (50 mg / kg bw / day) for 14 days against a single injection of Cisplatin (Cis) (7 mg / kg BW, intraperitoneal on day 8)</td>
<td>Cis triggers upregulation of NF-kB, TNF-α, IL-10, and IL-1β genes, decreased testicular germ cell proliferation, testicular steroidogenesis (expression of STAR, CYP11A1, 3β-HSD and 17β-HSD and protein), decreased stimulating hormone follicles, luteinizing, and testosterone. Cis also triggers decreased sperm count, motility, viability, morphology, and Johnsen score (68) However, induction with tBHQ is able to reduce oxidative stress by upregulating the Nrf2 gene, suppressing inflammation, apoptosis, and increasing testicular germ cell proliferation, steroidogenesis, and sperm quality.</td>
</tr>
<tr>
<td>7</td>
<td>Male mice</td>
<td>Vitamin D3 treatment in d-gal induced rats</td>
<td>Vitamin D3 can regulate testicular steroidogenic markers by increasing CYP19A1 and decreasing AR expression in the testis of old and normal mice with d-gal induction (69)</td>
</tr>
<tr>
<td>8</td>
<td>Puberty Sprague Dawley Rat</td>
<td>Dexametomidine (DEX) (0.015-1.5 µM) induction for 3 hours.</td>
<td>DEX is can inhibit the activity of steroidogenic enzymes and down-regulate the Cyp17a1 and Srd5a1 genes. An increase in ROS also occurs which causes a decrease in androgen production in immature Leydig cells in the process (70)</td>
</tr>
<tr>
<td>9</td>
<td>Male mouse</td>
<td>Male rats were induced by streptozotocin and nicotinamide (60 mg / kg + 120 mg / kg). Stevia rebaudiana Bertoni extract (400 mg / kg)</td>
<td>Decreased body weight, serum LH and testosterone levels, expression of genes associated with STAR Steroidogenesis, changes in testicular stereology, and increased levels of FBS in the diabetes group. Stevia rebaudiana Bertoni significantly increases body weight, testicular volume, sperm count, and motility and is a potential drug for the reproductive system (71)</td>
</tr>
</tbody>
</table>
Conclusion

Nearly 40% of the incidence of married couples’ infertility is caused by male factors. Steroidogenesis is an important aspect of male fertility. The important point of steroidogenesis is the transport of cholesterol as a base material for testosterone from the outer membrane to the inner mitochondria by the StAR protein. Testosterone as a result of steroidogenesis is a male sex hormone produced through steroidogenesis which functions to maintain the spermatogenesis process. Several factors can inhibit steroidogenesis, including Leydig cells, adrenal steroidogenesis defects, StAR enzyme activity, cytochrome P450, and exposure to free radicals.

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Ethical Approval: Ethical approval was not obtained to review this article because it did not involve participants, humans, or experimental animals.

Source of Funding: Self.

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Nurses’ Knowledge about MCH Services at Primary Health Care Centers in Rural Areas of Babylon Governorate - Iraq

Ibraheem Abdul-Hifid Hussein¹, Aysen Kamal Mohammed Noori²
¹MScN(C), ²Instructor, University of Baghdad, College of Nursing, Community Health Nursing Department, Baghdad, Iraq

Abstract

Background: If you truly believe that every mother and every child deserve the best possible care, whether or not the newborn comes into this world or during that time or after, that means that you are with us on the same train. The study aims to assess nurses’ knowledge about MCH; and determine the relationship between nurses’ knowledge and their demographic characteristics.

Methods: A descriptive study was used to guide this study. The study included a convenience sample of (98) nurses.

Data were collection using a self-report questionnaire. Data were analyzed using the statistical package for social science. The descriptive and inferential statistical measures were used.

The study results revealed that nurses have a moderate knowledge. Nurses’ education, years of experience, and training courses significantly correlate with their knowledge.

Conclusion: Nurses’ knowledge about maternal and childcare were moderate and their knowledge were influenced by educational level, years of experience, and training. Longer years of experiences in maternal and childcare and more training on MCH program help to increase their knowledge.

Keywords: Knowledge; Maternal and Child Health Services; Nurses

Introduction

Maternal wellbeing in most developed countries is a giant project. With the loss of 673/100,000 and 19,000 maternal deaths each year from mother’s lives, it makes the largest contribution to the death of all mothers around the world (¹). Under the country’s reproductive health coverage, maternal health is among the six most significant problem areas. A point out about the rise in women who from time to time receive maternal fitness treatment preferences. Nevertheless, the maternal health care searching for lady’s stuff to do is then again low down. One reason for terrible health penalties among women is the lack of the use of up-to-date health care providers with the assistance of a significant proportion of women in the country’s way of traveling (²). Around 1,200 girls face fatal pregnancy and birth problems every 12 months in the United States and 60 fatal problems - the cost of birth care in the United States reaches 60 billion in 2012 (³). “Globally, over 90% of births and infants die in developing countries, depending on the highest number of deaths due to population distribution. No contact or inability of maternal health priorities will increase the risk of pregnancy and childbirth problems, which may impact the high cost of maternity and death, reducing the chances of the unborn child. In Asia, the health care system does not meet the needs of women

Corresponding author:
Aysen Kamal Mohammed Noori
University of Baghdad, College of Nursing, Community Health Nursing Department, Baghdad, Iraq, Email: dr.aysin@conursing.uobaghdad.edu.iq
in healthcare. The maternal mortality rate in Asia remained inappropriately high, with more than 510 maternal deaths per 100,000 live births (4). Iraq as one of the developing countries has been subjected to some of the most complex emergencies, conflict and security situations in the world today”. Poor birth practices are the main causes behind this high rate; insufficient referral or availability of emergency obstetric care, a high level of anemia among pregnant women (35%), especially affecting rural women and those in the Central and Southern regions, and the lack of adequate health professionals and structural damage to facilities (5).

Verify the awareness, mindset, and practice of these women during the time of pregnancy about maternal and child health services. Awareness of complications of pregnancy, educational history and belief in women, providers of prenatal health care, transport and postnatal health care, and person, institutional, social and cultural qualitative data were established to determine a mean (6). Therefore, this study amid to assess nurses’ knowledge towards MCH; and determine the relationship between nurses’ knowledge and their demographic characteristics.

**Materials and Methods:**

A descriptive design was used to guide this study. The study included a convenience sample of (98) nurses who were recruited from five health sectors which are Hilla First, Hilla Second, Al-Musayyib, Al-Mahaweel, and Al-Hashimya for primary health care sector in Babylon Governorate, Iraq. Data were collected using a questionnaire constructed by the researchers which includes socio-demographic sheet and nurses’ knowledge.

**Data Analyses/Statistics**

Data were analyzed using the statistical package for social science (SPSS) for windows, version 26. The descriptive statistical measures of frequency, percent, and mean of score (MS), and inferential statistical measure “Chi-square test” was used.

**Results and Discussion**

**Table 1. Nurses’ demographic characteristics (N = 98)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Rating</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td></td>
<td>49</td>
<td>50.0</td>
</tr>
<tr>
<td>30-39</td>
<td></td>
<td>21</td>
<td>21.4</td>
</tr>
<tr>
<td>≥ 40</td>
<td></td>
<td>28</td>
<td>28.6</td>
</tr>
<tr>
<td>Mean + Sd.= 32.85+9.146</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>43</td>
<td>43.9</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>55</td>
<td>56.1</td>
</tr>
<tr>
<td>Marital Status</td>
<td>Single</td>
<td>27</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td>Married</td>
<td>65</td>
<td>66.3</td>
</tr>
<tr>
<td></td>
<td>Divorced</td>
<td>6</td>
<td>6.1</td>
</tr>
<tr>
<td>Educational attainment</td>
<td>Secondary school of nursing graduate</td>
<td>23</td>
<td>23.5</td>
</tr>
<tr>
<td></td>
<td>Diploma graduate</td>
<td>64</td>
<td>65.3</td>
</tr>
<tr>
<td></td>
<td>Bachelor’s graduate</td>
<td>11</td>
<td>11.2</td>
</tr>
<tr>
<td>Current job title</td>
<td>Technical nurse</td>
<td>20</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>Medical assistant</td>
<td>51</td>
<td>52.0</td>
</tr>
<tr>
<td></td>
<td>Skilled nurse</td>
<td>27</td>
<td>27.6</td>
</tr>
</tbody>
</table>
The descriptive statistics of the socio-demographic details of nurses are described in this table in terms of frequency and percentage. Of the (98) subjects that participated in this research.

![Graph](image)

**Figure 1. Overall Knowledge towards MCH**

Taking into account the statistical cut-off point, this figure indicates that the majority of nurses (60.2%) had moderate awareness at an average of $+ \text{Std.} = 1.68 + 0.5499$.

**Table 2. Relationship between nurses’ knowledge and their demographic characteristics**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Rating</th>
<th>Knowledge</th>
<th>Total</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Poor</td>
<td>Moderate</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>20-29 years old</td>
<td>17</td>
<td>30</td>
<td>2</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>30-39 years old</td>
<td>7</td>
<td>14</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>40 and older</td>
<td>11</td>
<td>15</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>35</td>
<td>59</td>
<td>4</td>
<td>98</td>
</tr>
</tbody>
</table>

$\chi^2_{\text{obs.}} = 2.006 \quad \chi^2_{\text{crit.}} = 9.488 \quad \text{P-value}=0.735$
<table>
<thead>
<tr>
<th></th>
<th>Gender</th>
<th>Marital status</th>
<th>Education attainment</th>
<th>Job title</th>
<th>Years of experience</th>
<th>Years of experience in PHC</th>
<th>Training course</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td>Single</td>
<td>Married</td>
<td>Divorced</td>
<td>No trained</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>18</td>
<td>35</td>
<td>10</td>
<td>25</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>33</td>
<td>59</td>
<td>17</td>
<td>36</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>55</td>
<td>98</td>
<td>43</td>
<td>65</td>
<td>6</td>
<td>52</td>
</tr>
<tr>
<td>P-value</td>
<td>0.179</td>
<td>0.186</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>$\chi^2$ obs. = 3.441</td>
<td>$\chi^2$ crit. = 5.991</td>
<td>P-value = 0.179</td>
<td>$\chi^2$ obs. = 6.187</td>
<td>$\chi^2$ crit. = 9.488</td>
<td>P-value = 0.186</td>
<td>$\chi^2$ obs. = 43.559</td>
</tr>
</tbody>
</table>

|                  | Total        |                  |                      |                  |                    |                           |                |
|                  | 35           | 17               | 0                    | 10               | 25                 | 0                         | 19             |
|                  | 59           | 33               | 4                    | 17               | 36                 | 6                         | 33             |
|                  | 4            | 4                | 0                    | 0                | 4                  | 0                         | 0              |
|                  | 98           | 52               | 32                   | 32               | 9                  | 5                         | 9              |

|                  | $\chi^2$ obs. = 12.136 | $\chi^2$ crit. = 9.488 | P-value = 0.016      | $\chi^2$ obs. = 2.835 | $\chi^2$ crit. = 9.488 | P-value = 0.586          | $\chi^2$ obs. = 8.480 | $\chi^2$ crit. = 12.592 | P-value = 0.205 |

|                  | $\chi^2$ obs. = 106.289 | $\chi^2$ crit. = 12.592 | P-value = 0.000      | $\chi^2$ obs. = 8.480 | $\chi^2$ crit. = 12.592 | P-value = 0.205          | $\chi^2$ obs. = 43.559 | $\chi^2$ crit. = 12.592 | P-value = 0.000 |

|                  | $\chi^2$ obs. = 43.559 | $\chi^2$ crit. = 12.592 | P-value = 0.000      | $\chi^2$ obs. = 8.480 | $\chi^2$ crit. = 12.592 | P-value = 0.205          | $\chi^2$ obs. = 43.559 | $\chi^2$ crit. = 12.592 | P-value = 0.000 |
“χ^2 obs.= Chi-square observer, χ^2 crit. = Chi-square critical, df = Degree of freedom, P-value= Probability value, S= significant, NS= non-significant, S= significant, HS= high significant”.

Findings indicate that there was no substantial association between maternal and childcare nursing expertise and demographic characteristics at p-value > 0.05, but that nursing education at p-value < 0.05 was significantly related. Their knowledge at p-value <0.01 has been closely correlated with years of experience and training courses.

**Discussion**

Knowledge tests, such as the one we performed in this report, may be a helpful complement to surveys on service availability and readiness evaluation to assess where and on what topics training is most needed. Where it is difficult to maintain routine training, treatment may be best concentrated in larger, better resourced facilities. If such an approach is to be considered, appropriate referral structures would need to be in place and existing overcrowding and staff shortages in larger primary health care centers in Babylon should be resolved to accommodate a larger number of customers.

Of the (98) subjects who participated in this study, their age ranged from (20-29) years of age and constituted (50 percent) of the study sample, as young workers, especially MCH, must be the primary health care staff to cover all duties. Our results come from research findings conducted at the Ibadan Primary Health Centre. The findings indicate that the majority of nurses are young adults (7).

It is evident from our findings that more than half of the study sample are female nurses. It represented (56.1 percent) of the total number of the study population, since most of the health care reviewers are women, so it includes female health workers, as in our findings. In our community, it is also normal for women nurses to be more than male nurses, since it is considered a feminine occupation by Iraqi society. These results are in line with the research conducted in Erbil City and address the expertise of nurses in primary health care. The majority of his research sample is stated to be females (59 percent) (8).

The distribution between married and single research samples. Where the proportion of married couples was the majority, the total amount accounted for 66.3 percent. It’s also, the small proportion of those divorced among those results, since most of these age groups are the marriage age. These results consist of a cross-sectional design carried out in Oman. Most of the participants were married and made up 92.0 out of 199 subjects in total. (9).

Regarding work variables, most of them medical assistants and graduates have 1-4 years of experience without training sessions, due to the diploma degree, due to the large number of institutions that graduate such degrees, the main proportion of staff nurses in health organizations are considered to be the diploma degree. The majority of nursing staff in primary health centers graduated with diplomas and lack of interest in training sessions were reported in the results of standard precautions among nurses in health facilities (10).

In order to minimize maternal and infant mortality, maternal and child health care is just as important. According to our study, the majority of nurses (60.2 percent) were moderately competent at mean + SD.= 1.68 + 0.5499. These results are in line with the findings of a study conducted in Nepal dealing with the awareness of rural areas of primary health centers in the Midwest. The results indicate that the participants were almost aware of maternal and child health due to the few years of work and discrepancies in educational levels (11). As well as serving in maternal and childcare, the Egyptian nurses wanted an effective educational program because of a lack of information about those programs (12).

“Another study also found that nurses interviewed showed a good to fair level of knowledge of maternity and routine newborn care management guidelines, but knowledge of small and sick newborn care guidelines was weaker and gaps in knowledge were reported”. Given the key role of nurses in providing day-to-day
treatment for maternal and newborn patients and the frequent shortage of specialist medical staff in the environment, it is important that nurses have appropriate expertise and skills to provide quality care (13).

In addition, nurses ranked highest for awareness of active mother management after birth and immediate routine neonatal treatment. In infant resuscitation, checking signs and symptoms of sick newborns, and controlling hypertension in pregnancy, output was worse. There was a particularly low overall awareness of treatment for sick newborns (score 0.62 of 1). Nurses who had undergone training after qualifying performed better than those who had not, in all areas evaluated. In contrast with better-resourced and busier facilities, poorly resourced and low case load facilities had lower overall information ratings (14). Several studies have shown that low resource environments have also found deficiencies in the expertise of health workers for maternal and newborn care (15)(16)(17).

At p-value <0.05, nursing education was significantly related. Their awareness at p-value <0.01 has been strongly correlated with years of experience and training courses. Nurses with a longer duration of work experience tended to understand more. Bases on findings of Al-Busaidi and others (2019), confirmed that those with less clinical nursing experience should be supported by potential educational initiatives and programs(9).

The importance of the total years of nursing experience and expertise was also established, as the knowledge increased with more years of experience. Also, Murphy and others (2019), Nurses with more years of experience and those who have undergone additional training since qualifying appeared to have higher awareness scores, confirmed in their results (14).

**Conclusion**

This research found knowledge in terms of maternal and childcare, nurses were moderately competent, and their knowledge was influenced by educational achievement, years of experience and training. More years of maternal and childcare experience and more training of MCH program workers by local officials help to increase the knowledge of nursing practitioners. Providing health services and the manipulation of young nurses’ energies, which actually helps to improve their skills.

**Conflict of Interest**: The researchers confirm that there is no any conflict of interest.

**Source of Funding**: This study is self-funded.

**Ethical Clearance**: The researchers obtained the ethical approval from the University of Baghdad, College of Nursing

**References**


Case Report: A Rare Case of Glioblastoma in Patient with HIV-AIDS

Ilham Munandar¹, M. Vitanata Arfijanto²

¹Resident Department of Internal Medicine Dr. Soetomo Hospital – Faculty of Medicine Airlangga University, Surabaya Indonesia, ²Doctor at Division of Tropical and Infection, Department of Internal Medicine, Dr. Soetomo Hospital – Faculty of Medicine Airlangga University, Surabaya Indonesia

Abstract

Patients with HIV_AIDS have an increased risk to develop neurological disorders include a complication of intracerebral mass. Primary CNS tumors in this condition are rare and difficult to diagnose because it has uncommon presentation, unusual tumor growth and manifests at a young age in a patient with HIV-AIDS. Advanced imaging techniques with contrast-enhanced magnetic resonance scans should be used to guide diagnosis in this condition. in a patient with HIV-AIDS, biopsy should be carried out if standard imaging showed atypical features or in a patient who has a poor response to empirical treatment for neurotoxoplasmosis. In this case, we reported a case of A 26 years old male with HIV-AIDS with neurological deficits who later diagnosed with glioblastoma.

Keywords: HIV-AIDS, Glioblastoma, Glioma.

Introduction

Patients with HIV-AIDS are at risk to develop either infectious or non-infectious complications. Neurological disorder occurred in up to 60% of patients with AIDS and 10% of patients have an intracerebral mass as a complication of its condition.

Glial central nervous system (CNS) malignancy found in 0.05 percent of all CNS tumors, and although this tumor recognized as the most common primary CNS tumors, it is very uncommon to found in the patient with HIV-AIDS and very difficult to diagnosed because they present with uncommon features of tumor localization and unusual tumor growth as well as presenting at a younger age in a patient with HIV-AIDS. Incidence of glioblastoma in HIV-AIDS patients increasing mortality risk that already substantially high in those populations. (1, 2)

This case report discussing a rare case of new diagnosed young HIV-AIDS patient with a glioblastoma who has neurologic deficit as initial manifestation.

Case Description

A 26 years old male was admitted to the emergency department of RS Dr. Soetomo, Surabaya, Indonesia because of loss of consciousness 2 days before admitted to the hospital. Heteroanamnesis from other family members revealed that initial symptoms developed fluctuating. This complaint is accompanied by limb weakness but no complaint of facial palsy. History of projectile vomit was denied but there was a history of prolonging headache and febrile. In the last 1 month, the patient frequently complained of fever that gone with over-the-counter medicine but denied any history of recurrent oral ulcer, profuse diarrhea, weight loss, or history of swollen skin or tumor. There was no history of hypertension or diabetes mellitus.

In the last two days, the patient started to feel shortness of breath with an occasional cough. There was no history of head trauma. The patient was admitted to the emergency department and then tested positive for HIV/AIDS. Head imaging showed a brain tumor and the patient was offered to undergo a surgical removal but the family denied any surgical approach. The patient...
worked as a metal worker and is currently not married with limited social interaction. The family didn’t know about a history of sexual activity but denied a history of alcoholism or drug abuse.

From the physical examination, the patient was somnolent with Glasgow Coma scale E2 V3 M4, the vital sign was blood pressure 11/70 mmHg, heart rate 96 beats per minute, respiratory rate 24-26 times per minute, body temperature was 37.2o. On head examination, the patient was dyspneic but was not anemic, not icteric, oral thrust and there was no lymphadenopathy. Chest examination was symmetric, with normal heart sound and crackle on pulmonary examination. Abdomen examination within normal limit without liver or spleen enlargement. There was no edema on extremity.

Laboratory examination was Hb 11.3 g/dl, HCT 32.1 %, MCV 81.5 fl, MCHC 35.2 g/dl, leukosit 7600 /mm³, neutrophil 85.3 %, lymphocyte 6.2 % mmol/l platelet 229.000/mm³, serum creatinin 0.43 mg/dl, BUN 13.0 mg/dl SGOT 57 U/l, SGPT 63 U/L, serum albumin 3.8 g/dl, Sodium 128 mmol/l, Potassium 3.8 mmol/l, Chloride 91 mmol/l, PPT 14.2 seconds dan APTT 28.8 seconds with non reactive HbsAg and anti-HCV. Blood gas analysis results was pH 7.45, pCO2 33 mmHg, pO2 131 mmHg, TCO2 23.9 mmol/L, base excess -1.1 mmol/l, HCO3 22.9 mmol/L, and SO2 99%. 3 methods HIV test was 3 positives and CD4 was 5 cells/µL (1.05%).

Chest radiography showed a diffuse pulmonary infiltration and normal cardiac imaging Head CT imaging with contrast showed a rim enhancing solid lesion 2.5x2.5x3.2 cm in internal capsule with an irregular inner wall and perifocal edema caused by narrowing down of right lateral ventricle dan midline shift to the left up to 1.9 cm suggesting a primary brain tumor (Glioblastoma). Later, evaluation with contrast-enhanced MRI of the head showed peripheral enhancing intra-axial, supratentorial lesion with hemorrhaging part and a necrotic area within internal capsule suggesting a glioblastoma.

The patient was assessed as HIV-AIDS, glioblastoma multiforme, suspected Pneumocystis Carinii Pneumonia, oral candidiasis, and mild hyponatremia. Patient treated with liquid diet 200 ml every 4 hours, IVFD NaCl 0.9% 1500 ml every 24 hours, 02 on a nonrebreathing mask 10 liters per minute, Ceftriaxone intravenously 1000 mg every 12 hours, Dexamethasone intravenous 5 mg every 6 hours tapered down based on clinical improvement, cotrimoxazole 960 mg every 12 hours, paracetamol tablet 500 mg every 6 hours and nystatin 2 drips every 12 hours. Laboratory examination planning was blood gas analysis evaluation, electrolyte serum, LDH serum, and smear sputum gram and culture and sensitivity of sputum. The patient denied to surgical approach for the tumor. 2 days after admission, the patient died in the hospital because of respiratory failure.

Discussion

HIV infection/AIDS is a global pandemic, with cases reported from virtually every country. It is estimated that 95% of people living with HIV/AIDS live in low-and middle-income countries; 50% are female, and 3.2 million are children aged under 15 years.(3)

Concerning HIV and malignancy, A study showed that HIV patients had a 5.4-fold higher incidence of malignancy compared to the general population. Even after removing the incidence of Hodgkin’s disease in the population with HIV-AIDS, there was still a 3.4-fold increase in the risk of malignancy. This includes an elevated risk of brain tumors, though not all of these were confirmed histologically.(4) Between 1986 and 1998, the population of patients with HIV-AIDS seen at Memorial Sloan-Kettering Cancer Center had a 45-fold higher risk to develop glioblastoma than normal patient.(5)

Glioma is a broad term used for primary brain tumors that are categorized based on their suspected cell of origin, for example, astrocytic tumors, ependymomas, oligodendrogliomas, and mixed gliomas. Account for almost 80% of all malignant primary tumors, it becomes the most common tumors of the central nervous system with glioblastoma multiforme as the most malignant and most frequent type of primary astrocytoma. Glioblastoma
was called glioblastoma multiforme because it varies in size and shape, but that term has not used nowadays. This type of tumors has a feature as highly invasive and infiltrating in brain parenchyma but typically limited in the central nervous system.\(^6, 7\)

Some studies to link this disease to particular environmental and occupational exposures still inconclusive and underpowered. Ionizing radiation is one of the few recognized risk factors for glioma progression that has been proven. Ionizing radiation is one of the few recognized risk factors as seen years after radiation indicated for therapeutic for another tumor or condition.\(^8\) Some specific genetic diseases are suspected to increase the risk of glioma development such as neurofibromatosis, tuberous sclerosis, Li-Fraumeni syndrome, and Turcot syndrome, but Glioma patients with a proven genetic disease account for fewer than 1% in all cases.\(^9\) Glioblastoma has not been confirmed to be caused by other environmental factors such as smoking, synthetic rubber, electromagnetic field, or nonionizing radiation from cell phones.\(^10\)

Gliomas develop from the malignant transformation of neuroectodermal-derived supporting cells and are presumed to link with mutation of tumor suppressor gene.\(^11\) The immunocompromised condition of the patient with HIV/AIDS may facilitate the development of neoplasms, as seen in primary cerebral lymphoma and Kaposi sarcoma.\(^12\) Evidence of interplay between immune system and pathogenesis of glioma in large epidemiological studies showed that patients with a history of the allergic disease have a lower risk for developing glioma, suggesting that a stronger immune system could be linked to a more effective intracranial response against certain neoplasm. Otherwise, immunosuppression caused by immunosuppressive drug therapy has been closely linked to a rise in the incidence of intracranial gliomas in organ transplant recipients.\(^1\)

Some of the hypotheses associated with an increased risk of patients with HIV-AIDS for to develop brain tumor were associated with HIV gene regulators such as \textit{nef} that alter astrocyte growth and morphology similar to neoplastic transformation in vitro studies. Besides, HIV infection also induces the secretion of proinflammatory cytokines such as IL-1, II-6, IL-8, and TNF alpha which can facilitate the development of glioblastoma.\(^1, 13\)

Initial symptoms of a patient with glioblastoma may vary depending on the size, location, and anatomic structure involved in the brain. The majority of typical symptoms are caused by increased intracranial pressure, such as headache and focal or progressive neurologic deficits.\(^14, 15\) Three mechanisms that account for glioblastoma signs and symptoms are the direct effect of the tumors that resulted from damage in brain tissue, by secondary effect of increased intracranial pressure, and symptoms related to specific tumor location.\(^6\) Concerning HIV-AIDS, a case report by Hall and Short showed most patients with HIV /AIDS who developed Glioblastoma were young (mean age is 38 years) with a CD4 count mean 400 cells/mm.\(^16\)

Glioblastoma in a patient with HIV-AIDS is difficult to diagnose because of its uncommon features of tumor localization, unusual tumor growth, and presence at a younger age in a patient with HIV-AIDS.\(^2\) referred non-invasive imaging techniques for brain tumors are MRI scans to visualizing the tumors. MR scans are the gold standard imaging because of their superior soft-tissue contrast to better visualization of complexity and heterogeneity of tumor lesion. CT scans are often recommended where a patient cannot perform an MR scan for several reasons, such as patients with pacemakers. T1–weighted MR scans can visualize hypointense lesions whereas proton density-weighted and T2-weighted images visualize hyperintense lesions. Gadolinium-enhanced MR scan of patients with glioblastoma typically shows a central area of necrosis, surrounded by white matter edema. Tumors are usually unifocal but can be multifocal. Latest imaging techniques with MR can also help to detect the hemodynamic changes, lesion architecture, and even cellular metabolism of tumors. This technique can be used to differentiate between active tumor or treatment effect on tumor itself.\(^7, 17\)
Figure 1. Patients head MRI with contrast showed a peripheral enhancing intra-axial, supratentorial lesion with hemorrhaging part and the necrotic area within the internal capsule suggesting a glioblastoma.

For intracerebral mass in a patient with HIV-AIDS, most protocols also recommend biopsy as the next diagnostic step if image features are atypical for neurotoxoplasmosis or after administration of empirical treatment not improved patient condition after 2 weeks. Current therapeutic options for glioblastoma in a patient with HIV-AIDS are similar to another patient, include surgery followed by radiotherapy and chemotherapy (temozolomide). The efficacy of specific antitumor therapies ultimately determines symptomatic treatment relief, but corticosteroids may temporarily help relieve neurologic symptoms caused by peritumoral edema.

Surgical resection should be considered in glioblastoma to reduce the mass effect, cytoreduction, and diagnostic procedures such as histologic and molecular characterization, even in suspected low-grade glioma to provide more reliable tumor grading. However, glioblastoma is known as a very invasive tumor that may relapse in approximately 80% of cases.

Radiotherapy following surgical resection has been shown to improve life expectancy, therefore this approach should be considered in all patients with glioblastoma. To improve patient survival, several chemotherapeutic agents have been tested for the treatment of glioblastoma. Temozolomide and biodegradable polymers containing the alkylating agent carmustine, implanted into the tumor bed after tumor resection used to improved survival glioblastoma. Because glioblastoma express a high level of vascular endothelial growth factors (VEGFs), protease inhibitor preferred to use in HIV-AIDS treatment since it promoted inhibition of VEGF and decreased angiogenesis.

In terms of prognosis, whether the tumor was a contributing factor for the accelerated progression to HIV-AIDS or, conversely, whether HIV-AIDS led to the faster growth of a tumor is still being studied. Despite a multimodal treatment, the prognosis is still grim and mostly determined by tumor progression.
Conclusion

It has been reported a young male recently diagnosed with HIV-AIDS with glioblastoma. The patient later died because of respiratory failure because of PCP. Because the increased incidence of non-AIDS-defining malignancies in patients with HIV-AIDS, it is important to include glioblastoma in the differential diagnosis of intracranial lesions, especially in patients presenting with neurological symptoms.

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References


Sensitivity and Specificity of a COVID-19 Lateral Flow Immunoassay Compared to RT-PCR in Pregnant Women at Arifin Achmad General Hospital Pekanbaru, Indonesia

Imelda EB Hutagaol1,2, Muhamad Yusuf1,2, NickoPisceki Kusika3,4, Suyanto5, Shinta Puja Tilusari6,7
1Consultant, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Riau, Pekanbaru, Riau, Indonesia, 2Consultant, Department of Obstetrics and Gynecology, Arifin Achmad Hospital, Pekanbaru, Riau, Indonesia, 3Assistant Professor, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Riau, Pekanbaru, Riau, Indonesia, 4Assistant Professor, Department of Obstetrics and Gynecology, Arifin Achmad Hospital, Pekanbaru, Riau, Indonesia, 5Assistant Professor, Department of Public Health, Faculty of Medicine, University of Riau, Pekanbaru, Riau, Indonesia, 6Resident, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Riau, Pekanbaru, Riau, Indonesia, 7Resident, Department of Obstetrics and Gynecology, Arifin Achmad Hospital, Pekanbaru, Riau, Indonesia

Abstract

Coronavirus disease 2019 (COVID-19) is significant global treat including to pregnant woman in Indonesia. A point-of-care diagnostic tool that is able to early diagnose and has a good sensitivity and specificity is critical during the pandemic. The aim of this study was to describe the clinical, laboratory and outcomes of pregnant women with presumptive COVID-19 and to compare the sensitivity and specificity between COVID-19 rapid test (lateral flow immunoassay) and real-time reverse transcription polymerase chain reaction (RT-PCR). Pregnant women with presumptive COVID-19 symptoms were recruited at Arifin Achmad General Hospital between April 2020 and December 2020. Demographic and clinical data were collected and patients were tested with COVID-19 rapid test as well as RT-PCR. Analyze was conducted to determine the sensitivity and specificity. We included 120 patients with presumptive COVID-19 of which 48 (40%) and 72 (60%) of patients were reactive for IgM and IgG antibody anti severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), respectively and 51 (42.5%) were reactive for both IgM and IgG. The sensitivity and the specificity of rapid test was 64.0% and 33.3%, respectively. The positive and negative predictive value was 61.5% and 35.7%, respectively. In conclusion, lateral flow immunoassay-based rapid test has relatively low sensitivity and specificity in diagnosing COVID-19 in pregnant women.

Keywords: Sensitivity, specificity, rapid test, RT-PCR, COVID-19

Introduction

On December 2019, coronavirus disease 2019 (COVID-19) cases, cause by a new coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), were firstly reported in Wuhan, China. World Health Organization (WHO) then declared COVID-19 as pandemic on March 11 2020. SARS-CoV-2 is a single-stranded ribonucleic acid (RNA) virus. The virus is able to survive at least for 12 hours on plastic surface, sensitive to sunlight and heat, easy to be inactivated with lipophilic substance such as ethanol and disinfectant contained chlorine, peroxyacetic acid and chloroform. The incubation of SARS-CoV-2 is about 5 to 7 days and is transmitted through droplet from symptomatic

Corresponding author:
Dr. NickoPisceki Kusika
Department of Obstetrics and Gynecology, Faculty of Medicine, University of Riau
Jl. Diponegoro No.1, SukaMulia, Kec. Sail, Kota Pekanbaru, Riau 28133, Indonesia
Email: pisceski@lecturer.unri.ac.id
nicko.pisceki@lecturer.unri.ac.id
persons.\textsuperscript{2,3,4} The natural reservoir of SARS-CoV-2 is bats; however, some evidence suggested that weasel or raccoon are also potential natural hosts.\textsuperscript{5}

COVID-19 causes an acute respiratory syndrome result in death which clinical and laboratory findings are similar between pregnant and non-pregnant woman. Pneumonia is the main finding in thorax CT-scan in patients with COVID-19 and some of frequent complications are acute respiratory distress syndrome (ADRS) and disseminated intravascular coagulopathy.\textsuperscript{2,5} A study in New York found that pregnant woman with COVID-19 were more likely to be hospitalized, required intubation, mechanical ventilation, and admitted to the intensive care unit (ICU) than non-pregnant woman.\textsuperscript{2} This is mainly because of the physiologic changes during pregnancy, such as decrease functional residual capacity, increased diaphragm, edema of respiratory mucosa, and change of immune system lead to increased susceptibility to virus infection.

In Indonesia, the first COVID-19 cases were identified in the capital Jakarta in March 2, 2020. As of March 26, 2021 more than 1.4 million cases and more than 40,000 deaths have been reported.\textsuperscript{6} COVID-19 has caused multisectoral disruptions including economics, healthcare system including children vaccination.\textsuperscript{7,8} Riau is one of the provinces in Indonesia with the highest COVID-19 cases.\textsuperscript{9} In Indonesia, COVID-19 rapid test is a screening tool that mainly to early detect COVID-19 and the result of rapid test will be confirmed with real-time reverse transcription polymerase chain reaction (RT-PCR). In the early of the COVID-19 pandemic, multiple brands of rapid test are available in the country with relatively limited in sensitivity and specificity assessments in the population. The aim of this study was to determine the sensitivity and specificity level of COVID-19 rapid test in comparison to gold standard RT-PCR in pregnant women.

**Methods**

A cross-sectional study was conducted from April 2020 to December 2020 at Arifin Achmad Hospital, Pekanbaru, Riau, Indonesia. Pregnant women with presumptive COVID-19 were tested with COVID-19 rapid test. In the first visit, data of demographic, exposure to COVID-19 cases and clinical data were collected. The COVID-19 rapid test was Healgen COVID-19 IgG/IgM Rapid Test Cassette (Healgen Scientific LLC, Houston, TX, USA). This kit detects both IgM and IgG antibodies to SARS-CoV-2 from whole blood serum or plasma and the test was conducted per manufacture protocol.

The patients with reactive for IgG/IgM rapid test then were re-tested with RT-PCR targeting at least two gene of SARS-CoV-2 using available kits at Arifin Achmad General Hospital Riau Indonesia (mainly using LiliF COVID-19 Real-time RT-PCR(iNtRON Biotechnology, Gyeonggi-do, Korea) or Live Rifer Novel Coronavirus COVID-19 (2019-nCoV) Real-Time Multiplex RT-PCR Kit (Shanghai ZJ Bio-Tech, Shanghai, China)). The samples for RT-PCR were nasal and oropharynx swabs. All run of RT-PCR were conducted per manufacture protocol.

The patients were also followed and data of outcome, time of stay in the hospital, intrapartum treatments or procedures were collected. The RT-PCR results of the new born baby was also collected. The sensitivity, specificity, positive prediction value (PPV) and negative prediction value (NPV) of rapid test were calculated.

**Results**

We included 120 patients with presumptive COVID-19. Characteristics of demographic, clinical, laboratory and outcomes of the patients are presented in Table 1. Almost 45% of the patients aged between 26–30-year-old (Table 1). There were 48.3% patients who were housewife, 28.3% general employees and 23.3% civil servants. More than two-third of the patients (76.6%) had caesarean section and only 13 (10.8%) had normal vaginal delivery. Our data suggested that less than 10% of the total patients had at least one of COVID-19 comorbidity.

Based on IgM and IgG antibody rapid test, 48 (40%) and 72 (60%) of patients were reactive for IgM and IgG anti-SARS-CoV-2, respectively (Table 1). Out of total
patients, 51 (42.5%) patients were reactive for both IgM and IgG anti-SARS-CoV-2.

Table 1. Characteristics of demographic, clinical, laboratory and outcomes of pregnant women with COVID-19 (n=120)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Details</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td>Level of education</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bachelor</td>
<td>24</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Senior high school</td>
<td>52</td>
<td>43.0</td>
</tr>
<tr>
<td></td>
<td>Junior high school</td>
<td>32</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td>Elementary school</td>
<td>12</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Age (year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18-25</td>
<td>17</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>26-30</td>
<td>52</td>
<td>43.3</td>
</tr>
<tr>
<td></td>
<td>31-35</td>
<td>29</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>36-40</td>
<td>1</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td>41-45</td>
<td>3</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Housewife</td>
<td>58</td>
<td>48.3</td>
</tr>
<tr>
<td></td>
<td>Civil servant</td>
<td>28</td>
<td>23.3</td>
</tr>
<tr>
<td></td>
<td>General employee</td>
<td>34</td>
<td>28.3</td>
</tr>
<tr>
<td>Clinical</td>
<td>COVID-19 screening score</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Less than 6</td>
<td>54</td>
<td>45.0</td>
</tr>
<tr>
<td></td>
<td>6-10</td>
<td>66</td>
<td>55.0</td>
</tr>
<tr>
<td></td>
<td>Hada fever (38oC) or had fever in 2 last weeks</td>
<td>12</td>
<td>10.0</td>
</tr>
<tr>
<td>Intrapartum management</td>
<td>Conservative</td>
<td>8</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>Vaginal delivery</td>
<td>13</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>Caesarean section</td>
<td>92</td>
<td>76.6</td>
</tr>
<tr>
<td></td>
<td>Vacuum extraction</td>
<td>5</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Un-hospitalized on own request</td>
<td>2</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Obstetric care’s length</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 day</td>
<td>10</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>2 days</td>
<td>13</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>3 days</td>
<td>97</td>
<td>80.8</td>
</tr>
<tr>
<td>Exposure</td>
<td>Direct contact with COVID-19 case in last 2 weeks</td>
<td>16</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>In the same room with a COVID-19 case within 1-2 meter for more than 15 minutes</td>
<td>20</td>
<td>16.6</td>
</tr>
<tr>
<td>Comorbidity</td>
<td>Had at least one comorbidity (diabetes, hypertension, heart disease, stroke, TBC, or cancer)</td>
<td>10</td>
<td>8.3</td>
</tr>
</tbody>
</table>
Table 1. Characteristics of demographic, clinical, laboratory and outcomes of pregnant women with COVID-19 (n=120)

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Immunology test</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM reactive</td>
<td>48</td>
</tr>
<tr>
<td>IgG reactive</td>
<td>72</td>
</tr>
<tr>
<td>IgG and IgM reactive</td>
<td>51</td>
</tr>
<tr>
<td>Swab RT-PCR</td>
<td></td>
</tr>
<tr>
<td>RT-PCR positive</td>
<td>52</td>
</tr>
<tr>
<td>RT-PCR negative</td>
<td>68</td>
</tr>
<tr>
<td>Total baby with positive swab test</td>
<td>4</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Sensitivity, specificity, positive prediction value (PPV) and negative prediction value (NPV) of rapid test compared to RT-PCR in pregnant women (n=120)

<table>
<thead>
<tr>
<th>Rapid test</th>
<th>RT-PCR</th>
<th>PPV / NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVID-19 (+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>48</td>
<td>61.5% (PPV)</td>
</tr>
<tr>
<td>Non-reactive</td>
<td>27</td>
<td>35.7% (NPV)</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>COVID-19 (-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Non-reactive</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Sensitivity / Specificity</td>
<td>64.0% (Sensitivity)</td>
<td>33.3% (Specificity)</td>
</tr>
<tr>
<td>PPV / NPV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We compared the sensitivity and specificity of rapid test with RT-PCR. Our data suggested that the sensitivity and specificity of COVID-19 rapid test was 64.0% and 33.3%, respectively (Table 2). The positive prediction value (PPV) and negative prediction value (NPV) of rapid test compared to RT-PCR was 61.5% and 35.7%, respectively.

**Discussion**

RT-PCR as a gold standard has a high sensitivity and specificity in detecting the presence of SARS-CoV-2 in specimens although in very small quantities. However, it requires high-skilled staff as well as high standard laboratory. Therefore, easy to use and cheap point-of-care diagnostic modality is critical during the COVID-19 pandemic particular in low- and middle-income countries. One of the rapid tests developed during the pandemic was lateral flow immunoassay that detects and differentiate IgM and IgG antibodies anti-SARS-CoV-2. We tested one of the lateral flow immunoassay rapid tests developed by Healgen Scientific LLC, Houston, TX, USA in pregnant women. Our data indicated that the sensitivity and specificity of COVID-19 rapid test was 64% and 33.3% with 61.5% and 35.7% PPV and NPV, respectively. The data suggest that the COVID-19 rapid test has relatively low power in diagnosing COVID-19 in pregnant women.

There are some possible reasons of low sensitivity and specificity of lateral flow immunoassay found in this study. Some of the patients in this study probably in the early phase of infection since the sensitivity of this kit for those in their early infection is unknown and negative.
results do not preclude acute SARS-CoV-2 infection. In addition, faults during nasal and oropharyngeal swab procedure could also be reasons since inadequate amount of virus could cause negative results during the rapid test. Some of the samples in this study were processed in virus inactivated viral transfer media and inactivation of the virus might affect the test results of rapid test. In addition, false positive results may occur due to cross-reactivity from pre-existing antibodies against other viruses.

**Conclusion**

The sensitivity and specificity of IgG and IgM lateral flow immunoassay in diagnosing COVID-19 of pregnant women at Arifin Achmad Hospital was 64% and 33.3%, respectively with PPV and NPV was 61.5% and 35.7%, respectively. Apart from sampling time and cross-reactivity, some other factors related to personnel and instrumental issues might associate with the results.

**Acknowledgement:** We would like to thank all patients and to the management of Arifin Achmad Hospital, Indonesia.

**Ethical Clearance:** The study protocol was approved by Arifin Achmad Hospital, Indonesia.

**Source of Funding:** Self

**Conflict of Interest:** Nil

**References**


Varicella Pneumonia in an Elderly Patient

Isidro Lumanpauw¹, Usman Hadi²

¹Resident, Internal Medicine, Faculty of Medicine, Airlangga University – Dr. Soetomo Academic General Hospital, Surabaya, Indonesia. ²Lecturer, Department of Internal Medicine, Faculty of Medicine, Airlangga University – Dr. Soetomo Academic General Hospital, Surabaya, Indonesia

Abstract

Varicella pneumonia is a common complication in adults, while it is a mild and self-limiting disease in children. Elderly patients may have severe manifestations compared to adults due to the weakening of their immune system in clearing the virus. Treatment with intravenous acyclovir and varicella-zoster immunoglobulin (VZIG) offers a good prognosis, while the use of steroids is still debated. Despite standard management, our case presents a fatality in an older woman with varicella pneumonia.

Keywords: Varicella pneumonia, ARDS, septic shock, elderly, Indonesia

Introduction

Varicella is a highly contagious disease with the airborne transmission. Adults have more chance to develop severe cases affecting multiorgan manifestations of which most common is liver and lung involvements[1]. Studies in England and Wales showed a death rate of 4-9 per 100,000 population, 80% more are adults[2,3]. Epidemiology study reported tropical countries to yield less immunity to varicella in adult general populations, with only 42-76% being immune[4]. With the elderly being susceptible to infection compared to healthy adults, more severe forms of complications might occur and potentially lethal[5]. It is imperative to encourage vaccination in the elderly who has not contracted varicella before as a preventive measure.

Case Report

A 65 years old female presented to us with a chief complaint of cough and shortness of breath. The patient also had a fever for three days, diffuse skin rash turning into fluid-filled vesicles for five days, nausea, and abdominal pain for one day before hospitalization. As an elderly, she was independent without known comorbid, and her grandchild was discharged two weeks prior due to varicella. The patient had no history of varicella infection nor vaccinated before.

Vital signs on her admission was as follow: BP 110/70, tachycardia 120 beats/min, tachypnea 32 times/ mins, fever at 38.7°C, pulse oximetry at 89% under high concentration mask at 12 L/m, bilateral basal rhonchi on auscultation with diffuse papulovesicular rash on her skin. The arterial blood gas before intubation and mechanical ventilation was as follows: pH 7.42, PaO₂ 74, PaCO₂ 27.9, BE 3.4, SO₂ 95% and after pH 7.36, PaCO₂ 42, pO₂ 108, HCO₃ 24.2, BE -1.4 SO₂ 98%. The laboratory showed hemoglobin 15 g/dL, leukocytosis 16160/µL, granulocyte 62.9%, lymphocyte 15.6%, thrombocyte 101000/µL, random blood glucose 98 mg/dL, albumin 3.28 g/dL, CRP 165 mg/L, AST 550 U/L, ALT 441 U/L, mildly increased direct bilirubin 1.27 mg/dL, SC 1.14 mg/dL, BUN 17 mg/dL and normal APTT 25.2 (23.5) and PPT 10.6 (10.9).

She was admitted to an isolation intensive care unit with an initial diagnosis of acute respiratory distress syndrome (ARDS) due to varicella pneumonia dd severe bacterial pneumonia with hepatic dysfunction due to varicella infection. Treatment of 800mg/8h intravenous acyclovir was started along with levofloxacin 750mg/24h and methylprednisolone 62.5mg/8h. Due to unavailability, VZIG was administered on the 2nd day of care. Tzanck smear showed multinucleated
giant cells. Abdominal ultrasound showed mild hepatic enlargement, the serologies viral hepatitis and HIV were negative.

Her chest X-ray showed worsening infiltrate. Leukocyte count was increased to 19,110/μL, granulocyte 80.5%, AST and ALT were lowered to 130 U/L and 104 U/L, direct bilirubin was normal 0.4 mg/dL, and lactate 1.9 mmol/L on the 3rd day. During the care we were able to reduce the FiO2 down to 0.6 from the initial 1.0 and the positive end-expiratory pressure (PEEP) at 12 down to 6 cmH2O with SIMV mode to maintain oxygenation. Later on that day, she had a decline in her condition, and vasopressor was started. On the 4th day, she passed away due to septic shock. Sputum and blood culture came back negative both on the 1st and 3rd day.
**Discussion**

Varicella or chickenpox is an airborne disease caused by the VZV. Despite primary infection usually occurring in childhood, adult cases are often seen with more severe manifestations. Symptoms usually begin 10-21 days after exposure\(^6\). The diagnosis of varicella is usually made by generalized vesicular skin rashes manifestation along with a history of exposure. IgM anti-VZV can be used to confirm the infection, but it may be negative on early infections\(^7\). Tzanck smear is a quick, accurate, and useful addition in confirming varicella diagnosis. Microscopically, multinucleated giant cells with or without intranuclear inclusion bodies can be seen\(^8\). The onset of symptoms, in this case, was around 9-15 days. The diagnosis of varicella was obvious by the skin manifestation and history of contact supported by Tzanck tests done showing multiple nucleated giant cells.

A previous study mentioned common complications in varicella infection are raised ALT, thrombocytopenia, pneumonia, secondary skin infection, and meningitis\(^1\). Pulmonary symptoms, which are shortness of breath, cough, pleuritic chest pain, and hemoptysis, may appear 1-6 days after the onset of the rash\(^9,10\). Hepatic involvement in varicella showed symptoms of vague epigastric pain, nausea, vomiting, and a possible increase in liver volume\(^11\). An increase in liver function test panel is expected, a couple of studies reported AST raised higher than ALT along with the increase of INR and bilirubin\(^12,13\). Risk factors for complications in varicella are newborns, adults, immunocompromised, males, smokers, and pregnant women. While references are not mentioning the elderly as immunocompromised, immunosenescence in the elderly causes susceptibility to infection due to thymic involution, altered innate, and adaptive immunity, which would result in more severe infection\(^5\). In this case, we suspected the infection had caused the liver involvement, excluding the cause of ischemic hepatitis and viral hepatitis.

Radiologic chest X-ray features of varicella pneumonia are multiple bilateral nodules 5-10mm with unclear margin and can be overlapped but not always present. High-resolution CT-Scan might enhance the feature along with ground glass halo appearance, which diffusely spread on both lungs. Lymphadenopathy, reticular opacity, and pleural effusion can be found on occasion. On recovery progress, calcification can be found\(^14\). In this case, we found only infiltrate on both lungs.

Intravenous acyclovir 10mg/kg/8h for 7-10 days is the standard treatment of severe varicella and should be started immediately after diagnosis is made. After 48 hours of rash onset, there is no evidence of the benefit of acyclovir\(^15\). The role of corticosteroid is still debated and is thought to alter the uncontrolled immune host response to the virus by inhibiting T cells function and neutrophil adherence to epithelial cells\(^16\). Some studies showed clinical improvement, but some mentioned at risk of superinfections\(^16,17,18,19\). Reports showed that use of steroids prior varicella infection might cause fulminant hepatitis\(^12,13\). Nevertheless, the use of steroids remains a clinical decision\(^19\). Our decision to add another antibiotic and stop the steroid was based on clinical judgment regarding the occurrence of bacterial superinfection. The increase of leukocyte and granulocytosis might be attributable to the use of the steroid. We found a reduction level in our liver test functions on the 3rd day with the use of high dose steroid in our initial therapy.

The use of VZIG for severe varicella may be useful if administered 72 hours after exposure and indicated for immunocompromised patients, seronegative VZV antibody, and after significant exposure to individuals with varicella\(^16\). A study showed severe varicella mortality is attributed to ARDS, MODS, septic shock, and fulminant hepatitis. Multiple organs involvement increases mortality and morbidity\(^18\). In this case, acyclovir and VZIG administration were way past the guidelines recommendation and resulted in unfavorable outcomes in this patient. Adults in Indonesia are presumed to be immune to varicella because most have contracted it in childhood. Hence, society often forgets the importance of vaccination, especially in the elderly.
Conclusion

We reported a fatal case of an elderly patient with no comorbidities with several complications due to the infection. Immunosenescence in the elderly may be the cause of this severe infection due to the weakening of the immune system in eliminating the virus causing severe complications. Vaccination is encouraged in the elderly who has no prior history of primary varicella infection and has not been vaccinated before to avert severe manifestation of the disease.

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References


A Review on the Detection of Skull by Forensic Analysis

J. Tracy Tina Angelina1, Bathula Murali Krishna2, A.R. Revathi3, S.R. Preethi4

1Scientist, Department of Microbiology, Biomedical Technology, K.J. Research Foundation, Chennai, 2Assistant Professor, Department of Electronics and Communication Engineering, Koneru Lakshmaiah Education Foundation (KL Deemed to be University), Vaddeswaram, Andhra Pradesh, 3Associate Professor, Department of IT, 4Assistant Professor, Department of ECE, SRM Valliammai Engineering College, Chennai

Abstract

Skull identification is extremely important in today’s society, especially in medico-legal circumstances. As a result, all technological advancements in this field can contribute to the growing need for precise and robust tools that allow for the establishment and verification of human identity. Identification of skulls when all other proof and evidence are destroyed or limited requires the usage of facial reconstruction for the forensic team. The facial graphics serve as an emphasis for public attention to specifics of the case to generate additional investigative guidance. The physical appearance of the face is at its best a significant cause for the purposeful identification and communication to the investigating authorities of knowledge. Human identification is one of the most remarkable approaches in the field of forensic medicine. The identification mission is performed in the field of forensic anthropology by the examination of the skeletal remains. Anthropologists have been paying attention to improve the techniques that allow accurate and clearer identification. Therefore, forensic identification has become an active field of study and skull detection has emerged as a vital source for identification. Skull identification is attracted and applied in many forensic areas. This review article is a summation of the various facial reconstruction approaches and their role in forensic science to identify the individual.

Keywords: Craniofacial superimposition (CFS), Forensic Identification, Skull Detection, Facial Approximation, Forensic Anthropological Analysis

Introduction

Anthropologists advocated forensic application in the early days. In the early 1990s, computerized face alignment approaches were initiated. The term “Facial Approximation” was used as a synonym for “Facial Reconstruction.” The morphological and anatomical correlations of the skull and face have been primarily studied in various fields of research such as maxillofacial and plastic surgeries, genetics, and prominently in the craniofacial reconstruction area. Therefore, several decades of consistent landmarks were identified in both the skull (craniometric) and face (cephalometric).

Numerous studies were conducted using data from different populations and techniques that measured the soft tissue depth between the relevant landmarks.

Egyptians implemented several measures to preserve many details of their ancestors. In the late 19th century, the similarity of the surface soft tissues of the face and the skull’s underlying ossic structure started to be studied by anatomists and anthropologists, and forensic odontologists. Modern times have seen the emergence of facial reconstruction to assist archaeologists in demonstrating the appearance of the early person. Recently, forensic science play a significant role in producing an image from the skull. For hundreds and millions of years, skulls can exist and offer incomparable means of identification. Identification will be needed if a skull is unintentionally restored from a backyard, forest,
etc. Conventional methods such as dental examination, radiography, DNA analysis, etc, can neither be employed nor have been unsuccessful. Forensic facial reconstruction can be applied as a major tool to aid in skull identification and to identify the individual³.

Skeleton-Based Forensic Human Identification

When other technologies fail, forensic facial reconstruction can be used to identify unknown human remains⁴. Numerous techniques are available for facial reconstruction, ranging from 2D graphics to 3D models. With the advancement of 3D technology, a fast and cost-effective computerized 3D forensic face reconstruction method has been developed, which reduces the level of errors previously encountered⁵. Forensic facial reconstruction is a combination of scientific methods and artistic skills. It can be used to reconstruct soft tissues on the skull to obtain images of individuals for identification and recognition⁶. Some commentators believe that forensic facial reconstruction is a method of facial approximation.

Various facial patterns can be created from the same skull. However, other researchers believe that each skull can only produce one face, so it is certain that a person uses the term “face reconstruction”⁷. Forensic facial reconstruction is used in both forensic science and archaeology. In forensic science, this method is used to identify individuals who are unsuccessful in conventional/common identification methods. In archaeology, it is used to identify the faces of people in the past, bone remains, embalmed bodies, etc.⁸. A person’s face has several different types of exclusive features, so it is crucial in recognizing a person. When a body is found, a facial photograph is taken. Sometimes the photo is digitally processed to make it suitable for witness identification or newspaper publishing that eventually leads to the identification of the body. The victim’s family, friends/acquaintances are visually identified, but not found to identify the only body part of identification is the face. Sometimes, due to animal damage, physical attacks, or environmental factors, the face of the deceased cannot be identified because the face cannot be recognized. In cases where little or no other evidence is available, forensic facial reconstruction is another method in the identification process⁹. Reconstruction techniques can be divided into two types: two-dimensional (2D) and three-dimensional (3D) techniques¹⁰. They can be performed manually or analyse specific software (computerized). The 3D manual methods used in forensic facial reconstruction are anatomical (Russia), anthropometric (US) and Manchester combined (UK) methods developed by Gerasimov, Krogman, and Neave, respectively¹¹,¹².

Forensic Anthropological Analysis

The active scientific identification of human remains recovered in the context of forensic medicine is the main goal of forensic anthropological analysis. Many aspects of anthropological activities, including search and recovery, identification of species, the estimation of gender, age of death, body size, time of death, detection of ancestry, and unique anatomical features, can be used to narrow the search for missing persons¹³. In the end, forensic anthropologists will contribute to active scientific identification directly or through a large amount of supplementary information provided. Direct contributions such as evaluating various anatomical features and compare them with pre-existing information are revealed through radiography and related images. The recognition types include provisional, environmental, presumptive, and positive types¹⁴. The first three types listed indicate that actual identities cannot be excluded, so these remains or other inspection evidence represent specific individuals. Research and case studies have shown that facial recognition is unreliable, especially when performing advanced decomposition¹⁵,¹⁶.

Positive recognition represents a higher level of probability and involves a two-step process. First, it is necessary to discover the anatomical features shared between the examined evidence and the known prior information about the specific individual. Second, the analyst should determine that the compared features are sufficiently unique to be able to be identified. Besides, any discrepancies should be recorded and explained.
Recognition errors are divided into two categories: 1 the difference is evidence of exclusion representing other factors; 2 the uniqueness is not fully considered when presenting shared features to support the recognition. There is a particular need for contributing forensic anthropologists to identification, and especially in the analysis of extensive decomposition and skeleton human remains. Experimental research reported by Sauerwein et al. pointed out that the process of decomposition can quickly destroy many indicators commonly used for identification, although the speed of destruction depends on many variables. In their study, fingerprints survived for 4 days after death at high temperature and survived for more than 50 days at low temperature. The time for iris recognition after death is 2 to 34 days, depending on the variable.

Accurate recovering, documenting, and evaluating the biological characteristics of human remains are important factors leading to active identification. To focus on the appropriate missing persons for identification, investigators must have meaningful information about the age of death, gender, ancestry, body size, and time since death. All evidence must be retracted together with detailed documents. It should be restored and analysed in a way that meets the requirements of legal procedures.

The unique function required by identification can be provided through surgical procedures, especially those that remain in bone tissue. For example, Hogge et al. detected postoperative defects associated with unilateral lambdoid combined resection, the body can be actively identified. The remains were identified as people who underwent neurosurgery for this rare congenital abnormality. Many orthopaedic devices with human remains provide information to the manufacturer. According to current laws, certain devices contain digital information that can be traced back to specific operating rooms or even individual patients.

Forensic anthropologists discovered these inorganic materials in their case. For example, Bennett et al. reported the identification of burned wreckage recovered from automobiles through inspections of internal fixtures. Radiographic examination of the recovered residue revealed that many wires were identified as osteostimulators. The serial number is not recorded, but it is a material related to the documented bone stimulator used to treat a patient's back injury to stimulate bone production during surgery.

Dental characteristics often provide the information needed for identification. Forensic odontologists explain dental restorations and other characteristics related to the dental practice. However, anthropologists and dentists have common interests and expertise in tooth morphology, which can provide evidence for positive scientific identification. Useful features for identification include the number of teeth present, missing front teeth, the pattern of tooth displacement, and pattern of abnormal rotation. Typically, comparative antemortem data are available via radiographs and related imaging. Murphy et al. recorded that 60 percent of the scientific identification in the St. Louis region of the United States resulted from radiographic evaluation in a 15-month period from April 1978 to July 1979. Most of the specific data used in positive scientific identification were provided by anatomical variants, disease alteration, and postoperative features. As noted by Fitzpatrick et al., to promote contrast, positioning, magnification, beam centring, angulation, and bone orientation techniques should be used properly.

Craniofacial Superimposition

A forensic technique for detecting the skull known as craniofacial superposition (CFS) is used to investigate the anatomical and morphological connection between a skull and the face. It includes overlaying the skull with a varying number of facial images. Most people now have images with identifiable faces and hence, this technique plays a significant role in skull detection.
craniofacial superimposition\textsuperscript{30}. From the viewpoint of recognition approaches, Yoshino et al. divided computer aided CFS into two groups. The first approach is to digitize the skull and face pictures\textsuperscript{31}. Image processing tools were employed to compare the two images morphologically. The second step is to perform a morphometric test to assess the match between the skull and facial images. The above contributions were made before the last decade’s image-processing boom\textsuperscript{32}. Damas et al. analyzed the current approaches contemplating a computing-based classification standard and are further concerned with the use of computers at various stages of CFS processing. To properly characterize any CFS method, and the authors have specified various stages involved in the craniofacial process\textsuperscript{33}. “These stages include face enhancement and skull modelling, skull-face overlay, and decision making.” The first stage entails creating a digital model of the skull and improving the image of the face. Instead of creating a 3D model of the skull, the oldest and most modern systems still obtain a photograph and a sequence of video shots of it.

Regarding face images, most modern systems employ a 2D digital image. This stage demands image-processing techniques to improve the quality of the face image that was given when an individual is missing. The skull-face overlay is the second level. It involves finding the best overlay of a 2D image of the skull/face or a 3D model of the skull/face obtained during the first stage. The final and third stage of CFS are decision making. Based on the skull-face overlay attained, the identification decision is made by examining the framework and soft-tissue thickness at different anthropometric landmarks, as well as anatomical details to determine the spatial relationships of the skull to face sections\textsuperscript{34}.

Forensic identification and technology involve two (or more) separate images/models/data and explore similar and compatible patterns. The data applied for comparison are the same ‘object’ in methods such as DNA, fingerprints, facial recognition, dental identity; for example, two DNA string comparison, fingerprint images, photographs of the face, and teeth x-rays. This is not the case for CFS, a human identification technique in which photographs of the face and skull are contrasted to determine the identity of a given subject. The entire CFS method can be divided into 3 subsequent steps: 1) The acquirement and processing, e.g., skull and ante-mortem (AM) face images and somatometric marks on both sides; 2) skull-face overlay (SFO) to perform the potential overlay for the skull and a single AM picture of the person that is missing. This is done in reiterative aspect with each photograph, attain different overlays; and 3) decision-making aimed at deciding the degree of match support, based on SFOs obtained in the earlier step\textsuperscript{35}. The technique of CFS identification has tremendous potential for use today because many people have photos (AM), where their faces are visible. A bone that deteriorates with the impact of fire, humidity, high or low temperatures, and distant future; is the counterpart skull.

Craniofacial superimposition the characteristics of the recovered skull with the ante-mortem picture of the missing person who may be represented by the remains. This technique can be used when it has not been positively identified by molecular analysis, dental reconstruction comparison, or anthropological radiological evaluation\textsuperscript{36}. Usually, this method can be used when a complete skull or crania can be used for comparison, but it is even tried using fragmentary evidence\textsuperscript{37}. Once a clear image of the compare crania that can be used to compare the recovery is found, forensic anthropologists must spend some time adjusting the orientation of the skull, usually using Q-tips as placement markers so that the images can be placed correctly on each other. Comparison techniques have become more complex and complicated, which mainly allow rejection rather than active scientific identification. Images are taken from police records, surveillance, or directly from relatives of possible individuals. The quality of the image corresponds to the accuracy of the exclusion process\textsuperscript{38}. Dorion pointed out that if used improperly, photographic superimposition can lead to recognition errors\textsuperscript{39}. He noted that this technology should not be used as the only way of identification. The research reported by Austin et al. supported this expression of
concern. They compared the front and side views of the three skulls with pictures of ninety-eight people. They reported a positive comparison with 9.6% and 8 of the side view and 5% of the front view. However, the consistency percentage is reduced to zero and 6% when using both front and side views.

Skull/photo video superimposition

Kahana defined this approach for the first time. This technique is effective if photographs of one or more probable progeny are available. On adjustable support is attached the skull to be marked. While the antemortem photograph is placed at right angles with a high-resolution video camera, the skull is aligned with a 2nd video camera. The lens center position should be on an equal level as the photograph's horizontal centre. Both the cameras process the two images in a vision mixer to achieve horizontal, vertical, super-imposed, and negative stimulus. In the case of the presence of teeth, the enlargement can be done until the teeth of the antemortem photograph intersect the teeth exactly in the super-imposed picture. If there are no teeth present, it is important to estimate the vertical height of the skull photograph.

Computerized 3D facial reconstruction

This approach uses computer programs to convert 3D skull images scanned with a laser into faces. Even though the findings are reproducible rather than sculpted reconstructions, certain subjectivity will continue to be added to the digitized skull matrix by a composite face image. A list of head models with their age, race, nutrition status, and other personal characters either skulls, faces, or soft tissue depths is essential. Remains of the dead are checked and used to pick the suitable skull and soft tissue models by the medical team and the evidence given. The skull is mounted in an upholstered head holder. The length varies as the skull rotates on the platform and with each latitude, the radius is determined. A wireframe with 256 x 256 radii must be converted to produce the basis of facial reconstruction using measurements of tissue depth. The facial characteristics unforeseen by the skull contour (nose, eyes, mouth) ought to be applied to the wireframe face to create a wire colour and texture.

Facial Approximation

Facial approximation means trying to generate the individual’s facial similarity from the skull. Although this method cannot be used directly for identification, the resulting images can be used to communicate with the public to collect information about missing persons who may be represented by recovered remains. Major advances in methodology include new soft tissue in-depth population data, new guidelines for evaluating facial features, and innovative computerized methods. Although several studies on the depth of soft tissues have been published, Stephen et al., note that the data show that there is no clear secular trend, and the value is increasing. They recommend that existing data be aggregated for use by adults. They also found similar results in sub-adult data and suggested that the data be divided into two age groups (0-11 years and 12-18 years old).

Although facial approximation is reported to be useful in collecting information related to recognition, Stephen and his team still expressed concern about the relative similarity level. Henneberg et al., published an experimental method to judge the recognition value and question the value for recognition. With the enhancement of information about the relationship between hard and soft facial tissues and more sophisticated computer technology, facial approximation technology is constantly improving. Despite these advances, the facial approximation does not represent a positive scientific identification method. However, the generated image proves to be helpful and assist the public in disseminating the remains of someone with specific visual and demographic characteristics.

Unique Cranial Evidence

Although other methods have led to preliminary identification, the unique features present on the skeleton allow for more definitive classification. Skulls provide anthropological analysis with unique information required for active scientific identification. There are two main reasons: (1) Historically, many studies have
focused on skulls, revealing huge differences in many anatomical features; (2) Front radiographs and related images are available for the head and contain multiple views. As described by Smith et al., skull images can present many unique features useful for recognition. In their case report, they pointed out that the detection of the frontal sinus, sphenoid sinus, ethmoid sinus mastoid air sacs, sagittal sutures, and internal protrusions of the occipital bone by computed tomography (CT) can be used for positive scientific identification. Culbert and his team provided extensive information on the use of the nasal cavity and mastoid system to identify deceased elderly patients in India. Sperry et al. offered further examples of detection using the frontal sinus and intracranial nervous system. Rogers et al. also adopted a cranial suture pattern, claiming that the path they followed for this activity fulfilled the legal requirements of the United States and Canada at that time.

Frontal Sinus Variation

While several features of the human skull show great differences and can be used for individual identification, many researchers have focused their attention on the frontal sinus. The sinus is located above the supraorbital bulge, higher than the nostrils, and shows significant changes, ranging from the smallest presence to the formation of large labia intussusception. Reflecting the environmental and developmental influence, even the same twins show morphological differences in the frontal sinus expression. Schuller specified the significance of the frontal sinus for positive scientific identification. Later, for comparative purposes, Asherson et al. established a method of using outlines of sinus expression. Ubelaker and colleagues in 1984 explained how frontal sinus comparison, combined with sella turcica morphology and other cranial characteristics, was used in a murder trial for positive scientific identification. Radiographs of cranial specimens at the Smithsonian Institution were also used by the team of scientists to show population variation of the frontal sinus. Angyalet al. presented cases from Hungary showing how frontal sinus radiography and features were performed. Though trends were recognized at the earliest, comparative studies on the frontal sinus morphology, metrical, and more complex statistical treatments were also implemented. Kirk et al. introduced a metric approach documenting the vertical and horizontal sinus expression. They declared the corresponding measurements to be less than 5 mm apart. The authors also documented using pattern recognition and metric analysis for positive scientific detection in their retrospective analyses of thirty-nine cases in Ontario’s Chief Coroner’s Office. Furthermore, it was noted that the probability of recognition using this function was not influenced by adult age, sex, and cause of death. Christensen and his team used Elliptic Fourier analysis to determine the individualization of the frontal sinus in the light of the increasing demands of the legal arena for increased quantifying and likelihood assessment of the features of identity. As indicated in the earlier publication, this application indicates a consistent methodology to human positive scientific identification by evaluation of the frontal sinus morphology.

Post Cranial Remains

There are also ample anatomical features used for the identification of skeletal remains from the post-cranial skeleton, where subsequent antemortem radiographs can be sited. Animal scavenging, and other post-mortem factors may influence post-cranial bones. Trabecular general bone patterns and ossic contours, abnormalities, and radiodensities may provide specific identifying features. Clavicle, thorax area, hand and wrist, patella, and foot defects were the key points for post-cranial detection approaches. Unconventional medical conditions are important because radiographs with skeletal anatomical information can be connected to them.

The Anthropometric American method/ tissue depth method

This technique was first introduced by Krogman in 1946. This method uses data from soft tissue depth collected by employing needles, X-rays, or ultra-sonic analysis. Muscles of the face are adequately recorded anatomically. This is not a favoured approach now a few
days because it needs highly skilled professionals\textsuperscript{56}.

\textbf{Conclusion}

Forensic analysis in skull detection and facial reconstructions is highly beneficial and an alternative approach in the identification process in generating images where no other evidence is offered and accessible. Nevertheless, the options taken by the team of investigators such as forensic anthropologists, forensic dentists are centred on the method of facial reconstruction. The facial reconstruction aided by computers has many advantages compared with conventional approaches. It simplifies the process and significantly reduces the time taken to propose a face model. Various models can be shifted in many ways improving the likelihood of individual recognition. Although the techniques employed for skull detection and facial reconstruction are dubious, these techniques also demonstrate to be a significant tool for the team of forensic analysts in detection when the source of substantiation is not available.

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\textbf{Ethical Clearance:} Ethical clearance and funding were not necessary as it is review article.

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Too Blue To Be True”: Indoxacarb Induced Methemoglobinemia- A Rare Case

Jerry Jacob¹, Karthik Reddy C H², Jobin James³, Nijila Janardhanan¹
¹Junior Resident, Emergency Medicine, St. Johns Medical College Hospital, Bengaluru, ²Assistant Professor, Emergency Medicine, St. Johns Medical College Hospital, Bengaluru, ³Senior Resident, Emergency Medicine, St. Johns Medical College Hospital, Bengaluru

Abstract

Indoxacarb is an oxadiazine insecticide, which is a sodium channel blocker in the nervous system of insects and causes mild tremors, cessation of feeding, and death in few hours.

Here we describe a case of novaluron and indoxacarb poisoning in a suicide attempt. The patient presented with cyanosis with saturation – paO2 gap indicating methemoglobinemia and was treated with methylene blue and other supportive measures.

Keywords: insecticide, toxin, indoxacarb, novaluron, methemoglobinemia, cyanosis, methylene blue, tissue hypoxia.

Introduction

Indoxacarb is an oxadiazine [¹] compound and a sodium channel blocker. It is a broad-spectrum insecticide [¹] widely used in commercial and farm planting for the control of certain insects, i.e. moth, leaf hopper and fruit worm. Contact with the substance can take place through ingestion, physical contact, pruning, and rewetting of surfaces.

We report a case of novaluron and indoxacarb toxicity with suicidal intent, presenting as a medical emergency. Acute methemoglobinemia is an emergency requiring prompt diagnosis and treatment with methylene blue.

Case Report: A 55 year old farmer hailing from Andhra Pradesh reported to our emergency department with alleged history of a feud at home and consumption of 100 ml of an unknown insecticide as shown in

The insecticide was identified to be a combination of novaluron (5.25%) and indoxacarb (4.5%) (trade name: SENORA) with no specific antidote as per the leaflet. The patient presented to us 4 hours after the ingestion with no first aid administered. He complained of vomiting, drowsiness, headache and abdominal pain.

On arrival he had a pulse rate of 84/min, blood pressure of 110/80 mm Hg, saturation of 80% on room air and 82% on 15 litres of oxygen via Non-rebreathing mask (NRBM), respiratory rate of 22/min. He was conscious but mildly drowsy with Glasgow Coma Scale of 14/15. His pupils were 3 mm, bilaterally equal and reactive to light.
Figure 1: Insecticide containing indoxacarb and novaluron (trade name: SENORA)

On general physical examination, the patient had central cyanosis involving tongue and mucous membranes as shown in Figure 2:

Figure 2: Central cyanosis involving tongue
On further eliciting history there was no history of congenital heart disease, fever, cough or respiratory distress in the near past or other comorbidities. His only habits were occasional ethanol consumption and smoking approximately amounting to 10 pack-years.

An arterial blood gas was obtained which showed a pH: 7.237, pCO2: 22.9, pO2: 141, Hb: 18.3, sO2: 97.8, Na: 143, K: 2.8, Cl: 109, lac: 18, HCO3: 9.4 interpreted as partially compensated metabolic with lactic acidosis with hypokalaemia.

To identify the cause of spO2 - paO2 gap and co-oximetry was ordered for.

The results came as follows: MetHB (methemoglobin) – 46.4%, COHb (carboxyhemoglobin) – 3.3%, leading us to the diagnosis of methemoglobinemia. Literature review led us to the conclusion of a rare case of methemoglobinemia caused by indoxacarb exposure.

On comparing the patient’s blood gas sample to a normal patient’s sample the characteristic chocolate brown colour of blood was appreciated as shown in Figure 3:

Our final diagnosis was methemoglobinemia secondary to pesticide ingestion: Indoxacarb and novaluron.

The patient was treated with anti-emetics and the antidote methylene blue. The patient’s weight was about 70 kg and he was treated with 70 mg of methylene blue intravenously (1mg/kg with each ml containing 10 mg of methylene blue). Saturation picked up to 94% shortly after and repeat ABG was as follows: pH 7.415, pCO2: 32, pO2: 101, HCO3: 20.1, sO2: 95.8, Na: 137, K: 3.11, Cl: 108, lac: 0.9, MetHb: 1.6.
Table 1 given below shows the comparison between the pre and post treatment blood gas analysis.

**Table 1: ABG comparison**

<table>
<thead>
<tr>
<th>Blood gas analysis</th>
<th>Pre-treatment</th>
<th>Post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.23</td>
<td>7.41</td>
</tr>
<tr>
<td>pCO2</td>
<td>22.9</td>
<td>32</td>
</tr>
<tr>
<td>pO2</td>
<td>141</td>
<td>101</td>
</tr>
<tr>
<td>HCO3</td>
<td>9.4</td>
<td>20.1</td>
</tr>
<tr>
<td>sO2</td>
<td>97.8</td>
<td>95.8</td>
</tr>
<tr>
<td>Na/K/Cl</td>
<td>143/2.8/109</td>
<td>137/3.11/108</td>
</tr>
<tr>
<td>MetHb</td>
<td>46.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Lactate</td>
<td>18</td>
<td>0.9</td>
</tr>
</tbody>
</table>

A repeat dose of 140 mg of methylene blue was given the following day as saturation dropped and MetHb levels was 24. He was also on treatment with supportive measures of vitamin c 500 mg once daily, intravenous fluids and thiamine as the patient had a history of an alcohol binge.

**Final diagnosis:** Indoxacarb and novaluron (pesticide) induced methemoglobinemia.

**Physiology**

*Normal physiological process is as shown below in Table 2:*

**Table 2: Physiological oxidization of hemoglobin**

*Formation of methemoglobin is as shown below in Table 3:*

**Table 3: Methemoglobin formation**
Formation of methemoglobin is as shown below in Table 3:

Table 3: Methemoglobin formation

Etiology

Table 4: given below lists the most common etiologies of methemoglobinemia.

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Methemoglobinemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local anaesthetics</td>
<td>Benzocaine, Procaine, Tetracaine, Lidocaine. [4]</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Dapsone, Rifampin, Sulfonamides (e.g., sulfamethoxazole), Antimalarials (chloroquine, primaquine). [4]</td>
</tr>
<tr>
<td>Environmental triggers</td>
<td>Pesticides, weed killers, Dyes, paints, thinner, rubber solvent. [4]</td>
</tr>
</tbody>
</table>

Table 4: Etiology of methemoglobinemia

CLINICAL FEATURES

Table 5given below illustrates symptoms with corresponding MetHb values.

<table>
<thead>
<tr>
<th>Methemoglobin concentration</th>
<th>Percentage of total haemoglobin</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.5 g/dl</td>
<td>&lt;10</td>
<td>None</td>
</tr>
<tr>
<td>1.5-3.0 g/dl</td>
<td>10-20</td>
<td>Cyanosis</td>
</tr>
<tr>
<td>3.0-4.5 g/dl</td>
<td>20-30</td>
<td>Anxiety, headache</td>
</tr>
<tr>
<td>4.5-7.5 g/dl</td>
<td>30-50</td>
<td>Fatigue, confusion</td>
</tr>
<tr>
<td>7.5-10.5 g/dl</td>
<td>50-70</td>
<td>Coma, seizures, acidosis</td>
</tr>
<tr>
<td>&gt;10.5 g/dl</td>
<td>&gt;70</td>
<td>Death</td>
</tr>
</tbody>
</table>

Table 5: Symptoms of methemoglobinemia with corresponding values

CLINICAL DECISION MAKING

The Figure 7 given below illustrates an algorithm to approach a patient with cyanosis.

**ED**

![Algorithm Image]

**Figure 7: Approach to cyanosis in ED**


**Diagnosis**

**Arterial blood gas analysis**

Methemoglobinemia may cause chocolate-brown discoloration of the blood. As a bedside test, if blood is dropped on a piece of white gauze, it will remain brown as it dries (in contrast, normal deoxygenated blood will absorb oxygen and become red). [5]

There is an obvious mis-match between the PaO2 (which is >>100 mm) versus the pulse oximetry (which is typically ~80-90% saturated). This is known as PaO2-saturation gap, and it is indicative of some sort of hemoglobinopathy (most often methemoglobinemia). [5]

Co-oximetry is the gold standard of detecting methemoglobinemia with spectrophotometry. Methemoglobinemia has a peak absorbance of light at 630 nm which is characteristic. [6]

**Discussion**

Methemoglobinemia refers to the oxidation of ferrous iron (Fe++) to ferric iron (Fe+++ within the haemoglobin molecule. [1] This reaction impedes the ability of haemoglobin to transport oxygen, leading to tissue hypoxemia. Methemoglobinemia most commonly results from exposure to an oxidizing chemical, but may also arise from genetic, dietary, or even idiopathic etiologies. [2, 3]

**TREATMENT**

**Methylene blue**

Methylene blue (MB) is a reducing agent. MB exerts its reductive effects by activating the dormant but volatile hexose monophosphate (HMP) shunt to regenerate NADPH. Dextrose should be co-administered in order to increase NADPH formation. If MB therapy is ineffective and life-threatening shock is imminent, exchange transfusion should be initiated.

Methylene blue is provided as a 1% solution (10 mg/mL). The dose is 1 to 2 mg/kg (0.2 mL/kg of a 1% solution) infused intravenously over 3 to 5 minutes. The dose may be repeated at 1 mg/kg if MB does not resolve within 30 minutes. Methylene blue should reduce MB levels significantly in less than an hour. The maximum dose is up to 7 mg/kg within 24 hours. [7]

Dextrose should be co-administered in order to increase NADPH formation. If MB therapy is ineffective, exchange transfusion should be considered. Ascorbic acid, part of the minor reduction pathway of methemoglobin, may be useful in patients in whom MB
therapy is contraindicated. The dose is 1-3 gm every 8th hourly. [8] Hyperbaric oxygen is also effective in MB levels above 50% and in patients with no response to other treatment modalities. Other agents considered were N-acetyl cysteine and high dose cimetidine in dapsone induced methemoglobinemia. [9]

**Conclusion**

To conclude we report a rare case of toxin (indoxacarb) induced methemoglobinemia with a MB level of 46%, treated successfully with methylene blue and ascorbic acid and discharged after 5 days of hospital stay. Early diagnosis and initiation of methylene blue aided in significantly reducing the morbidity and need for mechanical ventilation in this patient. During this pandemic of COVID-19, importance of understanding that other etiologies might present with hypoxemia and ordering for co-oximetry when appropriate is of paramount importance.

**Ethical Clerance:** Taken from the institutional ethical committee.

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**Conflict of Interest:** Nil.

**References**


Knowledge and Awareness towards Dental Management of Post-Cardio-Thoracic Surgery Patients among Dental Students

Johara Maria Cruz¹, Keerthi Narayan. V², Vasumathi.D³

¹Professor, Department of Prosthodontics, Sathyabama Dental College and Hospital (Deemed University) Jeppiar Nagar, Shollinganallur, Chennai, ²Senior Lecturer, Department of Oral Pathology and Microbiology, Thai Moogambigai Dental College and Hospital, Dr. M. G. R. Educational and Research Institute, Chennai, ³Lecturer, Department of Public Health Dentistry, Thai Moogambigai Dental College and Hospital, Dr. M. G. R. Educational and Research Institute, Chennai

Abstract

Background of the Study: Oral health care professionals must be able to identify various cardiac diseases and its associated post-operative dental emergencies to adopt the appropriate measures to treat them efficiently and effectively. A comprehensive dental treatment plan should be constructed keeping in view all the possible complications related to the post-operative cardio-thoracic surgical factors.

Aim: This study was aimed to determine the knowledge, attitude and awareness towards dental treatment and their management following Cardio-thoracic surgeries among the dental students at undergraduate and postgraduate level.

Methodology: A cross sectional survey was conducted using self-administered questionnaires, through Google forms among 100 dental students from Tamil Nadu India. Statistics has been done and results are tabulated.

Results: The overall knowledge towards various cardio-thoracic surgical procedures carried out and their dental management among dental students was only 65% and 45% for postgraduates and interns group respectively.

Conclusion: The present study showed lack of awareness with limited knowledge and attitude towards various cardiac-thoracic surgical procedures carried out and their corresponding dental management among the dental students especially at the undergraduate level. Hence enlightening and educating the students on several post-surgical management phases at the undergraduate level is essential for effective and efficient treatment outcome and increase the quality of life.

Keywords: Awareness, Cardio-thoracic Surgery, Cardiac Pacemaker, Dental Extraction, Oral Health Problems.

Introduction

Cardiovascular disease is one of the most common medically compromised conditions that can be challenging to manage in terms of oral complications, oral rehabilitation with dental treatment and emergency care. Oral health care professionals play a crucial role in managing this state and are believed to have a sound knowledge, attitude and awareness towards medically compromised condition and treating the same [1].

Several authors recommended that if emergency dental procedure must be performed on a patient who has received a CABG (Coronary Artery Bypass Grafting), pacemaker, or stent before the appropriate time (three to six month) interval has elapsed, that patient should be pre-medicated. After the time interval, no premedication is indicated for those procedures since coronary artery bypass graft surgery is not associated with a long-term risk of infection, antibiotic prophylaxis for dental procedures
is not needed in individuals who have undergone such surgeries. Antibiotic prophylaxis is recommended only for patients with conditions such as Cardiac valvulopathy in a cardiac transplant recipient, congenital heart disease, patients with previous infective endocarditis and Prosthetic cardiac valve who are undergoing dental procedures that involve manipulation of gingival tissues or periapical region of teeth, or perforation of the oral mucosa [2, 3, 4]. Over the years several studies have shown that invasive dental procedures may contribute to the development of infective endocarditis in patients known to present a high incidence of cardiovascular disease and to be at high risk of morbidity and mortality associated with the disease [5].

A multidisciplinary approach while treating medically compromised dental patients is required to reduce complications and to enhance the prognosis. At the same time, the cardio-thoracic surgeon should refer to a dental surgeon preceding any cardiac surgery in view to prevent infection from the Oral and Para-oral region seeding structures within the heart [6]. To the best of our knowledge, there are very few studies initiated with a key emphasis on knowledge about various cardiac surgical procedures carried out and their corresponding dental management among dental students. Hence the present cross-sectional study was performed to determine the knowledge, attitude and awareness towards dental treatment and their management following Cardiac surgeries among the dental students at undergraduate and postgraduate level.

**Methodology**

This survey was done to evidently assess the knowledge, attitude and awareness of undergraduate and postgraduate dental students about dental treatment and their management following Cardiac surgeries. It was randomly done among the dental students residing in Chennai through Google forms. The links of the Google forms were circulated via social media to reach 100 dental students within a time of 3 weeks. This study was based on self-applied questionnaire composed of 10 questions, the questions were based on students own perception hence it helps to assess the actual scenario about the knowledge and behaviour of students. The actual purpose behind the study was well-explained to the participants. The questionnaire covered all the important aspects of oral health problems with special emphasis among post-cardiac surgery patients.

**Results**

Non-probability, convenient sampling technique was employed that yielded information from 100 dental students. The study comprises of 27 male and 73 female participants. Relevant questionnaire was prepared; responses were noted among the selected population group under the study and evaluated for statistical analysis by SPSS software Version 20.0. The level of statistical significance was set at a $P$ value less than 0.05. On statistical evaluation it was observed that all 100 samples were valid for the study with Cronbach’s alpha reliability score being 0.846 (Significant score). On evaluation, 100 participants were distributed category wise comprising of 50 intern (undergraduate) students followed by 50 postgraduate students. The questions were individually subjected to chi square test. The Overall chi-square statistic is 277.985. The $p$-value is .0001. The result is significant at $p < .05$.

On evaluating the responses about knowledge on various cardiac surgical procedures among the undergraduate and postgraduate dental students it was observed only 76.02% of the total study participants were aware, among which 58.4% were postgraduates. About 33.8% of the study population were familiar about dental problems associated with cardiac pacemakers, 24.6% about CABG (Coronary Artery Bypass Graft) and 41.6% were aware of dental problems associated with heart valve replacement (Stent) procedure (Figure 1). On assessing the distribution of responses about knowledge on medications prescribed and their mechanism of action following cardiac surgical procedures it was observed only 46.5% of postgraduates and 31.1% of undergraduates were aware of aspirin given to prevent blood clots (66.4%) followed by beta-blockers (85%) and ACE inhibitors to help lower blood
pressure (57.6%).

On assessing the responses about attitude and awareness on Indications and Contra-indications of various dental procedures following cardiac surgeries it was noted 64.7% of the post-graduates and 52.3% of the under-graduates were aware of the recommended dental procedures, However majority of the study participants (94.3%) prefer referring to physician or cardiac specialist before starting any dental procedures.

On evaluating the responses about knowledge on emergency dental procedures to be followed it was observed 79.4% were aware of role of Local anaesthesia with Vasoconstrictors for extraction procedures (Figure 2) and only 43.3% were aware of guidelines for safe dental practice in this high-risk group such as antibiotic prophylaxis for various cardio-thoracic surgeries (Figure 3), and 57.36% were aware of dental guidelines for minor dental procedures such as use of hand scalers rather than ultrasonic scalers. No significant differences in responses were obtained among the study groups when asked about the oral manifestation of patients following cardiac surgeries.

Descriptive statistics were used to summarize the responses to the questionnaire, with the results being presented as frequencies and percentages. Chi-square tests were used to compare between groups, and the level of significance was set at \( P \leq 0.05 \). In the final analysis, “yes” or correct responses were given a score of 1 and “no” and “maybe” responses were given a score of 0; the scores were summed to obtain the overall scores in each group and they were then converted into percentages for all the questions. These percentages were classified into four levels: ≤ 50 (“poor”), 50-70 (“moderate”), 70- 90 (“good”), and ≥ 90 (“very good”). The overall knowledge towards various cardiac surgical procedures carried out and their dental management among dental students was only 65% and 45% for postgraduates and interns group respectively (Table 1).

![Cardio-thoracic procedures with dental problems (%)](image)

**Figure 1**: Pie-chart showing responses on knowledge about various cardio-thoracic surgeries with dental problems
Figure 2: Pie-chart showing responses on awareness about use of Vasoconstrictors

![Local anaesthesia with Vasoconstrictors](image1)

Figure 3: Pie-chart showing responses on awareness about antibiotic prophylactic guidelines for safe dental practice

![prophylactic antibiotics safety guidelines](image2)

Table 1: Table showing the total score analysis of knowledge about cardiac surgical procedures carried out and their dental management among dental students (%)

<table>
<thead>
<tr>
<th>OVERALL</th>
<th>Undergraduate (Internship)</th>
<th>postgraduates</th>
<th>X2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤50 (low)</td>
<td>18 (36.0)</td>
<td>8 (16.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-70 (average)</td>
<td>27 (54.0)</td>
<td>32 (64.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70-90 (good)</td>
<td>3 (6.0)</td>
<td>7 (14.0)</td>
<td>27.798</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>≥90 (very good)</td>
<td>2 (4.0)</td>
<td>3 (6.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50 (100.0)</td>
<td>50 (100.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>median score</td>
<td>45</td>
<td>65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*p<0.05-significant)
Discussion

Patients with cardiovascular disease who had undergone surgeries constitute a high-risk group in dental practice, particularly with a short-span following surgery (Less than six months). It is therefore important for dental surgeons to know their medical condition, cardio-thoracic surgery performed, associated treatments received, dental complication and medicaments, and the possibilities for dental treatment [7]. In the present study on evaluating the responses about knowledge on various cardiac surgical procedures among the undergraduate and postgraduate dental students it was observed only 76.02% of the total study participants were aware, among which 58.4% were postgraduates. About 33.8% of the study population were familiar about dental problems associated with cardiac pacemakers, 24.6% about CABG (Coronal Artery Bypass Graft) and 41.6% were aware of dental problems associated with heart valve replacement (Stent) procedure. Kamath et al [6]. Pacaric et al [8], Cruz-Pamplona et al [9] and Cotti et al [10] suggested modified dental treatment and procedural considerations in cardiac disease patients with recent cardio-thoracic surgeries.

On assessing the distribution of responses about knowledge on medications prescribed and their mechanism of action following cardiac surgical procedures it was observed only 46.5% of postgraduates and 31.1% of undergraduates were aware of aspirin given to prevent blood clots (66.4%) followed by beta-blockers (85%) and ACE inhibitors to help lower blood pressure (57.6%). Chaudhry et al [11] similar to our study also recommended the prerequisite of knowledge about several drugs used in treatment of cardiovascular diseases, mechanism of action and the potential adverse effects of drugs.

On assessing the responses about attitude and awareness on Indications and Contra-indications of various dental procedures following cardiac surgeries it was noted 64.7% of the post-graduates and 52.3% of the under-graduates were aware of the recommended dental procedures. Deppe et al [12]. Singh et al [13] evaluated the long-term need for dental treatment and concluded that non-radical dental treatment modes prior to cardiac valve replacement can only be successful over the long-term if adequate postoperative dental care is provided suggesting the need for emergency dental treatment even after surgery however in the present study majority of the study participants (94.3%) lack confidence in treating emergency cases and prefer referring to physician or cardiac specialist before starting any dental procedures.

On evaluating the responses about knowledge on emergency dental procedures to be followed it was observed 79.4% were aware of role of Local anaesthesia with Vasoconstrictors for extraction procedures. However, different studies have shown that no significant increases in arterial pressure are induced by the use of anaesthesia with a vasoconstrictor in dental treatments. Silvestre et al [14] observed no significant changes in systolic blood pressure before, during or after dental extractions – the lowest pressures being recorded at the end of the procedure, and the highest at the time of extraction. Laragnoit et al [15] in coincidence with other studies in patients with heart diseases, reported that the administration of 2% lidocaine with epinephrine (1: 100,000) induces no significant changes in the hemodynamic parameters during dental treatment suggesting that its use is safe in minor dental operations, provided a good anesthetic technique is performed and the treatment prescribed by the cardiologist is maintained.

Tubiana et al [16] assessed the relation between invasive dental procedures and infective endocarditis in patients with prosthetic heart valves and concluded proper understanding of the various antibiotic regimes is mandatory for effective dental treatment whereas in the present study only 43.3% were aware of guidelines for safe dental practice in this high-risk group such as antibiotic prophylaxis for various cardio-thoracic surgeries and 57.36% were aware of dental guidelines for minor procedures such as use of hand scalers rather than ultrasonic scalers suggesting lack of awareness and knowledge. In dental practice a minimum safety period of 6 months has been established before any oral surgical
procedure can be carried out. In this time, dental treatment should be limited to emergency procedures aimed at pain relieving extractions, the drainage of abscesses and pulpal therapy preferably carried out in the hospital setting \[17\]. After this safety period, the treatment decision should be established on the basis of the condition and medical status of each specific patient. Thus a comprehensive dental treatment plan should be constructed keeping in view all the possible complications related to the post-operative cardio-thoracic surgical factors.

**Conclusion**

The present study showed lack of awareness with limited knowledge and attitude towards various cardio-thoracic surgical procedures carried out and their corresponding dental management among the dental students especially at the undergraduate level. Most of the study participants specified that they would prefer referring to physician before starting any procedures however were unsure and lack confidence to treat in emergency situations. Hence enlightening and educating the students in several post-surgical management phases at the undergraduate level is essential for effective and efficient treatment outcome and increase the quality of life.

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**Conflict of Interest:** None Declared

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Forensic Medical Assessment of Thanatogenesis in the Experience of Gunshot Injuries

Julia V. Zbrueva

1Associate Professor of the Department of Forensic Medicine of the Federal State Budgetary Institution “Astrakhan State Medical University” of the Ministry of Health of the Russian Federation, 414000, Astrakhan, ul. Baku, bld

Abstract

Gunshot injury remains relevant at the present time and requires serious study. During the examination of a gunshot injury, it is necessary to answer the investigator’s questions about the cause of death, the type and rate of thanatogenesis, the distance of the shot, the sequence and mechanism of damage formation, and the severity of the injury. The aim of the study was to determine the type of thanatogenesis in death from various types of gunshot injuries. Standard methods of sectional research, forensic histological methods, morphological and statistical analysis, supplemented with semi-quantitative technologies of thanatogenetic analysis were used in the study. The material for this study was 85 cases of death from gunshot injuries, which was distributed by gender, age, location of the injury, type and rate of thanatogenesis, type of gunshot injury (bullet and shotgun wounds), the distance of the shot and the duration of hospital stay.

Keywords: type of thanatogenesis, gunshot injury, post-traumatic period, momentum of death, cause of death

Introduction

Gunshot injuries account for the smallest proportion of violent deaths. Examination of a gunshot injury has certain features, is complex and not always well studied. Great attention should be paid to the specifics and severity of the gunshot injury.

During the examination of a gunshot injury, it is necessary to answer the investigator’s questions about the cause of death, the type and rate of thanatogenesis, the distance of the shot, the sequence and mechanism of damage formation, and the severity of the injury.

The Aim of the Study is to determine thanatogenesis as a result of death from various types of gunshot injuries.

Materials and Methods

The study used standard methods of sectional research, forensic histological methods, morphological and statistical analysis, supplemented by semi-quantitative technologies of thanatogenetic analysis.

The material for this study was cases of death from gunshot injuries from various types of weapons. This group is made up of 85 observations.

The material was distributed by gender, age, injury localization, type and rate of thanatogenesis, type of gunshot injury (bullet and shotgun wounds), distance of the shot, and duration of hospital stay.

Results and Discussion

We examined the materials of 85 victims who died as a result of gunshot injuries under various circumstances.

By gender, the material was distributed as follows: men – 77 (90.6%), women – 8 (9.4%). In the age aspect, the following indicators were noted: under 19 years – 2 (2.4%), 20-29 years – 21 (24.7%), 30-39 years – 22 (25.9%), 40-49 years – 23 (27.1%), 50-59 years-12 (14.1%), 60-69 years – 5 (5.9%). Alcoholemia was observed in 26 cases, which corresponds to 30.6%. A forensic chemical blood test for the presence of...
ethyl alcohol was not performed in 11 (12.9%) cases, since death occurred in a hospital with a duration of hospitalization of more than 1 day. Out of 26 (30.6%) studies, mild alcohol intoxication was observed in 8 (9.4%) cases, average – 9 (10.6%) cases, high – 4 (4.7%) cases, and severe – 5 (5.9%) cases. Shotguns were used in 37 (43.5%) cases, while bullets were used in 48 (56.5%) cases. When studying the close-range shot following data were obtained when the shot was noted in 30 (35.3%), within the scope of the additional factors of a shot – 48 (56.5%), outside the scope of the additional factors shots – 7 (8.2%). According to the localization of the injury, the material was distributed as follows: isolated gunshot injury was observed in 51 (60.0%) cases, concomitant injury - in 33 (38.8%), combined injury - in 1 (1.2%) case, where a combination of both gunshot and stab injuries was established.

21 victims out of the 85 cases of gunshot cases died in the hospital at various times of the hospital period. We used the following time periods: the period of 1 day, 2-3 days, 4-7 days, more than 7 days. 9 (10.6%) victims were observed during the 1st day, 3 (3.5%) on the 2nd-3rd day, 3 (3.5%) on the 4th – 7th day, and 6 (7.1%) on more than 7 days.

Studying thanatogenesis in gunshot injuries, it was found that the combined type prevailed, which made up 62 observations, corresponding to 72.9%, and the isolated type – 23 (27.1%).

<table>
<thead>
<tr>
<th>Type of thanatogenesis</th>
<th>Ab</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral</td>
<td>20</td>
<td>23.5</td>
</tr>
<tr>
<td>Cardiac</td>
<td>1</td>
<td>2.4</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>2</td>
<td>1.2</td>
</tr>
<tr>
<td>Pulmonary+cardiac</td>
<td>11</td>
<td>12.9</td>
</tr>
<tr>
<td>Cerebral+cardiac</td>
<td>6</td>
<td>7.1</td>
</tr>
<tr>
<td>Cerebral+pulmonary</td>
<td>18</td>
<td>21.2</td>
</tr>
<tr>
<td>Cerebral+cardiac+pulmonary</td>
<td>27</td>
<td>31.8</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The thanatogenetic analysis revealed the predominance of the combined type of thanatogenesis, which was represented by a combination of three components (cerebral+cardiac+pulmonary), which accounted for 27 (31.8%) of observations. The combination of cerebral, cardiac, and pulmonary components was manifested by the presence of perivascular and recellular edema of the brain, neuronal ischemia, neurocyte lysis, neurocyte karyolysis, neurocyte gliosis, and neuron swelling. The pulmonary component of this type of thanatogenesis was manifested by ARDS (hyaline membranes and fibrin), pulmonary edema, pneumonia, bronchial spasm, atelectasis, and emphysema. The cardiac component of this type was manifested by fragmentation, cytolysis of cardiomyocytes, tortuosity of cardiomyocytes, karyolysis, hypertrophy, and contracture injuries. Among the isolated type of thanatogenesis, the cerebral
component of 20 (23.5%) observations prevailed. The cerebral type of thanatogenesis was accompanied by destructive and recellular edema of the brain, hemorrhages under the membranes and substance of the brain, neuronal ischemia, lysis, karyolysis and gliosis of neurocytes, and swelling of neurons with gross destruction of the brain substance, as well as with damage to the skull bones. The described type of thanatogenesis is explained by localization of injuries or massive blood loss leading to cerebral edema in this group\textsuperscript{10,12,13}.

### Table 2 - variants of the momentum of death from gunshot injuries

<table>
<thead>
<tr>
<th>Variant of the momentum of death</th>
<th>Ab</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fulminant (15-30 minutes)</td>
<td>41</td>
<td>48.2</td>
</tr>
<tr>
<td>Fast (30 to 2 hours)</td>
<td>28</td>
<td>32.9</td>
</tr>
<tr>
<td>Average (more than 2 to 6 hours)</td>
<td>4</td>
<td>4.7</td>
</tr>
<tr>
<td>Slow (more than 6 to 12 hours)</td>
<td>2</td>
<td>2.4</td>
</tr>
<tr>
<td>Long-term (more than 12 hours)</td>
<td>10</td>
<td>11.8</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>100.0</td>
</tr>
</tbody>
</table>

When studying the momentum of death, we obtained the following distributions, which revealed the prevailing lightning-fast rate of death, which was characterized by an agonal period not exceeding 15-30 minutes, which corresponded to 41 (48.2%) cases \textsuperscript{11}.

As an example, we present the observation of an isolated type of thanatogenesis, which is represented by the brain component.

The body of Mr. H., 43 years old, was found near the cafe “Dream****”. At the time of examination of the scene, the corpse is located on the front surface of the body, arms spread out to the sides, slightly stretched up, legs stretched out. Clothes on the corpse: a cotton jacket, gray, contains brown blots in the upper sections of the item; knitted T-shirt, black, contains similar blots mainly in the collar area; blue jeans; knitted panties, black; knitted socks, black. According to the degree of development of early postmortem changes, death had occurred about 2-4 hours before the start of the examination. In the occipital region of the head, a wound is detected, the skin and hair are contaminated with brain matter and liquid blood. After an examination of the crime scene, the body was sent for medical forensic examination.

An external examination of the corpse found that the postmortem changes correspond to the first day after death. The corpse of a man of the correct constitution, satisfactory nutrition. Body height is 178 cm. The hair on the head, in the occipital region, is profusely stained with dark red liquid blood with an overlay of gray brain detritus. The hair on the head is black with a single gray, straight, up to 4 cm long. In the frontal area on the left, an unevenly expressed abrasion, irregular oval shape, 3x2 cm in size, oriented from bottom to top, from left to right, located 4.5 cm from the conditional median line of the face and 4 cm from the left brow arch. In the frontal area on the left, immediately above the left brow arch, there is an irregular oval abrasion, 1.5x1.0 cm, oriented
longitudinally, located 5 cm from the conditional median line of the face. In the eyelids of the right eye, mainly in the lower eyelid, with a spread to the right half of the nose and the right zygomatic region, on a 6x3 cm section oriented longitudinally, multiple wounds are discernible, with the edges of the linear shape reduced, from 0.2 cm to 0.4 cm long. Their ends are sharp, the edges are relatively smooth, comparable, and have no abrasions. In the inner corner of the right eye wound, when the edges of the linear length of 0.6 cm, focused on the numbers 1 and 7 hours conditional dial in the lower eyelid of the right eye wound, when the edges of the linear length of 1.0 cm, oriented transversely. The ends of the above-described wounds are sharp; the edges are uneven, wavy, have no abrasions; the walls are uneven, soaked in blood. The lumen of these wounds contains soft tissues and damaged skull bones. The remaining wounds are superficial, 0.1 cm deep, and in the nose area, wounds of the type of cracking, up to 0.1 cm deep; in their lumen, deep layers of skin and subcutaneous fat. The lower edge of the above-described area is located 165 cm from the level of the plantar surface of the right foot. In the occipital region of the scalp, in the projection of the top of the occipital protuberance, there is an extensive wound with a defect “minus tissue” of an irregular oval shape, 3, 3x2, 5 cm, oriented longitudinally. The lower pole of the edge of this defect is located 173 cm from the level of the plantar surface of the left foot. The edge of the defect is uneven, wavy, with discernible breaks oriented on numbers 12, 2, 3, 4, 7, 8, 11 conventional watch face, length from 0.2 cm to 0.5 cm. The edge of the defect is unevenly aligned throughout the entire width from 0.1 cm to 0.3 cm. Minor defects are visible around the defect: right 0.4 cm in diameter located at a distance of 0.4 cm; diameter 0.4 cm, located at a distance of 0.3 cm from the large defect; bottom, 0.3 cm in diameter, located at a distance of 0.2 cm from extensive defect; 0.4 cm in diameter, situated at a distance of 1.5 cm from the primary defect; 0.4 cm in diameter, located at a distance of 0.8 cm from extensive defect; left, 0.4 cm in diameter, located at a distance of 0.2 cm from an extensive defect. The properties and nature of these defects resemble those described above, their edges are aligned to a width of 0.2 cm to 0.3 cm, throughout. The bruise described above is bluish-red, with indistinct contours, without soft tissue swelling. The surface of the above-described abrasions is red-brown, dried, sinking, without a characteristic pattern and flaps of detached epidermis. The thickness of the bones on the cut: frontal – 0.9 cm, temporal – 0.5 cm, parietal – 0.6 cm, occipital – 0.6 cm. In the soft tissues of the head in the occipital region, there is an extensive dark red hemorrhage of an irregular oval shape, 10x7 cm, oriented longitudinally. Blood permeates the skin-aponeurotic flap to its entire thickness. In the center of the hemorrhage is the above-described defect on the skin. The walls of this defect is dyed in a light red color. There is a defect of the occipital bone at the top of the occipital protuberance 3.5 cm in diameter, from it there are multiple lines of fractures that spread in different directions, sometimes combine with each other to form many fragments of various shapes and sizes, which are held by the underlying soft tissues. These lines of fractures in their course damage the base of the skull throughout, in all cranial pits. At the bottom of the defect, multiple fragments of the bones of the arch, the base of the skull and the facial skeleton, flaps of the dura mater, fragments of the crushed substance of the brain and partially preserved brain, and foreign objects made of white metal (shot) are detected. There are multiple cracks forming bone fragments of various shapes and sizes. The parietal, occipital, temporal, frontal, sphenoid, and latticed bones are damaged. Intense hemorrhage in the ligaments and muscles of the atlanto-occipital joint. The large occipital foramen is not damaged. There are intense hemorrhages in the fat tissue of the orbits. Multiple fractures of the bones of the facial skull are determined. Bones are damaged, shattered. The right zygomatic bone is fragmented, easily mobile, and sinks into the thickness of soft tissues. The left zygomatic bone is intact. The bones of the nose are shattered. The resulting bone fragments are held by soft tissues, are poorly comparable with each other, and some of them are missing. The soft tissues of the face at the level of fractures are soaked with blood. The maxillary sinuses and other sinuses of the facial skull are filled with blood. The upper jaw is mobile, easily
shifted to the sides. The dura mater is preserved over the preserved part of the brain; pale gray, smooth, shiny, not tense, in places tightly soldered to the bones of the cranial vault, in the sinuses of its dark red liquid blood. The soft brain membranes at the level of crushing are preserved as separate small flaps of gray-red color. The pattern of sulcus and convolutions at the level of crushing is not distinguishable. In the substance of the brain at this level, multiple gray-red, pinpoint hemorrhages are discernible, spreading in the form of a wedge directed by the base to the occipital lobes. In the posterior part of the large hemispheres of the brain, at the level of crushing, the lateral ventricles filled with dark red blood are distinguishable, as well as poorly distinguishable subcortical nuclei. On the preserved part of the brain, the soft meninges are pale gray, translucent, and moderately full-blooded. The wound channel is directed from the back to the front, from left to right and somewhat from top to bottom. This wound channel communicates with the wounds of the right eyelid. A fragment of gray plastic resembling a wad container was found. On the basal surface of the preserved part of the brain, under the soft meninges, there is a dark red hemorrhage of an irregular oval shape, 14x11 cm, oriented longitudinally. The blood spreads to the bottom of the furrows and covers 1/3 of the height of the convolutions. The soft meninges are intact at this level. The pattern of furrows and convolutions is somewhat smoothed and flattened. There are no hemorrhages in the brain substance at this level. For the rest of the length, the pattern of furrows and convolutions is expressed satisfactorily.

During the forensic histological examination of the brain substance, it was found that areas with a lost modified structure with perifocal infiltration by a few red blood cells and the imposition of microparticles of bone fragments are determined. Pronounced perivascular and pericellular edema of the substance. Focal venous paresis, with their fullness with perivascular exudation of red blood cells. Burned-out neurons: cell-shading, shrinkage and loss of neurons, neuronophagia.

During the forensic histological examination of soft tissues from the wound area, it was found that infiltrating hemorrhages consisting of contoured, partially sludged red blood cells with an admixture of single white blood cells, edema of myocytes are detected; vessels are spasmodic, mainly anaemic.

Thus, the death of Mr. H., 43 years old, came from gunshot blast wounds to the head with injuries of bones of vault and base of the skull and right zygomatic, maxilla and nasal bone, shells and substances of the brain, crush the big hemispheres of a brain is accompanied by focal-diffuse subarachnoid hemorrhage to the basal surface preserved brain and hemorrhages in the soft tissue of the occipital region and face.

This morphological picture corresponded to the brain type of thanatogenesis. The momentum of death is lightning fast.

**Conclusion**

The results of the study showed that the largest number of victims of gunshot injuries were men – 77 (90.6%), in the age group of 40-49 years – 23 (27.1%). Alcoholemia was observed in 26 (30.6%) cases. Based on this indicator, the prevalence of the average degree of alcohol intoxication was revealed – 9 (10.6%). Assessing the morphology of the injury, the prevalence of isolated injuries was revealed – 51 (60.0%). Among the types of firearms, the largest number of injuries was caused from a bullet weapon – 48 (56.5%). The highest number of deaths occurred at the scene – 64 (75.3), due to the severity of the victim’s condition. The number of victims who died during the hospital period during various periods of stay was 21 (24.7%), with 10 (11.8%) fatalities prevailing on the first day.

Evaluating the variants of the course of thanatogenesis in gunshot injuries, it was found that the combined type prevailed in 62 (72.9%) cases. The combined type included markers of three components of the cerebral, cardiac and pulmonary, which were manifested by the corresponding morphological features.

Studying the momentum of death, it was found that the predominance of fulminant, which was characterized by an agonal period of no more than 15-30 minutes,
which corresponded to 41 (48.2%).

The data obtained confirm the relevance of the study of forensic medical examination of gunshot injuries. It is important to note that it is promising to use new research methods to analyze such observations2,3,5.

Ethical Clearance – Samarkand State Medical Institute

Source of Funding – N/A

Conflict of Interest – Authors declare no conflict of interests.

References


A Study to Assess the Prevalence of Anemia and Knowledge Regarding Anemia among Adolescent Girls of Selected Schools of Gurugram, Haryana

Jyoti¹, Varinder Kaur², Arindam Chatterjee³
¹PG Tutor, SGT University, Gurugram, Faculty of Nursing, SGT University, Budhera, Gurugram, ²Assistant Professor, MM University, Solan, College of Nursing, MM University, Solan, ³Medical Officer, MBBS, MD(FMT) DCH Sonipat

Abstract

Background: Anemia is a major nutritional health problem in developing countries like India because of poor dietary habits and malnutrition. The prevalence of anemia is inordinately higher among adolescents as they are signalized by marked physical activity and rapid growth spurt; therefore they need additional nutritional supplements and are at utmost risk of developing nutritional anemia.

Materials and Methods: A quantitative research approach with descriptive Research design was adopted for the study. A total of 86 adolescent girls studying in Vinay public school, Gurugram, Haryana who met the inclusion criteria were selected using total enumerative sampling technique. The tools consists of demographic variables, knowledge questionnaire and Sahli’s hemoglobinometer to assess the knowledge and prevalence regarding anemia. Reliability of the tool was established by Cronbach’s alpha method and it was found to be 0.84. Ethical permission was obtained from ethical committee SGT University and the written informed consent was taken from each participant before administering the tool.

Results: Findings of the present study revealed that majority 81% adolescent girls have poor level of knowledge followed by 19% adolescent girls have moderate level of knowledge regarding anemia. Overall prevalence and knowledge regarding anemia has range value varied from of 3-16 with mean 9.8 and standard deviation 2.88. The area of residence had significant difference on knowledge score regarding anemia in adolescent girls when P value is ≤ 0.05.

Conclusion: Knowledge level affects the prevalence rate of anemia in adolescent girls. Majority of adolescent girls have poor level of knowledge. Most of adolescent girls fall under moderate category of anemia.

Key Words: prevalence, anemia, knowledge, adolescent girls.

Introduction

Anemia is the condition of having a lower-than-normal number of red blood cells or quantity of haemoglobin. Anemia diminishes the capacity of the blood to carry oxygen. Patients with anemia may feel tired, fatigue easily, appear pale, develop palpitations, and become short of breath. Children with chronic anemia are prone to infections and learning problems.¹

Adolescents are vulnerable to both macro and micro nutrient deficiencies. Iron requirements increase due to growth during adolescence. Adolescent girls are particularly vulnerable group as their requirement of iron...
as well as its loss from the body is high which may be due the blood loss during menstruation and poor dietary habits. The average monthly menstrual blood loss is about 45 ml and causes the loss about 22mg of iron as a result as peak in the prevalence of iron deficiency anemia frequently occurs among females during adolescence.

The world health report of World Health Organization (WHO) states that the world wide mortality rates of iron deficiency anemia is 60,404,000 in 2017. It also states that the world wide morality rate of iron deficiency anemia’s 13,704,953 cases in India 2017 moreover the risk of anemia is further aggravated by poverty illiteracy, ignorance and lack of knowledge.²

According to National Family Health Survey (NFHS)–IV (2015-16), the prevalence of anemia among women aged 15 to 49 years is 53%. According to National Family Health Survey (NFHS)–IV (2015-16), the prevalence of anemia among adolescent girls aged 15-19 years is 54%. The disaggregate data of adolescent girls suffering from anemia in the country, urban and rural-wise is not available. In order to tackle this health problem, some initiative has been launched in the country. The initiative is targeted at all adolescents across the country with the aim for achieving haemoglobin level of 12 gm% by the age of adolescence by 2018. The important element of the initiative are as follows as capacity building, health and nutrition education, Increasing control through periodic, De-worming, Appropriate immunization.³

In India the prevalence of anemia among adolescent girls were 56% and this amount to an average 64 million girls at any point in time. Studies conducted in different regions of India shown that prevalence of anemia was 52.5% in Madhya Pradesh, 37% in Gujarat, 41.4% in Karnataka, 85.4% in Maharashtra, 21.5% in Shimla, 56.3% in Andhra Pradesh, 58.4% in Tamilnadu.

Need of the Study:

Chaudhary SM, DhageVR.(2018) conducted a cross sectional study on anemia among adolescent females in the urban area of Nagpur. Data was collected from 296 adolescent females aged between 10–19 years through simple random sampling method. Results depicted that the prevalence of anemia was found to be 35.1%. A significant association of anemia was found with socio-economic status and literacy status of parents. Mean height and weight of subjects with anemia was significantly less than subjects without anemia. Hence it is concluded that a high prevalence of anemia among adolescent females was found, which was higher in the lower socio-economic strata and among those whose parents were less educated. It was seen that anemia affects the overall nutritional status of adolescent females.⁴

Nutritional problems during Adolescents period are causing impact not only in girl’s quality of life, also affect her family members and community as well. Anemia in adolescent age is one of the important public Health Problem which also increases morbidity significantly. Therefore the investigator thought that Nutritional Anemia is the most frequent among adolescent girls so they thought to do a research on assessment of the prevalence of anemia and knowledge regarding anemia among adolescent girls of selected schools of Gurugram.

Statement of Problem

A study to assess the prevalence of anemia and knowledge regarding anemia among adolescent girls of selected schools of Gurugram

Objectives of the Study

• To assess the prevalence of anemia among adolescent girls.

• To assess the knowledge regarding anemia among adolescent girls.

• To determine the association of knowledge score regarding anemia with selected demographic variables.

Review of Literature

A. noviani, D. Indarto, A. probandari (2017) conducted a cross sectional study on high prevalence
anemia in female adolescents with low intake of vitamins and minerals. Data was collected from 120 female adolescents girls aged between 13-17 years. Results illustrated that 37.5% female adolescents was found anemic and the result of chi-square analysis indicated a positive association between folic acid (OR=1.6), Magnesium (OR=1.17) and Manganese (OR=1.97) and anemia, but it was not significant. Inadequate vitamin B12 intake increased 1.78 times anemia risk. Thus, it is concluded that higher risk of anemia was found in female adolescents with inadequate intake of Vitamin B12 and Magnesium.5

Ahankari A.S, Myles P.R, Fogarty A.W, Dixit J.V, Tata L.J (2017) conducted a cross-sectional study on 13 to 17 years old adolescent girls living in 34 villages of Osmanabad district. Data was collected from 1010 adolescent girls on individual health, dietary, socio demographic factors, and anthropometric measurements were taken. Logistic and linear regressions were used to identify risk factors associated with IDA and Hb levels, respectively. Results demonstrated that 87% had anemia. The prevalence of anemia was 17% were having mild anemia followed by 65% were having moderate while 5% were having severe anemia. Therefore, it is concluded that anemia prevalence was extremely high among adolescent girls in rural area of Maharashtra. There is urgent need of comprehensive preventive intervention for the adolescent girl’s population.6

A.K. Arya, P. Lal, N. Kumar, S. Barman (2017) conducted a cross-sectional study to determine the prevalence of anemia among adolescent girls in an urban slum of Kanpur, Uttar Pradesh. Data was collected from 400 adolescent girls aged between 10-19 years. Results depicted that the prevalence of anemia among adolescent girls was found to be 78.5%, of which 40% had mild followed by 33% had moderate while only 5.5% had severe anemia. Thus it is concluded that anemia was significantly higher among the adolescent girls whose mothers were either illiterate or having only primary education and those belonging to lower socioeconomic status. A special attention is needed to improve their haemoglobin status through proper implementation of intervention programs such as iron and folic acid supplementation and dietary modifications along with special emphasis on health education.7

Material and Methods

The study was conducted from October 2019-December 2020. A quantitative research approach with descriptive Research design was adopted for the study. A total of 86 adolescent girls studying in Vinay public school, Gurugram, Haryana who met the inclusion criteria were selected using total enumerative sampling technique. The conceptual framework of the study is based on General system model. The tools consists of demographic variables, knowledge questionnaire and Sahli’s hemoglobinometer to assess the knowledge and prevalence regarding anemia. Reliability of the tool was established by Cronbach’s alpha method and it was found to be 0.84. Ethical permission was obtained from ethical committee SGT University and the written informed consent was taken from each participant before administering the tool. The data was analyzed and interpreted in terms of objectives of the study. Descriptive and inferential statistics were utilized for the data analysis. A p value ≤ 0.5 was considered as significant for the present study.
Findings

**TABLE 1: Frequency and % Distribution of adolescent girls in terms of selected demographic variables**

N= 86

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Demographic variables</th>
<th>f (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age(years)</td>
<td>13-14</td>
<td>37 (43%)</td>
</tr>
<tr>
<td></td>
<td>15-16</td>
<td>47 (54%)</td>
</tr>
<tr>
<td></td>
<td>Up to 18 years</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>2. Education</td>
<td>8th</td>
<td>16 (19%)</td>
</tr>
<tr>
<td></td>
<td>9th</td>
<td>30 (35%)</td>
</tr>
<tr>
<td></td>
<td>10th</td>
<td>21 (24%)</td>
</tr>
<tr>
<td></td>
<td>11th</td>
<td>13 (15%)</td>
</tr>
<tr>
<td></td>
<td>12th</td>
<td>6 (7%)</td>
</tr>
<tr>
<td>3. Religion</td>
<td>Hindu</td>
<td>85 (98%)</td>
</tr>
<tr>
<td></td>
<td>Muslim</td>
<td>1 (2%)</td>
</tr>
<tr>
<td></td>
<td>Christian</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>-</td>
</tr>
<tr>
<td>4. Type of family</td>
<td>Nuclear</td>
<td>33 (39%)</td>
</tr>
<tr>
<td></td>
<td>Joint family</td>
<td>49 (57%)</td>
</tr>
<tr>
<td></td>
<td>Extended family</td>
<td>2 (2%)</td>
</tr>
<tr>
<td></td>
<td>Single parent</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>5. Mother's educational status</td>
<td>No formal education</td>
<td>24 (28%)</td>
</tr>
<tr>
<td></td>
<td>Primary</td>
<td>39 (45%)</td>
</tr>
<tr>
<td></td>
<td>Secondary</td>
<td>14 (16%)</td>
</tr>
<tr>
<td></td>
<td>Undergraduate and above</td>
<td>3 (4%)</td>
</tr>
</tbody>
</table>
Cont... TABLE 1: Frequency and % Distribution of adolescent girls in terms of selected demographic variables

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Father’s educational status</td>
</tr>
<tr>
<td></td>
<td>a. No formal education</td>
</tr>
<tr>
<td></td>
<td>b. Primary</td>
</tr>
<tr>
<td></td>
<td>c. Secondary</td>
</tr>
<tr>
<td></td>
<td>d. Higher secondary</td>
</tr>
<tr>
<td></td>
<td>e. Undergraduate and above</td>
</tr>
<tr>
<td></td>
<td>Family income per month</td>
</tr>
<tr>
<td></td>
<td>a. less than Rs.10000</td>
</tr>
<tr>
<td></td>
<td>b. 10000-20000</td>
</tr>
<tr>
<td></td>
<td>c. 20000-30000</td>
</tr>
<tr>
<td></td>
<td>d. More than 30000</td>
</tr>
<tr>
<td></td>
<td>Area of residence</td>
</tr>
<tr>
<td></td>
<td>a. Urban</td>
</tr>
<tr>
<td></td>
<td>b. Rural</td>
</tr>
<tr>
<td></td>
<td>Type of diet</td>
</tr>
<tr>
<td></td>
<td>a. Vegetarian</td>
</tr>
<tr>
<td></td>
<td>b. Non vegetarian</td>
</tr>
<tr>
<td></td>
<td>c. Eggetarian</td>
</tr>
<tr>
<td></td>
<td>Onset of menarche</td>
</tr>
<tr>
<td></td>
<td>a. Yes</td>
</tr>
<tr>
<td></td>
<td>b. No</td>
</tr>
<tr>
<td></td>
<td>History of taking iron supplements</td>
</tr>
<tr>
<td></td>
<td>a. yes</td>
</tr>
<tr>
<td></td>
<td>b. no</td>
</tr>
<tr>
<td></td>
<td>Source of health information?</td>
</tr>
<tr>
<td></td>
<td>a. newspaper/ magazine</td>
</tr>
<tr>
<td></td>
<td>b. radio/ television</td>
</tr>
<tr>
<td></td>
<td>c. friends/ relatives</td>
</tr>
<tr>
<td></td>
<td>d. health professionals</td>
</tr>
<tr>
<td></td>
<td>Do you have knowledge regarding anemia?</td>
</tr>
<tr>
<td></td>
<td>a. Yes</td>
</tr>
<tr>
<td></td>
<td>b. No</td>
</tr>
</tbody>
</table>
Data presented in table 1 depicted that nearly half (54%) of adolescent girls belongs to age group 15-16 years. 35% of adolescent girls were studying in class 9th. Majority (98%) of adolescent girls belongs to Hindu religion. Most (57%) of adolescent girls were having joint family. Nearly half (45%) of adolescent girl’s mother had secondary education 36% of adolescent girls father were having secondary education. 38% of adolescent girls’ family income per month was Rs.10000-20000. Most (73%) of adolescent girls were from rural area of residence and most (70%) of adolescent girls were having vegetarian diet. Most (74%) of adolescent girls had attained onset of menarche and majority (84%) of adolescent girls had no history of taking iron supplements. Majority (61%) of adolescent girls were having no knowledge regarding anemia.

Findings revealed that majority of the participants (81%) of had inadequate level of knowledge followed by 19% with moderately adequate level of knowledge.

**TABLE 2: Prevalence and severity of anemia among adolescent girls.**

<table>
<thead>
<tr>
<th>Severity of anemia</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (10-11gm/dl)</td>
<td>37.5</td>
</tr>
<tr>
<td>Moderate (7-10gm/dl)</td>
<td>55.6</td>
</tr>
<tr>
<td>Severe (&lt;7gm/dl)</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Findings represented that 37 participants had moderate anemia followed by 27 participants with mild and only 5 with severe anemia. Findings also revealed that knowledge score ranged from 3-16. The mean knowledge score & standard deviation was 9.8±2.88. It is also concluded that area of residence had significant difference on knowledge score regarding anemia in adolescent girls. Whereas selected demographic variables of adolescent girls age, education, religion, type of family, mother’s education status, father’s education status, family income per month, type of diet, onset of menarche, history of taking iron supplements, sources of health information were not statistically significant at 0.05 level of significance.

**Discussion**

Findings in the present study revealed that half (54%) of the girls were between the age group 15-16 years. Nearly half (35%) of girls were studying in class 9. Most (73%) of adolescent girls were from rural area of residence. Most (57%) of adolescent girls were having joint family. Most (74%) of adolescent girls had attained onset of menarche while 26% of adolescent girls had not attained onset of menarche. The findings are consistent with the findings of the study conducted by Toppo M, etal\(^8\) where it was found that the most of girls (36.4%) belong to age group 14-16 years. Mostly (80%) of girls had attained menarche.

The result shows that majority (81%) of adolescent girls have poor level of knowledge followed by 19% adolescent girls having moderate level knowledge regarding anemia. Not a single girl had good level of knowledge regarding anemia. The findings are consistent with the findings of R.Sridevi\(^9\) where it was found that the maximum 43 (61.4%) adolescent girls are having inadequate level of knowledge regarding anemia, 26 (37.1%) adolescent girls are having moderate level of knowledge regarding anemia and 1 (1.5%) adolescent girls are having adequate level of knowledge regarding anemia.

**Conclusion**

The following conclusions were drawn from the findings of the study:

- Knowledge level affects the prevalence rate of anemia in adolescent girls.
- Majority of adolescent girls have poor level of knowledge followed by few who had moderate level of knowledge regarding anemia.
- Most of adolescent girls were from rural area of residence while some were from urban area.
Demographic variables mother’s educational status, father educational status, family income per month, had significant difference on knowledge score regarding anemia in adolescent girls.

Mostly of adolescent girls falls under moderate category of anemia.

Implication of The Study

The findings of the study have several implications which are discussed under the following areas.

Nursing Practice

- Nurses are the backbone of every health care set up of any country. The nursing practice has gone under many evolutions in the recent past. The expanded role of professional nurse is to emphasize the activities which include the promotive, preventive, curative and the rehabilitative aspects. The nurse educator has the responsibility to update the knowledge of adolescent girls. They should be able to identify and understand the knowledge regarding prevention of anemia in adolescent girls.

- The nursing personnel working in the community unit must be well acquainted with knowledge regarding the prevention of anemia in adolescent girls of age group 13-18 years.

- The study has an important implication in the nursing education and other fields. Nurse educator has the responsibility to upgrade the knowledge regarding prevention of anemia in the adolescent girls of age group 13-18 years.

- In service and continue education programs needs to be planned and implemented for the health care personnel to upgrade the knowledge regarding prevention of anemia in adolescents’ girls age group 13-18 years.

- The nursing educator/clinical instructor should be encouraged the adolescent’s girls and their parents to improve their knowledge regarding prevention of anemia among the adolescent’s girls age group 13-18 years.

- By adopting the different teaching strategies like lectures, seminars, related video and workshops regarding prevention of anemia can be disseminated effectively.

Nursing Administration

- Administrator should include the questionnaire for the adolescent girls to assess the knowledge regarding prevention of anemia among adolescent girls age group 13-18 years.

- The nurse as an administrator organizes and conducts teaching programs for the adolescent girls regarding prevention of anemia among adolescent girls age group 13-18 years.

- Nurse administrator should be aware about the general problem faced by the adolescent girls regarding prevention of anemia.

- Nurse administrator should ensure that there are adequate facilities of using different types of strategies to conduct the programs on prevention of anemia among adolescent girls age group 13-18 years.

Nursing Research

- Nursing research is a strong foundation for the evidence-based nursing. Hence nursing staffs and students should be encouraged to conduct research. Research provides knowledge regarding the prevention of anemia among adolescent girls age group 13-18 years.

- Research studies need to be explored in the other health care setting to make timely referral of parturient in order to save their lives as women’s health is Nation’s health.

Recommendations

Ø Similar studies can be undertaken on a large sample for making a more valid generalization.

Ø Study can be conducted on different samples.
Ø A comparative study can be conducted to assess effectiveness of structured teaching program with other instructional methods.

Ø A correlation study can be conducted to analysis of knowledge regarding prevention of anemia among adolescent girls age group 13-18 years.

Ø The study can be replicated on large sample size.

Ø A similar study can be done to assess the prevalence and knowledge regarding anemia in adolescent girls of urban population.

Ø A similar study can be performed for various diseases.

Ø A similar study can be done to compare the prevalence and knowledge regarding anemia in adolescent girls of urban and rural population.

Ethical Consideration: Ethical approval to conduct this study was obtained from DRC and the ethical committee of the Faculty of Nursing, SGT University.

Conflict of Interest: There is no conflict of interest among the authors.

Source of Funding: Self

References


Effects of Medical Hand Washing on Hand Flora among Staff Nurses

Kabita Dhami¹, Padmapriya S², Sasi Kumar S³

¹Lecturer, Nobel Medical College Teaching Hospital, Kanchanbari, Biratnagar, Morang, Nepal, ²Associate Professor, ³Professor, Yenepoya Nursing College, Yenepoya Deemed to be University, Mangaluru, Karnataka, India

Abstract

Background: One of the leading causes of hospital associated infection is providing patient care with contaminated hands as a result of transmission of microorganism from health care provider to patients and others. Hand washing is the most important and simple act of reducing the spread of transmission of infectious agent and reducing hand flora. Materials and Methods: The study was done to evaluate the effects of medical hand washing on hand flora among staff nurses working in a selected hospital, Bengaluru. One group time series design was used. Swabs were taken from a randomly selected sample of 25 nurses working in medical ward for hand flora analysis. Results: Study results revealed that before hand washing there were many colonies of hand flora were found. After medical hand washing there was a significant reduction in the number of colonies of the hand floras between the pre-test mean (O₁= 10.02) and post-tests mean (O₂= 4.23, O₃ = 4.02 and O₄ = 2.36) and was found to be statistically significant at H= 83.288 and P value = 0.001. Conclusion: Medical hand washing is an effective technique to reducing the hand flora among the staff nurses working in the different medical wards of hospital.

Key words: Medical hand washing, hand flora, Hand hygiene, Health care providers.

Introduction

Hand hygiene is an important infection control procedure which limits the spread of micro-organism by breaking the transmission. World Health Organisation (WHO) emphasise that inappropriate hand hygiene practices is a leading cause of health care-associated infections (HCAI). It has been recognised as a significant contributor to outbreaks of infectious diseases and is responsible for increase in the cost of treatment, prolong the hospital stay, and increase the cost of healthcare, all over the world¹-⁴. The nurses are the health care providers should learn to adopt hand washing before and after giving patients care as well as after contact with any possible source of contamination⁵. Nursing practices, such as direct touching, contact with bodily fluids, and wound care, can result in high levels of microorganism contamination. Even during relatively clean procedures, such as taking the pulse, measuring arterial blood pressure and taking temperature, nurses’ hands can become contaminated with anywhere from 100–1000 Colony Forming Units (CFU) of Klebsiella, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Enterococcus faecalis after patient contact. Transient flora on the hand is mostly acquired from the hospital environment or poor hygiene and is responsible for cross-infections. The number of microorganisms found on the hands of nurses as they are performing patient care procedures such as providing medication, dressing, sterilization and disinfection. The microorganisms that colonize the external layer of the skin temporarily are eradicated and reduced when hands are washed with soap and alcohol-based agents. But hand washing habits of nurses are thought to be poor.

Corresponding author:
Dr. Sasikumar S
MOB- 9620410946
E-mail: sashwya@rediffmail.com
for many reasons\textsuperscript{6-8}. This study was done to evaluate the extent to which medical hand washing practices are helpful on reduction of hand flora among staff nurses in a selected hospital.

**Materials and Methods**

The study was conducted after getting the written approval from the Institutional Ethics Committee Review Board. Individual permission and consent was obtained. The study was conducted at selected hospital, Whitefield, Bengaluru. The formal and written permissions were obtained from the hospital medical and nursing superintendent before conducting the study. Study used one group time series design based on the study objectives. The sample size was 25 staff nurses selected from different medical ward of the hospital using simple random sampling techniques. The sample size was estimated by use of power analysis with 90% confidence level and 5% significant level. The data was collected using structured questionnaire for demographic profile and observation checklist for assessing the steps of medical hand washing which prepared based on WHO guide lines. The hand floras were collected in a sterile manner using sterile cotton swab from the four different sites of the nurse’s hands that is from nail beds, in between fingers, palm and dorsum before and after hand washing each using different swab. After hand washing the hand floras were collected four times respectively at the interval of one and half hour each in the shift. In which the pre- test swabs were collected at the starting of the shift before which the nurse start their duty with hand washing, where the first non-participant observation was done and swabs were collected and then the procedure of proper medical hand washing was demonstrated by the researcher to the nurses and respectively the other three post-test swabs were collected at the interval of 1 and half hour. The swabs after collection were inoculated in the nutrient agar media in Biosafety cabinet by dividing the Petridis into four quadrants each, and then the media was incubated in an incubator for overnight at 37\textdegree C. Next day, the growth of hand flora was observed and number of colonies of hand floras was counted and each site different slides were prepared by investigator. Then smearing of slides was done using normal saline in biosafety cabinet and Grams staining of slides were done by investigator with the help of microbiologist the slides were observed in microscope to identify the hand floras and if hand floras were found as staphylococcus the further test was done to differentiate whether it was staphylococcus aureus or staphylococcus epidermidis with coagulases test by investigator and at last methicillin-resistant staphylococcus aureus (MRSA) test was done. The sample size estimation, data entering, analyzing, and graph plotting were carried out using SPSS 23.0 Version.

**Results**

Demographic characteristics: Table 1 reveals that frequency and percentage distribution of staff nurse demographic characteristics. Maximum numbers of staff nurses 21 (84%) were in the age group of 20-30 years. In case of sex 23 (92%) were female and 2 (8%) were male. With regard to qualification, majority of staff nurses 16 (64%) were GNM and 9 (36%) with BSc Nursing. The maximum number of years of experience of registered nurses, 21 (84%) were having below 8 years of experience and 4 (16%) were having above 8 years of experience. Majority of the staff nurses that is 19 (76%) have attended in-service education on hand washing. Figure 1 shows that percentage distribution of staff nurses base on following the hospital standard protocol. Out of 25 nurses most of the selected registered nurses follow standard protocols of the hospital that i.e,16 (64%) follows always, 8 (32%) follows sometimes and 1 (4%) never follows the standard protocol of hand washing of hospital.

The frequency distribution of staff nurses according to hand flora analysis: Table 2 shows that in pre-test (O\textsubscript{1}) observations maximum hand floras were Staphylococcus epidermidis 27, Micrococci 18, Gram positive cocci in pairs 9, Gram positive cocci in singles 7, Gram positive bacilli 7, Gram positive cocci in chains 6, Streptococci 3, Spore bearer bacilli 2, Staphylococcus aureus 2, Pneumococci 2, and the least were Diptheroids 1,Acienito bacter 1, and MRSA 1. Similarly in the first
post test (O2) observations the maximum hand floras were Staphylococcus epidermidis 19, Micrococci 18, Gram positive cocci in pairs 9, Streptococci 7, Gram positive cocci in singles 6, Gram positive cocci in chains 6, Entero cocci 4, Diptheroids 2, Gram positive bacilli 2, and the least were Spore bearer bacilli 1 and Gram negative bacilli 1. The second post-test(O3) observations the highest number of hand floras were Staphylococcus epidermidis 13, Gram positive cocci in pairs 7, gram positive cocci in chains 7, Gram positive cocci in singles 6, Gram positive bacilli 5, Spore bearer bacilli 4, Streptococci 4, Pneumococci 2, and the least were Micrococci 1, Diptheroids 1, Entero cocci 1, and Candida 1. Similarly, the third post-test(O4) observations the maximum number of hand floras were Micrococci 16, Staphylococcus epidermidis 13, Gram positive cocci in pairs 7, Gram positive bacilli 7, Gram positive cocci in singles 6, Gram negative bacilli 4, MRSA 3, Entero cocci 2, Staphylococcus aureus 2 and the least were Diptheroids 1 and streptococci 1.

Effectiveness medical hand washing on hand floras Table 3 shows that mean, standard deviation, standard error of the mean and Kruskal wallis test (H) value. It was found that there was a significant reduction in the number of colonies of the hand floras after medical hand washing between the pretest mean (O1= 10.02) and posttest mean (O2= 4.23, O3 = 4.02 and O4 = 2.36) and was found to be statistically significant at H= 83.288 and P value = 0.001. It revealed that medical hand washing was effective to reduce the hand flora.

Conclusion and Discussion

Hand hygiene is an important and simple way to prevent the spread of micro-organism. Proper hand washing reduces the 50% of hospital associated infection by breaking the transmission of infection from one person to other9. According to World Health Organization (WHO) per second 1,400,000 people worldwide suffer from the side effects of hospital infections. In developing countries, the rate of preventable infections induced by healthcare services was estimated 40% higher10. Hand hygiene and hand washing are considered to be a first step to control infections11. Some studies show that various factors such as knowledge, beliefs and attitudes can affect the hand hygiene compliance rates12. The spread of germs between humans can occur directly through hands, or indirectly through an environmental source by having contact with them (e.g. clinical equipment, opening of doors, toys etc.)13 Nurses are often exposed to the risk of coming in contact with biohazards. Even health professionals could also be vehicle for disease transmission. Nurses required hand hygiene as they are part of the health care workforce come into contact with patients14,15. Hand hygiene has now been recognised as one of the most effective intervention to control the transmission of infections in a hospital and education is an important tool to ensure its implementation. The study demonstrates that transient bacteria are present on the hands of health care workers but majority could be removed by proper hand hygiene. Research has shown that may healthcare-acquired infections (HAIs) can be prevented with good hand hygiene. In the present study different types of hand flora were identified in the nurses hands such as staphylococcus epidermidis, spore bearer bacilli, micro cocci, diptheroids, enterococi, gram positive cocci in singles, gram positive cocci in pairs, gram positive cocci in chains, gram positive bacilli, gram negative bacilli, acienitobacter, staphylococcus aureus, candida, pneumonia, streptococci and methicillin resistant staphylococcus aureus. Medical hand washing is performed to removes both transient microorganisms that colonize the superficial layers of the skin and normal flora that is microorganisms that live under the superficial cells of the stratum corneum. The numerous study have reported that hand hygiene was effective on reduction of rates of nosocomial infection in hospital and communities16-18 in which majority were causing both community and hospital acquired infections19. In the present study it was found that there was a significant reduction in the number of colonies of the hand floras after medical hand washing. The finding was also supported by the similar study that hand washing with plain soap and water for 15 seconds reduced transient floral load on the skin by 0.6 - 1.1 log10, whereas washing for 30 seconds reduced counts by 1.8 - 2.8
log_{10} 20. It’s the nurse’s responsibility to ensure that his or her patients receive the best care possible, which includes care provided with clean hands.

**Acknowledgement:** The author would like to thank all ethical committee members, participants for their support and cooperation.

**Conflict of Interest:** The authors declare no conflict of interest.

**TABLE 1: Frequency and percentage distribution staff nurses according to hand flora analysis**

<table>
<thead>
<tr>
<th>sl.no</th>
<th>Variables</th>
<th>Frequency</th>
<th>Percentage( %)</th>
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<td>&gt;30</td>
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<td></td>
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<tr>
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<td>GNM</td>
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<td>B.Sc</td>
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<td>36</td>
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<td>Years of experience</td>
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<tr>
<td></td>
<td>&lt;8 years</td>
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</tr>
<tr>
<td></td>
<td>&gt;8 years</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>In service education attended</td>
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<td></td>
</tr>
<tr>
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<td>Yes</td>
<td>19</td>
<td>76</td>
</tr>
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</table>

**Figure 1.** Bar diagram shows nurses who follows standard protocol of hospital N=25
### Table 2: Frequency distribution of microorganism between different observations

<table>
<thead>
<tr>
<th>HAND FLORAS</th>
<th>OBSERVATIONAL FREQUENCY</th>
<th>Pretest O1</th>
<th>Posttest1 O2</th>
<th>Posttest2 O3</th>
<th>Posttest3 O4</th>
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<tr>
<td>Staphylococcus epidermidis</td>
<td></td>
<td>27</td>
<td>19</td>
<td>13</td>
<td>13</td>
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<tr>
<td>Spore bearer bacilli</td>
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<td>2</td>
<td>1</td>
<td>4</td>
<td>3</td>
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<tr>
<td>Micrococci</td>
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<td>18</td>
<td>18</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Diptheroids</td>
<td></td>
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<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Entero cocci</td>
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<td>4</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Gram positive cocci in singles</td>
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<tr>
<td>Gram positive cocci in pairs</td>
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<td>9</td>
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<td>7</td>
</tr>
<tr>
<td>Gram positive cocci in chains</td>
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<td>6</td>
<td>6</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Gram positive bacilli</td>
<td></td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>7</td>
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<tr>
<td>Gram negative bacilli</td>
<td></td>
<td>4</td>
<td>1</td>
<td>-</td>
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<tr>
<td>Acienitobacter</td>
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<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Candida</td>
<td></td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Pneumococci</td>
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<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Streptococci</td>
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<td>7</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>MRSA</td>
<td></td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 3. Effectiveness of medical hand washing on hand floras.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Observations</th>
<th>Mean ±SD</th>
<th>SEM</th>
<th>Median</th>
<th>H Value</th>
<th>‘P’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand floras</td>
<td>Pretest O1</td>
<td>10.02±20.807</td>
<td>2.081</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posttest1 O2</td>
<td>4.23±12.418</td>
<td>1.242</td>
<td>1</td>
<td>83.288</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>Posttest2 O3</td>
<td>4.02±11.482</td>
<td>1.148</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posttest3 O4</td>
<td>2.36±4.464</td>
<td>0.446</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*significant at 0.05 level of significance
References


Effect of Helichrysum Oil on Toothache

Kamalli.M¹, Gayatri Devi R², Jothi Priya.A², Saravana Kumar.S³

¹Undergraduate Student, ²Assistant Professor, Department of Physiology, Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, ³Assistant Professor, Department of Anatomy, SEGI University, Malaysia

Abstract

Introduction: Toothache is the pain or inflammation in the tooth. A symptom of toothache includes sensitivity, gum bleeding, cavities etc. Toothache can occur due to improper dental care and oral hygiene practice which is one of the commonest reasons. The genus Helichrysum consists of about 600 species which are flowering in the family of sunflower. The other names are Italian strawflower and Immortelle. It grows on dry, rocky and sandy regions in the Mediterranean. It has anti-inflammatory, antispasmodic and analgesic properties. The aim of the study is to determine the effect of helichrysum oil on toothache.

Materials and Methods: The sample size of the study was 15 individuals with toothache who visited the dental clinics. Participants having periodontal problems, cardiovascular problems were excluded from the study. To every 2 drops of helichrysum oil, 1 ml of coconut oil was added. The intensity of the pain was measured by using the pain scale. The participants were given to apply it on the gingival area of the painful tooth. The patients were followed to know the intensity of pain after a few hours. The results were tabulated and then statistically analysed.

Results and Discussion: 27% of the participants have moderate pain after a few hours of application of oil. 26% of them have less pain. 13% of participants have very little pain. 27% of them do not feel any change in the intensity of pain. 7% have high pain.

Conclusion: From this study it was evident that helichrysum oil had an effect in reducing the toothache as helichrysum possess anti-inflammatory properties.

Keywords: Helichrysum oil; toothache; intensity of pain; anti-inflammatory

Introduction

Toothache is the pain or inflammation in the tooth. Symptoms of toothache include sensitivity, gum bleeding, cavities etc. Toothache can occur due to improper dental care and oral hygiene practice which is one of the commonest reasons. Generally, salt water rinsing is preferred as the first treatment for toothache as it is a natural disinfectant and reduces the inflammation followed by using a hot pack. Regular brushing and flossing will reduce the risk of toothache. Root canal treatment is required in case of intense toothache as it affects the nerves. Proper identification of severity of toothache is necessary as the infection may spread to other parts of the face.

The genus Helichrysum consists of about 600 species which are flowering in the family of sunflowers. The other names are Italian strawflower and Immortelle. It grows on dry, rocky and sandy regions in the Mediterranean. It has anti-inflammatory, antispasmodic and analgesic properties. There is a fact that Helichrysum was considered a very complex genus (¹). Antifungal property was exhibited by the species of Helichrysum (²). Helichrysum is an aromatic plant in the Asteraceae family of plants (³). Helichrysum being

Corresponding Author:
Dr Gayatri Devi R
Assistant Professor, Department of Physiology, Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Phone: +91 8248016505 Email: gayatri.physio88@gmail.com

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a traditional medicine, it possessed choleretic, diuretic and expectorant \(^{(4)}\). Helichrysum also showcases the properties of antibacterial activity which can be turned into medicinal usefulness. The Helichrysum decoction which is obtained by heating the roots of the plant can be used for the treatment of asthma \(^{(5)}\). Anti-inflammatory properties of Helichrysum italicum included inhibition of enzymes and scavenging activity \(^{(6)}\). In the genetics study of Helichrysum species aided the discovery of various chemotypes \(^{(7)}\). Anti viral and Anti-oxidant properties of the species were also identified \(^{(8)}\). Other species exhibited properties of a purgative and it can be used as herbal medicine for cold, cough, flu and fever etc \(^{(9,10)}\).

The primary aim of this study is to find the effect of Helichrysum oil on toothache. The analysis is based on different parameters like the effect on tooth ache, ability to reduce the pain and the duration of relief of pain determined by using pain scale.

**Materials and Methods**

The sample size of the study was 30 individuals with toothache who visited dental clinics. They were randomly selected. Participants having toothache only were included. Exclusion criteria which included are participants having periodontal problems, cardiovascular problems, diabetes. The Oil which was used for this study was Helichrysum italicum essential oil. To every 2 drops of helichrysum oil, 1 ml of coconut oil (carrier oil) was added. The intensity of the pain was measured by using the pain scale before the application of the oil. The participants were given to apply it on the gingival area of the painful tooth. The patients were followed to know the intensity of pain after a few hours of application. The results were tabulated and then statistically analysed.

**Pain scale:**

0-Nil, 1-Very mild, 2- mild, 3-Moderate, 4 – severe, 5- Very severe

**Results**

From this study, 27% of the participants have moderate pain after a few hours of application of oil. 26% of them have less pain. 13% of participants have very little pain. 27% of them do not feel any change in the intensity of pain. 7% of the participants have high pain. The time of relief of pain differs from individual to individual (Fig 1).

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**Figure 1: Pain sensation after usage of Helichrysum oil**

![Effect of Helichrysum oil](image_url)
Discussion

There are many studies done on pharmacological activities of Helichrysum. In the study by the authors Daniel Antunes Viegas et al., they reported that the promising pharmacological activity of Helichrysum italicum can be converted into useful medicines. The pharmacological activities i.e the anti-inflammatory properties and antimicrobial are best studied. The authors mentioned that it has considerable effect on inflammation in skin and gums (1). In the present study, it shows a positive effect in relieving the pain and inflammation in gums as mentioned in the previous study. The authors Angel Rumbero et al., in their study concluded that the results finalized that the co-occurrence of acidic phenolic hydroxyls and lipophilic residues is a significant chemical feature for the expression of antifungal property in Helichrysum species (2). In the present study, the time of relief varies from individuals and there is no constant time of relief that can be reported. In the study by Angioni et al., it was concluded that two chemotypes of the species showed an interesting antifungal activity (7). In the study by J.Mastelic et al., it was reported that there was an exhibition of antimicrobial activities related to the terpene fraction (11). The authors A Bianchini et al. concluded that there was an exhibition of different compositions (12). In the study by JB Hinou et al., it was reported that Helichrysum oil exhibited medicinal usefulness (13). Nostro et al., in their study concluded that Helichrysum showed anti-allergic properties as well (14). In the study by Teodara Jankovic et al., it was reported that Helichrysum can be used as an hepatoprotective agent which is significant for detoxification (15). In the study by Lourens et al., they reported that extracts of helichrysum exhibited promising activity in the anti-microbial tests (16). In the study by Juliano et al., they reported in their results that helichrysum as a therapeutic alternative in the treatment of opportunistic infections and stated that topical application has strong inflammatory action. In the present study, the topical application of oil aids in reduction of inflammation and pain which coincides with the statement of the previous literature (17).

Conclusion

From this study, it was evident that Helichrysum oil had an effect in reducing the toothache as it has anti-inflammatory properties and this study can be utilized as a basis for further studies.

Acknowledgement: We thank Saveetha Dental College for providing us the support to conduct the study.

Conflicts of Interest: The authors declare that there are no conflicts of interest in the present study.

Funding: None

Ethical Clearance: SRB (Scientific review board) of Saveetha dental college

References


Disorders of Kidney Function in Chronic Heart Failure

Kamilova Umida¹, Abdullaeva Charos², Atakhodjaeva Gulchehra², Beknazarova Saida², Masharipova Dilyafruz³, Ikramova Feruza³

¹Doctor of Science, Professor, ²Doctor of Science, ³Researcher, Department of Cardiology, SI “Republican Specialized Scientific and Practical Medical Center of Therapy and Medical Rehabilitation”, Osiyostreet 4, Uzbekistan Tashkent, Uzbekistan

Abstract

The formation of cardiorenal syndrome (CRS) in patients with chronic heart failure (CHF) is a natural manifestation of a functionally interrelated process at the organ level. Moreover, impaired renal function is a common and independent factor in the progression of the disease, a high incidence of cardiovascular events and death in a population of patients with asymptomatic and/or clinically manifested CHF, which is due to the pathogenetic features of the formation of cattle in patients with CHF of ischemic genesis. The article provides an overview of studies on the pathogenesis of kidney damage in CHF, the role of various markers in the early diagnosis of cattle. A decrease in glomerular filtration rate (GFR) and an increase in urinary albumin excretion are currently considered as “renal” markers of a poor prognosis within the cardiorenal continuum. In this regard, for stratification of patients by stages of chronic kidney disease (CKD) and, consequently, for assessing the risks of increased overall and cardiovascular mortality in patients with CHF, the accuracy of calculating GFR, as the main indicator reflecting the severity of formed kidney damage, is of great importance. Microalbuminuria is one of the early signs of CKD, the main cause of which is glomerular capillary endothelial dysfunction that occurs in most patients with CHF. The presence and persistence of subclinical renal dysfunction during treatment, even with the achievement of risk factors (RF) control and regression of organ lesions, can adversely affect the patient’s prognosis. Assessment of the functional state of the kidneys is important for early diagnosis, selection of effective preventive measures and treatment. The therapeutic strategy in patients with CHF in combination with CKD should be based on the nephroprotective effects of drugs.

Keywords: chronic heart failure, renal function, cardiorenal syndrome

Introduction

Chronic heart failure (CHF) due to the significant prevalence, high mortality rate is not only a medical, but also a social problem. Chronic heart failure (CHF) due to the significant prevalence, high mortality rate is not only a medical, but also a social problem. Impaired renal function is the most important predictor of poor prognosis in patients with CHF, even more significant than the severity of CHF and left ventricular ejection fraction (LVEF). The prevalence of impaired renal function in chronic heart failure (CHF), according to various studies, ranges from 25% to 60%.

Proteinuria and decreased glomerular filtration rate (GFR) are seen as a marker of a poor prognosis for cardiovascular disease (CVD), which is in line with the well-established concept of cardiorenal relationships. Proteinuria causes an increase in the synthesis of angiotensin II, endothelin and profibrinogenic growth factors, which participate in the development of renal damage in CHF.

Corresponding author:
Kamilova Umida,
Doctor of Science, professor, Department of Cardiology, SI “Republican Specialized Scientific and Practical Medical Center of Therapy and Medical Rehabilitation”, Osiyostreet 4, Uzbekistan Tashkent, Uzbekistan Phone: +998909798940
Email: umida_kamilova@mail.ru

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factors, transformed by mesenchymal and tubular cells. With GFR < 60 ml/min/1.73 m², the risk of mortality increases 2.1 times, with reduced left ventricular (LV) systolic function, the risk of death in patients with kidney failure (KF) increases 3.8 times, with unchanged systolic function - 2.9 times. LV ejection fraction in CHF, as the main indicator of LV dysfunction, determining the prognosis of CHF, so the level of creatinine is also an independent predictor of a poor prognosis of LTP.

The formation of cardiorenal syndrome (CRS) in patients with CHF is a natural manifestation of a functionally interrelated process at the organ level. Moreover, impaired renal function is a common and independent factor in the progression of the disease, a high incidence of cardiovascular events and death in a population of patients with asymptomatic and/or clinically manifested CHF, which is due to the pathogenetic features of the formation of CRS in patients with CHF of ischemic origin.

The kidneys are an integral and significant part of the microcirculatory system of the body, an important organ of metabolism and humoral regulation of various processes.

In the formation of CRS, the leading role is played by the activation of SAS, circulating RAAS, and renal tissue RAS, which trigger neurohumoral and microcirculatory mechanisms of damage to the glomeruli, tubules, and tubulointerstitial tissue in the kidneys.

It is known that ATII enhances sodium reabsorption, leads to contraction of smooth muscle cells of the arterioles in and out of the glomeruli and, consequently, to a decrease in renal blood flow. The narrowing of the efferent arterioles prevails over the narrowing of the efferent arterioles; therefore, in the early stages of CHF, despite a decrease in renal blood flow, renal perfusion pressure and filtration fraction increase, and normal GFR values remain.

Hyperfiltration promotes a decrease in hydrostatic pressure, an increase in oncotic pressure in the peritubular capillaries and an even greater increase in water reabsorption in the proximal tubules and the ascending part of Henle's loop. With the progression of CHF and a pronounced decrease in cardiac output, renal blood flow decreases so much that renal perfusion pressure, GFR decrease and the concentration of Cr in the blood serum increases.

In addition to the effect on renal hemodynamics and reabsorption of sodium and water, ATII, as shown in animal experiments, stimulates the production of transforming growth factor P by mesangial cells of the glomeruli, which increases the synthesis of extracellular matrix components such as biglycan, type I collagen and fibronectin. The accumulation of the glomerular matrix can lead to the development of nephrosclerosis, a morphological substrate of chronic kidney failure (CKF). Aldosterone, the synthesis and release of which is enhanced by ATII, like the latter, promotes sodium reabsorption, but works at the level of the distal tubules and collecting ducts. It should be noted that normally high doses of mineralocorticoids first increase renal sodium retention and extracellular fluid volume, but after 3-5 days' renal sodium retention ceases and sodium balance is restored.

This is the so-called mineralocorticoid-mediated sodium retention (escape phenomenon) mechanism, the development of which is due to an increase in sodium delivery to the sites of aldosterone action in the collecting ducts. In patients with CHF, the sodium-sparing action of aldosterone does not escape. This is due to a decrease in sodium intake to the distal tubules and collecting ducts due to an increase in its reabsorption under the action of ATII and adrenergic stimulation.

Microalbuminuria is one of the early signs of chronic kidney disease (CKD), the main cause of which is glomerular capillary endothelial dysfunction that occurs in most patients with CHF II – IV FC NYHA. This is especially true for persons with CHF of ischemic origin, which is accompanied by endothelial dysfunction of large arterial vessels and veins, coronary vessels, pulmonary vessels and vessels of the peripheral microvasculature.
The leading role in its formation is played by systemic oxidative stress, an increase in the content of circulating pro-inflammatory cytokines, which cause a loss of negative charges of the endothelium of glomerular capillaries and an increase in the permeability of the glomerular filter.

Another cause of kidney dysfunction (KD) is glomerular hypertension, which is detected at an early stage of HF progression and is associated with impaired renal hemodynamics. The resulting increase in pressure in the glomerular capillaries causes mechanical damage to the endothelium, glomerular basement membrane and podocytes, leading to an additional increase in the permeability of the glomerular filter.

A large number of works are devoted to the diagnostic role of β2-microglobulin and microalbuminuria in various diseases. The greatest value of β2-microglobulin has been proven in nephrology. With the help of this marker, it became possible to early detection and determination of the localization of damage, selective assessment of filtration, resorptive function, and determination of the degree of damage to the glomeruli and tubules. According to the literature, microalbuminuria is an indispensable criterion for early diagnosis of diabetic nephropathy.

The influence of CKD on the prognosis of CHF patients is closely related to the severity of renal function damage, which is manifested by the degree of decrease in the level of the calculated GFR. According to current recommendations, the group of persons with mild KD includes patients with a GFR level of 60–70 ml / min / 1.73 m², a serum creatinine content of 115–133 μmol / L (1.3–1.5 mg / dL) and 107–124 μmol / L (1.2–1.4 mg / dL) for men and women, respectively. The observation period was at least one year. During this period, 38% of patients died in the first group and 51% of patients in the second. The relative risk of total mortality in the group of people with mild KD increased 1.56 times (p <0.001) and 2.31 times in the second group. The results of the study showed that mortality in the general population of patients with CHF increases by 15% with each subsequent increase in serum creatinine by 44.3 μmol / L (0.5 mg / dL), starting from 88.6 μmol / L, and by 7% with another decrease in the level of the calculated GFR by 10 ml / min / 1.73 m².

In recent years, biomarkers of renal tubular damage have been used to detect renal dysfunction. A retrospective analysis of data obtained in 85 controlled studies, which included about 550 thousand patients with CKD of various origins, showed that GFR is critical for damage to the heart and blood vessels at about 75 ml / min / 1.73 m², below which an increasing increase in cardiovascular vascular morbidity and mortality. In this regard, for stratification of patients by stages of CKD and, therefore, for assessing the risks of increased overall and cardiovascular mortality in patients with CHF, the accuracy of calculating GFR, as the main indicator reflecting the severity of the formed kidney damage, is of great importance. Previously, for this purpose, the Cockcroft – Gault and MDRD formulas were used.

A decrease in GFR and an increase in urinary albumin excretion are currently considered as “renal” markers of a poor prognosis within the cardiorenal continuum. Microalbuminuria is one of the early signs of CKD, the main cause of which is glomerular capillary endothelial
dysfunction that occurs in most patients with CHF II – IV FC NYHA. This is especially true for persons with CHF of ischemic origin, which is accompanied by endothelial dysfunction of large arterial vessels and veins, coronary vessels, pulmonary vessels and vessels of the peripheral microvasculature. The leading role in its formation is played by systemic oxidative stress, an increase in the content of circulating pro-inflammatory cytokines, which cause a loss of negative charges of the endothelium of glomerular capillaries and an increase in the permeability of the glomerular filter.

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According to the literature, microalbuminuria is an indispensable criterion for early diagnosis of diabetic nephropathy. The CHARMS sub-study (n = 2310) showed a significant independent worsening of prognosis for the combined endpoint (overall mortality, cardiovascular mortality, and hospitalizations for acute decompensated heart failure (ADHF)) in patients with heart failure and microalbuminuria (risk ratio (RR) was 1.43 (1.21-1.69); p <0.0001 and 1.75 (1.39-2.20); p <0.0001, respectively) 28. The GISSI-HF study (n = 2131) also confirmed the role of micro- and macroalbuminuria as a predictor of overall mortality in patients with CHF with LVEF 33 ± 9% (RR = 1.42 (1.11-1.81); p = 0.005) and RR = 1.70 (1.16-2.50); p = 0.006) 9, 22.

In recent years, there is also more and more data on the importance of assessing the state and tubulointerstitial tissue of the kidneys, which, according to some researchers, is involved in the pathological process in cardiovascular diseases often before the glomerular apparatus 15. It is known that the cause of proteinuria can be not only increased glomerular protein filtration, but also impaired tubular reabsorption of the filtered protein. There is evidence that in the absence of proteinuria in 75% of diabetic patients, antibodies to the antigen of the nephritogenic fraction of the epithelium of the brush border of the proximal tubules are found in the serum25, 29.

Thus, the presence and persistence of subclinical renal dysfunctions during treatment, even when risk factors (RF) control is achieved and organ lesions regress, may adversely affect the patient’s prognosis. Assessment of the functional state of the kidneys is important for early diagnosis, selection of effective preventive measures and treatment. The therapeutic strategy in patients with CHF in combination with CKD should be based on the nephroprotective effects of drugs.

Conflict of Interests: None.

Ethical Clearance – Not applicable.

Sourse of Funding – Self

References


A Review of Environmental Screening on Strategic Formulation in Health Care Sector

Kasih Puji Utami¹, Nur Wening²

¹Doctoral Student, ²Associate Professor, Post Graduate Program of Management, University of Technology Yogyakarta

Abstract

This paper is a literature review that discusses the screening of the business environment in strategy formulation. The purpose of the review is to explore and connecting theories from various research. This paper identified 22 articles with related topics published the last decade. The results of this study show that screening of the external environment that is important to do is the general environment (politics, economy, socio-culture, technology, demography, and epidemiology) which plays a role of 66.66%. While internal environmental analysis that is important to do is resources (finance, physical, technology, human resources, innovation and reputation) and value chain management (infrastructure, HRM, technology development, purchasing, inbound logistics, operations, outbound logistics, sales and marketing, and services) played a role of 33.33%. Based on a summary related to research on internal and external factors that have been carried out in health facilities, it is known that industrial environmental factors and environmental factors of competitors are still minimally studied so that they can become opportunities for future research.

Keywords: Screening Business environment, internal environmental, external environmental

Introduction

High market competition and strict regulations related to the health sector make the implementation of strategic management important¹. There are many problems faced by health services such as patient dissatisfaction with the health sector to implement the best strategy in ensuring the quality of services². Changes in the business environment that take place very quickly in the health sector require the health sector to carry out environmental screening properly to create the right strategy formulation to achieve company targets³,⁴. Previous research has shown that environmental screening is important in supporting the formulation of strategies in the health sector. Previous research also shows that most health facilities have not implemented strategic management in their companies, namely 71.4% who have not implemented strategic formulations so that this research can help health facilities in examining what matters are important in screening the business environment⁵. However, there are not many studies that discuss environmental factors that are important to be analyzed in the strategy formulation process. His article contains a review of research that discussed environmental screening in the strategy formulation process. Article searches were carried out using the google, google scholar and mandeley databases. The review process was carried out on articles that discussed environmental screening in the strategy formulation process. The articles reviewed are articles published in the last 10 years. This review identified approximately 51 articles that fit the topic and were published in the last 10 years for further analysis and conclusion in this paper.
Literature Review

Freed David formulated that the process of creating a business plan was carried out in several stages, namely environmental screening in strategy formulation, strategy implementation and evaluation. Environmental screening includes internal and external environmental screening. The organization must continuously observe changes in the internal and external environment because changes in the internal and external environment will change the formulation of the company’s strategy, so the company must be adaptive to environmental changes. The development of increasingly advanced technology makes the process of screening the business environment easier, among others, by using Decision Support Systems which enables a company to achieve a competitive advantage. Health facilities are very complex and pluralistic organizations so that organizational knowledge, legitimacy and social capacities greatly influence the screening of the internal and external environment in health facilities. Besides, political influence also plays an important role in environmental screening at the stage of strategy formulation in health facilities.

Previous research has shown that there are several factors that are important in screening the business environment, including political factors, economic factors, socio-cultural factors, technological factors and epidemiological factors. Internal environmental factors that are important to be studied in business processes include resources (financial, physical, technology, human resources, innovation and reputation) and value chain analysis (infrastructure, HRM, technology development, purchasing, inbound logistics, operations and bound logistics, sales and marketing, and service). While the external environment that is important in screening the business environment includes the general environment (political, economic, socio-cultural, technological, demographic, epidemiological), industrial environment (threat of new entrants, bargaining power of suppliers, bargaining power of buyers, threats of substituted products), and the environment of competitors. These factors in the business environment are important to analyze in order to make sound and wise strategic decisions and solutions. The company must be able to analyze and control both internal and external factors to make strategic management successful.

The organization’s ability to conduct internal and external environmental screening will enable the organization to quickly extract information that is important for strategic management for the company. In the management of internal and external factors, the existence of external factors is the most difficult to predict and control by a company because they are outside the company, while internal factors are easier to screen, predict and control because they are in the company’s internal environment. Several studies have shown that screening of the company’s internal and external environment can have a positive impact on the performance of health facilities.

Method

This paper is a literature review that discusses the screening of the business environment in strategy formulation. The articles collecting by the google database, google scholar and mendeley. A review was conducted on articles with topic. Screening of the business environment in strategy formulation. We identified approximately 22 articles that were published in the last decade then analysis and drawing conclusions.

Results and Discussion

Based on a summary of various studies, it shows that the internal and external environments that are important to be screened for strategy formulation include the internal environment, namely resources and value chain management. Resources include finance, physical, technology, human resources, innovation and reputation. While value chain management includes infrastructure, HRM, technology development, purchasing, inbound logistics, operations, outbound logistics, sales and marketing, and services. If it is made in the form of a description of the composition of the internal and external environment in the formulation of a strategy based on research that has been done previously, it is as
follows

**Table 1. Summary of percentage of internal and external environmental factors that have been studied in health facilities.**

<table>
<thead>
<tr>
<th>Type of Environment</th>
<th>Type of analysis</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal Environment</td>
<td>Resource (finance, physical, technology, human resources, innovation and reputation)</td>
<td>22.22%</td>
</tr>
<tr>
<td></td>
<td>Value chain management (infrastructure, HRM, technology development, purchasing, inbound logistics, operations, outbound logistics, sales and marketing, and services)</td>
<td>11.11%</td>
</tr>
<tr>
<td>External Environment</td>
<td>General environment (politics, economy, socio-culture, technology, demography, epidemiology)</td>
<td>66.66%</td>
</tr>
<tr>
<td></td>
<td>Industrial Environment (threat of new entrants, bargaining power of suppliers, bargaining power of buyers, threat of substitute products)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Competitor Environment</td>
<td>-</td>
</tr>
</tbody>
</table>

Source 5,12,15,17–22

Based on table 1, it is known that the external environment has a greater composition than the internal environment in the environmental screening process for the formulation of process strategies in health facilities. External environmental screening (politics, economy, socio-culture, technology, demography, and epidemiology) holds a composition of 66.66% while internal environmental screening (finance, physical, technology, human resources, innovation and reputation, infrastructure, HRM, technology development, purchasing, inbound logistics, operations, outbound logistics, sales and marketing, and services) is only 33.33%. External environmental factors are the environment outside the company that continues to change rapidly, is unpredictable and difficult to control so that it plays a bigger role and is more difficult to analyze than the internal business environment within the company.

**Conclusion**

The internal and external environmental screening stage is an important stage in the strategy formulation process. Internal environmental factors consist of resources (finance, physical, technology, human resources, innovation and reputation) and value chain management (infrastructure, HRM, technology development, purchasing, inbound logistics, operations, outbound logistics, sales and marketing, and services). While external environmental factors consist of general environment (politics, economy, socio-culture, technology, demography, epidemiology), industrial Environment (threat of new entrants, bargaining power of suppliers, bargaining power of buyers, threat of substitute products), competitor Environment. Based on this review paper, it is known that external environmental screening has a more role in the strategy formulation process which has a 66.66% share, while internal environmental analysis has a 33.33% portion, this happens because the external environment is an environment outside the company that changes rapidly, not predictable and controlled so that the external environment screening while the internal environment of the company is in the company. In the end, the process of screening the external and internal business environment is a very important process for the formulation of strategies in achieving company goals, including in health facilities.
**Ethical Clearance:** No Need Ethical Clearance on this Research

**Source of Funding:** This research was funded privately and did not receive funding from any party.

**Conflict of Interest:** The author confirm that there are no conflicts of interest to disclose.

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Basal Cell Adenoma – A Report of a Rarity in a Relatively Rare Site

1Reader, 2Senior Lecturer, Department of Oral Medicine and Radiology, Madha Dental College and Hospital, Kundrathur, Chennai, Tamil Nadu, India, 3Senior Lecturer, Department of Oral Medicine and Radiology, Tham Moogambigai Dental College and Hospital, Dr.M.G.R Educational and Research Institute, Chennai, Tamil Nadu, India, 4Senior Lecturer, Department of Oral Medicine and Radiology, Ragas Dental College and Hospital, Chennai, Tamil Nadu, India

Abstract

Basal cell adenoma is a rare benign neoplasm of the salivary gland. It preferentially occurs in the parotid gland and upper lip during the sixth and seventh decades of life. The clinical presentation most frequently seen is a slow-growing, asymptomatic, movable, round or oval, normally coloured submucosal mass measuring less than 3.0 cm in diameter, encapsulated and well circumscribed. Histologically, the tumor consists of a proliferation of the terminal duct epithelial cells forming islands and sheets supported by a fibrous stroma, and also the presence of myoepithelial cells. There are 3 subtypes of basal cell adenoma: solid, tubulo-trabecular, and membranous. Although recurrence is rare, the membranous subtype, which is a hereditary variety of basal cell adenoma, has a 25% to 37% recurrence rate, possibly related to its multifocal nature, which impairs complete removal. [5]

Keywords: Basal cell adenoma, Monomorphic adenoma, Palate

Introduction

Basal cell adenoma is a very rare benign neoplasm of the salivary glands and name derives from the basaloïd appearance of tumor cells. It represents 54% of monomorphous adenomas and 1-3% of major salivary gland tumors and has an incidence of 7.5% among primary epithelial parotid gland tumors. [1] It occurs more common in the parotid gland and upper lip during the sixth and seventh decades of life, with female predilection (1:1.02). Palate is a rare site of occurrence. [2,3,4] Clinically, it presents as slow-growing, asymptomatic, round or oval, normally coloured well circumscribed submucosal mass. [1] Histologically, the tumor consists of a proliferation of the terminal duct epithelial cells forming islands and sheets supported by a fibrous stroma, and also the presence of myoepithelial cells. There are 3 subtypes of basal cell adenoma: solid, tubulo-trabecular, and membranous. [5] Although recurrence is rare, the membranous subtype, which is a hereditary variety of basal cell adenoma, has a 25% to

Report of a Case:

A 52 yrs old female patient reported with a chief complaint of slow growing asymptomatic swelling in the left side of the palate of 3 months duration. On General examination, patient was healthy. There was no abnormalities of TMJ and lymph nodes. No abnormalities on extra-oral examination. Intraoral examination (Fig 1) revealed a well-defined oval shaped smooth surfaced swelling on the left posterior hard palate, extending antero-posteriorly from the mesial aspect of 25 to the soft palate, Medio-laterally from palatal gingiva of 25-28 to 0.5 cm away from the midline, measuring approximately about 5x3 cms with no secondary changes and no surface pulsations. On palpation, the swelling was non tender, uniformly soft to firm in consistency, smooth, non-fluctuant, non-compressible, no blanching and does not yields on pressure, fixed to
the underlying tissues with well-defined borders with no discharge. Associated teeth 25, 27 were non-tender and vital. On aspiration, hemorrhagic tissue smear showed small clumps of plump spindle cells over a background of hemorrhage. Provisional diagnosis of **benign minor salivary gland tumor** in relation to the left posterior hard palate were considered. Differential diagnosis of Pleomorphic adenoma, monomorphic adenoma, Warthin’s tumour, Palatal abscess in relation to 27, Palatal mucocele were included.

![Image](image1.png)

**Fig 1.** Intra oral Picture showing swelling in the left side of the palate

On radiological investigations, IOPAR, Maxillary cross sectional occlusal view (Fig.2), OPG (Fig.3) and PNS (Fig.4) showed no radiographic abnormalities.

![Image](image2.png)

**Fig 2.** IOPA-24, 25, 26 region and Maxillary cross sectional occlusal view showing no abnormalities.
All laboratory findings were found to be within normal limits. Incisional biopsy of the lesion revealed relatively uniform, small, dark basaloid epithelial cells in the stroma, and is surrounded by a fibrous connective tissue capsule. Palisading at the periphery of the epithelial nests result in a ‘basaloid’ appearance (Fig. 5) and is diagnosed as Basal cell adenoma in the minor salivary gland of hard palate.
Discussion

Basal cell adenoma is a type of monomorphic adenomas. The salivary gland tumours are uncommon, which constitutes less than 3% of all neoplasms of head and neck. Although basal cell adenoma is the most common variant in the group of “monomorphic adenomas,” it represents only 1% of all salivary tumors. It is most prevalent in the sixth and seventh decades of life [6,7,8,9], however, in our case, the patient was in the fourth decade of life, in contrast to the literature and has...
female predilection. The basal cell adenoma occurs more frequently in the parotid gland, followed by upper lip. The development of these tumors in the buccal mucosa, palate, or lower lip is unusual. [2, 6, 7, 8] in the current case; the palatal location of the tumor did not fit the more frequent sites. Clinically, it presents as painless mass, enlarging slowly and frequently measures about 3-8 cms [10]

Malignant transformation to basal cell adenocarcinoma is rare but is suggested by some authors. Recurrence is rare, with the membranous subtype has a recurrence rate of 25% to 37% due to its multifocal nature, which impairs complete removal.[11] The treatment used in this case is the same proposed in the literature, consisting of complete surgical removal with an extra capsular limit. The patient had a satisfactory postoperative period, with complete healing of the operated area, and presents no signs of local recurrence 18 months after surgery

**Conclusion**

The present case, Basal cell adenoma is an uncommon salivary gland tumour presented in a relatively rare site. These rare cases should provide us an insight to the biologic behaviour and clinical course of the tumours and should be correlated with radiological & histopathological findings which help in accurate and timely diagnosis that can go a long way in the overall meticulous rehabilitation of the patient.

**Conflict of Interest-** Nil

**Source of Funding-** Self

**Ethical Clearance-** Nil (as it is a case report)

**References**


Molecular Characteristics of Iraqi *Lactobacillus plantarum* Isolates and Evaluate its Natural Bacteriocin (plantaricins) Antimicrobial Activity against Pathogenic Bacteria

Khadeeja S. Madhi¹, Mohammed H. Khudr¹, Rasha M. Othman¹

¹College of Veterinary Medicine, University of Basrah, Basrah, Iraq

Abstract

In this study, local strains of *Lactobacillus plantarum* bacteria were isolated from traditional Iraqi raw milk and milk products. The present study was aimed to study the molecular aspects of Iraqi *Lactobacillus plantarum* isolates and to evaluate its natural plantaricins antimicrobial activity against pathogenic bacteria. For complete detection for *Lactobacillus* spp., the partial sequencing of 16S rDNA gene result was analyzed and compared with those in GenBank to find out the different in the sequence using the BLAST program (http://www.ncbi.nlm.nih). The result was 17 samples diagnosed as *Lactobacillus plantarum*. Moreover, 14 isolates of local *Lactobacillus plantarum* bacteria were show 100% similarity with those previously recorded in GenBank, while 3 isolates were found to display more than 99% similarity with *L. plantarum* strains were previously listed in GenBank. Moreover, the study also reveals that all *L. plantarum* strains showed antibacterial activity against three studied indicators bacteria and the *plnEF* gene (530bp) was detected in all 17 isolates of *L. plantarum*.

**Key words:** *Lactobacillus plantarum*, molecular, bacteriocin, antimicrobial, pathogenic bacteria

Introduction

Bacteria in their life produce a different types of substances some of these substance have antibacterial activity. Plantaricins is a bacteriocin which is a unique group of substances, which contains of aminopoly-peptides with a bactericidal and moderately narrow anti-bacterial spectrum ¹ ². Currently, the newly purified bacteriocins have a wide range of anti-microbial uses particularly in food preservation besides the fields of medication ³ ⁴. Moreover, the lactobacilli are consider a common microorganisms which have been applied in manufacturing and human health, including food preservation and probiotics ⁵ ⁶. Additionally, the Lactic Acid Bacteria (LAB), are broadly used in the food production, especially in the fermented foods manufacture. Lactic acid bacteria are Gram positive bacteria, non-sporulating, anaerobic fermentative bacteria ⁷. In addition to the previous application of antimicrobial bacteriocin and bacteriocin-like inhibitory substances (BLIS), that produced by lactic acid bacteria, they also show a good health promoting, for enhancing the nutrition rate, regulator the infection of intestinal, and reduce of pathogenic microorganisms ⁸. Among the best significant LAB which are applied for making of fermented food products for example (meat, grass, and vegetable) is *Lactobacillus plantarum*. Several types of bacteriocins substances which produce by *L. plantarum*, was already described, as plantaricin (A), plantaricin (B), plantaricin (C), plantaricin (F), plantaricin (S) and plantaricin T ⁹.

*Lactobacillus plantarum* strains are produced plantaricin belong to class (IIb). These include two-peptide of bacteriocins, where in corresponding peptides for instance, PlnE/F and PlnJ/K which have a
synergistic influence in contrast to the target bacteria. The majority of antibiotics have specific targets at the site of the bacteria. The bacteriocins, by destructive the cytoplasmic membrane or inhibition of bacterial cell wall, or by both actions. The bacteriocins have a complex mode of action, development of resistance against bacteriocins is mostly not reportable. One of the significant limits within the widespread usage of most of the bacteriocins from Gram positive microorganisms is their incapability to eradicate the Gram-negative pathogens. On the other hand, many previous studies attempt to isolate the Lactobacillus plantarum from worldwide a variety of traditional cheese products, such as several Iranian, Italian cheese varieties, etc. on the basis of antimicrobial substances production and harmless characteristics, L. plantarum could be the suitable applicants for natural antimicrobial agent. Therefore, the present study was aimed to study the molecular aspects of Iraqi Lactobacillus plantarum isolates and to evaluate its natural plantaricins antimicrobial activity against pathogenic bacteria.

**Materials and Methods**

**Samples collection and isolation Lactic Acid Bacteria (LAB)**

One hundred twenty-three samples of raw milk and traditional milk products (cheese and yogurt) were used in this study. The samples of milks from cows and goats’ milk were collected from farms located at the local north of Basrah province, while the cheese and yogurt samples were collected from local markets. The samples have been collected under aspect condition in sterile bottles and kept in an ice-box and instantly transported to the laboratory of Microbiology. About 1 ml of milk sample was mixed thoroughly with 9 ml of sterilizedringer’s solution (1:4) for 60 s. A serial dilution has been made, then 100 µl from each diluteness were spreading on duplicate plated of MRS agar (HiMedia, India). The MRS inoculated culture were incubated under anaerobic conditions at 37°C for 48 h. Then ten colonies from inoculated plates which analogous to the maximum diluting were picked and subculturing for further tests.

On the other hand, cheese and yogurt samples were collected from different local markets in which 1 gm. of sample was inoculated into 9 ml of MRS broth (HiMedia, India) and incubated at 37°C for 48 h. under anaerobic conditions. Then 1 loopful of broth culture was spread on MRS agar plates and incubated at 37°C for 48 h. under anaerobic conditions at 37°C.

For further characterization of LAB, both Gram staining and catalase test were applied on all suspected isolates from raw milk, cheese, yogurt samples. Only Gram-positive and catalase-negative isolates were taken as presumptive LAB and stored at 4°C in MRS agar plates.

**DNA extraction and PCR analysis**

All suspect LAB isolates were initially cultured in MRS broth at 37°C for 48 h. Three ml of overnight culture were centrifuged at 14000 × g for 2 min., the supernatant was discarded and the pellet cells were collected. The bacterial DNA were extracted by using the Geneaid microbial DNA isolation Kit (Geneaid Biotech Ltd, China), following the instructions of the manufacturer.

For confirmation and identification of L. plantarum, the PCR was used on previously extracted DNA samples. The PCR reaction was completed in 20 µl reaction mixtures with 1 µl (10 pmol/µl) for each primer, 5 µl ready to use master mix (Bioneer/Korea) and the 5 µl of DNA template. The final volume was adjusted by adding 8 µl of sterile water to each reaction tube. The PCR conditions were initial denaturation of 95°C for 5 min. followed by 34 cycle of denaturation of 95°C for 30 s, annealing of 45°C for 45 s, and final extension at 72°C for 10 min. The PCR amplicons were then run on 1.5% agarose gel staining with ethidium bromide to check the successful of amplification. Agarose gel was used and visualized using a gel documentation system (E - graph – ATTO -Japan). Fragment size of approximately
319bp. positive gene was verified as positive for *L. plantarum* bacteria.

16S rRNA gene sequencing and phylogenetic analysis

The 5′ end variable region of the 16S rDNA was PCR amplified using 27F and 1525R primers (table 1). The PCR amplification reaction was performed in a total volume of 50μl, contains of 10μl master mix supplied from Bioneer /Korea and 10μl of genomic DNA, then 1μl of each primer was added to the reaction and the final volume was adjustment by adding the nucleus free water. Amplifications steps were carried out in a Thermal Cycler (Thermal Cycler, MJR), using the following program:

ThePCR condition was achieved with first denaturing phase of 95 °C for (5min) then 30 PCR cycles of 94°C (15s); 52°C(30s) and 72°C (2min). The final elongation cycle was conduct at 72 °C for (5min). The PCR amplification products then electrophoresis using 1.5% agarose gel already staining with ethidium bromide and subsequently visualize using gel documentation system. The DNA ladder was used as a molecular mass marker. The PCR product then sequenced by (Macrogen Inc./Seoul,South Korea Korea).The blast analysis are used then to distinguish comparable sequences in the https://www.ncbi.nlm.nih.gov (NCBI) database. The phylogenetic tree were constructed by the neighbor -joining method using the software MEGA (Version 10).

Identification of genes encoding bacteriocin production

The oligonucleotides primers used in the current study are mentioned in table 1. The PCR was achieved on17 DNA samples using a master mix from Bioneer /Korea under the following conditions: the initial denaturation at 94°C for 3 min, then 35 cycles of another denaturation step at 94°C (1 min.); annealing step at 58.5°C(1 min) and elongation step at 72 °C.(90 s.) and final elongation at 72°C (6 min.). The amplicons of PCR were electrophoresed on 1.5% agarose gel.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5′-3′)</th>
<th>Size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16rRNA: L. plantarum</td>
<td>-TCGGGATTACCAACATCAC-</td>
<td>319 bp</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>-CCGTTTATGCGGAACACCTA-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16rRNA: 27F and1525R</td>
<td>27F: 5′-AGAGTTTTGTATCCTGGCTCAG-3′</td>
<td>1525bp</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>1525R: 5′-AAGGAGGT GWT CCARCGCA-3′</td>
<td></td>
<td></td>
</tr>
<tr>
<td>planEF forward</td>
<td>-AGAGCAGCTATAGGTAGTAAATAGCTGTGA-</td>
<td>530 bp</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>-AAATAACATCATACAA GGGGATTATT-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1: The sequences of oligonucleotide primer used in the current study**

**Evaluation the ability of identified strains to produced active bacteriocin**

All 17 isolated were showed a significant bacteriocin producing, we select only one strain for further studies related to antibacterial activity and bacteriocin producing. Production of crude bacteriocin from isolated strain was tested according to method described previously 33.
The antibacterial effect of bacteriocin were screened in vitro for their antibacterial activity against: *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* using the paper disc-agar diffusion technique on Muller Hinton agar as a culture medium for antibacterial activity. The filter paper disks (6 mm in diameter) impregnated with different volume of crude bacteriocin (20, 40, 60 μl) and placed on the Petri plates. The plates were incubated for 24 hrs. at 37 °C. The inhibition zone diameters were measured in millimeters of inhibition zone around the disc. The pathogenic bacteria was supplied from Department of Microbiology, College of Veterinary Medicine, University of Basrah, Iraq. The percent bacteriocin activity was calculated using the following formula:

\[
\frac{B-A}{A} \times 100
\]

Bacteriocin activity (%) =

A = Disc diameter

B = Inhibition zone diameter

**Results**

A total of 28 Lactic Acid Bacteria (LAB) were isolated from the 123 collected samples of locally produced raw milk and traditional milk products (cheese and yogurt). These samples originated from different animal sources, including cows and goats. All of the isolates were Gram positive and catalase negative (table 2). On the other hand the PCR technique was done for these samples. The PCR results of 16rRNA specific primers revealed that out of 28 only 23 isolates were diagnosed as *Lactobacillus plantarum* (figure 1) which reveals the bands of 319bp.s was clear observed on the 1.5% agarose gel analysis. Moreover the DNA of the 23 isolates were sent to Macrogen (Seoul, South Korea) with the primer (27F-1525R), Averagely 1525bp was obtained per sequence, which was then compared with those in GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov). The result was 17 sample diagnosed as *lactobacillus plantarum* (table 2). In this table 14 isolates of local *Lactobacillus plantarum* bacteria were show 100% similarity with those previously recorded in GenBank, while 3 isolates were found to display more than 99% similarity with *L. plantarum* strains were previously listed in GenBank (table 3). The 16S rDNA gene sequences results of 17 isolates were used to construct the phylogenetic tree using MEGA10 software. The Neighbor -Joining method was used for tree constructed and for confirmed the results of the homology analysis with the higher query cover (above 99%) of national samples as shown in figure (2). For recognize between Iraqi samples and national sample from NCBI information a recording code was put before national sample with the country were it isolate. Finally the PCR results of plnEF gene specific primers revealed that all of the 17 isolates of *Lactobacillus plantarum* were have the plantaricin gene (figure 3). According to this figure the bands of 530bp was observed on the gel and represented the plnEF gene.
Table 2: The number and percentage of *lactobacillus plantarum* recovered from milk & milk products samples.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Sample no.</th>
<th>No. of MRS culture</th>
<th>No. of PCR Positive</th>
<th>No. of positive sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Milk &amp; milk products</td>
<td>123</td>
<td>28</td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>%</td>
<td>-</td>
<td>%22.76</td>
<td>82.14%</td>
<td>73.91%</td>
</tr>
</tbody>
</table>

Table 3: The identification results of 17-strains of *Lactobacillus plantarum* by 16S rRNA Sequences

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Source</th>
<th>Identities</th>
<th>GenBank ID</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lactobacillus plantarum</td>
<td>99.39%</td>
<td>MT020400.1</td>
<td>China</td>
</tr>
<tr>
<td>2</td>
<td>Lactobacillus plantarum</td>
<td>100.00%</td>
<td>CP034997.1</td>
<td>Slovakia</td>
</tr>
<tr>
<td>3</td>
<td>Lactobacillus plantarum</td>
<td>100.00%</td>
<td>MG754629.1</td>
<td>China</td>
</tr>
<tr>
<td>4</td>
<td>Lactobacillus plantarum</td>
<td>100.00%</td>
<td>MF369880.1</td>
<td>China</td>
</tr>
<tr>
<td>5</td>
<td>Lactobacillus plantarum</td>
<td>100.00%</td>
<td>MT109312.1</td>
<td>Egypt</td>
</tr>
<tr>
<td>6</td>
<td>Lactobacillus plantarum</td>
<td>100.00%</td>
<td>MT109312.1</td>
<td>Egypt</td>
</tr>
<tr>
<td>7</td>
<td>Lactobacillus plantarum</td>
<td>100.00%</td>
<td>MT109312.1</td>
<td>Egypt</td>
</tr>
<tr>
<td>8</td>
<td>Lactobacillus plantarum</td>
<td>100.00%</td>
<td>MT109312.1</td>
<td>Egypt</td>
</tr>
</tbody>
</table>
The plantaricins antimicrobial activity was tested in three volumes against each pathogenic bacteria. Plantaricins revealed highest antimicrobial inhibitory activity in 40 and 60μl concentration against pathogenic tested bacteria: Staphylococcus aureus (136% and 276.66% respectively) followed by Pseudomonas aeruginosa (1991.66%, 252.66%) while the inhibitory activity against E.coli was 166.66% and 219.33% respectively (table 4,5 and 6).
<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>crude plantaricins (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus</td>
<td>20µl 40 µl 60 µl</td>
</tr>
<tr>
<td>Mean Diameter of inhibition zone (in mm)</td>
<td>13.5 14.16 22.6</td>
</tr>
<tr>
<td>Mean diameter(mm) of inhibition zone (Ampicillin 25µg/ml)</td>
<td>12 13 20</td>
</tr>
<tr>
<td>Plantaricins activity%</td>
<td>125% 136% 276.66%</td>
</tr>
</tbody>
</table>

The disk diameter 6.0 mm.

Table 5: Antimicrobial activity of crude plantaricins at (20 µl,40 µl, 60µl) against E.coli.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>crude plantaricins (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>20µl 40 µl 60 µl</td>
</tr>
<tr>
<td>Mean Diameter of inhibition zone (in mm)</td>
<td>12.5 16 19.16</td>
</tr>
<tr>
<td>Mean diameter(mm) of inhibition zone (Ampicillin 25µg/ml)</td>
<td>10 13 15</td>
</tr>
<tr>
<td>Plantaricins activity%</td>
<td>108.33% 166.66% 219.33%</td>
</tr>
</tbody>
</table>

The disk diameter 6.0 mm.

Table 6: Antimicrobial activity of crude plantaricins at (20 µl,40 µl, 60µl) against P.aeruginosa.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>crude plantaricins (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.aeruginosa</td>
<td>20µl 40 µl 60 µl</td>
</tr>
<tr>
<td>Mean Diameter of inhibition zone (in mm)</td>
<td>12.83 17.5 21.16</td>
</tr>
<tr>
<td>Mean diameter(mm) of inhibition zone (Ampicillin 25µg/ml)</td>
<td>13 15 18</td>
</tr>
<tr>
<td>Plantaricins activity%</td>
<td>113.83% 191.66% 252.66%</td>
</tr>
</tbody>
</table>

The disk diameter 6.0 mm.

Discussion

The raw cows and goats milk and their derivative products are considered a good source for isolation of lactic acid bacteria (LAB) which having the probiotic properties and have the ability to produce a novel bacteriocinogenic materials like plantaricins. In this context, there is a several previous studies were focused on isolation and molecular characterization of lactic
acid bacteria and study their activity against other pathogenic bacteria \(^{34-36}\). In the present study we try to isolate the local strain of *Lactobacillus plantarum*, and study their potential antibacterial activity. The results of primary isolation indicated that 28 out of 123 were isolated based on culturing De Man Rogosa Sharpe (MRS) medium and catalase test, and 23 isolated on the bases of PCR. This results were in line with the results of \(^{37,38}\). They reported that MRS agar was used as routinely medium for isolation of LAB and suitable for lactobacilli bacteriocin assay.

On the other hand the final diagnosis were based on sequencing results in which 17 isolates were confirmed as *L. plantarum*. Since the genome sequencing plays an important role in the accurate identification of bacteriocinogenic LAB \(^{39}\). The phylogenetic tree was constructed based on 16S rDNA gene of local bacterial isolated and the results of sequences analysis were closely linked to those previously registered on the NCBI (National Centre of Biotechnology Information) from Slovakia, China, India and Egypt (figure.3). The phylogenetic analysis categorized all isolates into two main roots and each roots have different sub-roots or branching. In the figure (3) the first branch have shown the distribution of the national samples among with the Iraqi samples S9 and S2 in which S9 show extreme similarity with sample from Slovakia, while S2 share the same ancestor but it has a special branch. On the other hand the *Lactobacillus plantarum* local strain (S7 and S8) show more nearby with sample from Egypt. While *Lactobacillus plantarum* local strain S14 also share the same ancestor but it has the special branch. While the *lactobacillus plantarum* local strain (S5,S12,S3) were lacks to the national samples lead to distributed in special branch. Moreover, figure (3) also reveals that most *lactobacillus plantarum* local strain (S17,S16,S6,S4, S13 ,S10, S15,S11,S1) show similarity with sample from China and this result was in line with \(^{40}\).

In all genetics and molecular studies the phylogenetic tree was consider a good tool to see the kinship between species based on similarities or differences in physical properties such as sequence or genetic sequence of DNA or amino acid and protein \(^{41}\). So the differences in the distribution of *lactobacillus plantarum* local strain could be attributed to the mutation in 16S rDNA gene that led to some variation in the Iraqi isolates. This mean the Iraqi isolates of *lactobacillus plantarum* were extreme similar to the national strains and share the same sequence of (16S rDNA) gene because this gene is more constant and have a slight mutation rate and evolution and need very long time to change so it is used in the classification of the microorganisms in the world. Detection based on 16S rDNA is consider a reference means for bacterial identification and taxonomic studies. Additionally the using of 16S rRNA gene as housekeeping genetic marker for taxonomic and bacterial phylogeny analysis could be attributed to the exist of 16S rRNA gene in almost all bacteria, moreover the 16S rRNA genes (1500 bp) are huge enough for informatics and genetic analysis purposes \(^{43}\).

In the present study the primary screening for the ability of local *L. plantarum* isolates to produce antimicrobial activity (crude plantaricins) was assessed against three studied indicators *S.aureus*, *E.coli* and *P.aeruginosa* (table 4,5,6). In these tables the highest inhibitory activity was observed at 40μl and 60μl concentrations against *S. aureus* (166.66%, 219.33%) followed by *P. aeruginosa* (191.66% and 252.66%) while inhibitory activity reported against *E. coli* was (166.66% , 219.33%) for 40μl and 60μl respectively. The antimicrobial activity of crude bacteriocins have been reported to be inhibitory against several other bacteria \(^{44-46}\) and \(^{33}\). The results described in the present study are in agreement with the observations of \(^{20,21}\) who reported a highest inhibitory activity of bacteriocin producing *Lactobacillus* spp. against *S. aureus*, \(^{33}\) and against*S.aureus, E.coli* \(^{47}\). Furthermore, \(^{35}\) was observed that *L.plantarum* isolates from raw cow milk samples had a powerful antimicrobial activity against a set of indicator microorganisms.

Moreover, the plantaricins-encoding genes was detected in all *L.plantarum* isolates by polymerase chain reaction (PCR). The results were revealed that all
tested *Lactobacillus plantarum* strains (no = 17) hold plnEF genes. The results were agree with the results of 48 based on the observation of the PCR product by using specific primers for amplified the plnEF in *Lactobacillus plantarum* bacteria.

**Conclusion**

We concluded that *lactobacillus plantarum* local strains have share the same DNA sequences similarity with samples from Slovakia, China, India and Egypt. Moreover all *L.plantarum* isolates were have the genes responsible for production of bacteriocins which a provide a natural substitute for antibiotics. However, more further studies are necessary to improve quality and safety of these bacteriocins.

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**Conflict of Interest:** The authors state that there is no conflict of interest.

**Source of Funding:** self-funded

**Ethical Clearance:** Ethical clearance was taken from Research Ethical Committee of College (RECC)

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1572


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Outcomes of Fissurectomy and Advancement Flap in Management of Chronic Anal Fissure

Khairallah Muzhir Gabash1, Saleh Omair2, SarabMuhammed Zeki Radhi3

1Assist. Prof. Department of Surgery / College of Medicine / Wasit University, 2Lect., Department of Anatomy and Biology / College of Medicine / Wasit University, 3B.D.S.MSc Oral and Maxillofacial Radiology Wasit health Directorate

Abstract

Chronic anal fissure is a painful linear tear in the mucocutaneous junction of the distal part of the anal canal and involves the full thickness of mucosa, causing severe pain may last even hours after defecation. Usually, it occurs along the midline either anterior or posterior; if it presents off the midline, other pathology might be associated with. Unhealed anal fissure after 6-8 weeks is defined chronic, with the presence of other criteria, it unlikely heals with medical treatment, and surgery is mandatory after pharmacological treatment failure. Lateral internal sphincterotomy had high healing rate, but unfortunately, with risk of incontinence. Fissurectomy with complete excision of the fissure keeping the internal anal sphincter intact, and an advancement flap of well vascularized tissue is transferred to cover the base of fissure and improve healing. This study mainly aiming to analyze the outcome of fissurectomy with advancement flap procedure in the treatment of chronic anal fissure and highlight its advantage in terms of healing rate and complications especially anal incontinence. During a period of about 5 years 345 patients (260 males and 85 females), have been included in this study and were diagnosed as cases of chronic anal fissure. After administration of local anesthetic, fissurectomy with advanced flap was performed. Patients had been followed up regularly for 6-24 months. Data collected and statistical analyses by using SPSS. About 75% of patients were males while, 25% were females, their ages were between 20-59 year, most of the patients were in thirties, nevertheless most of the females were in twenties. Mean time for wound healing was 10 days. Whilst complete fissure healing process lasting approximately one month. About 11.9% of the patients take more than one month to complete healing process. Healing rate was 100% after the follow up period of 6-24 months. Recurrence developed only in 2% of the patients. None of the patients developed incontinence, anal stenosis, key hole deformity, necrosis or urine retention. From this study concluded Fissurectomy with advancement flap in the treatment of chronic anal fissure is cost effective procedure, can be done under LA with least time-off work, with low complication, low recurrence, and no incontinence.

Key words: Fissurectomy, Chronic, Anal canal, Fissure, Tissue and Flap

Introduction

Fissure has been known as a painful, linear tear in the mucocutaneous junction of the distal part of the anal canal, which usually involves the epithelial layer only in acute stage and involves the full thickness of mucosa in chronic stage1. It develops with equal frequency in both males and females, but it tends to have predilection for younger and middle age group1,2. Anal fissure causing severe pain may last even hours after defecation and it is accompanied either with constipation or diarrhea1. The exact etiology is not understood, yet, but physical trauma stands at the top list(2,3). Anal fissure mainly occurs in the midline either posteriorly or anteriorly, if it presents off the midline, other pathology might be associated such as crohn’s disease, sexually transmitted disease, granulomatous disease and neoplasms4,5. Unhealed anal fissure after 6-8 weeks is considered chronic, with the presence of other criterialike skin tag, hypertrophied papilla, raised margin and exposed internal sphincter.
muscle fibers\textsuperscript{4,5,6}.

Chronic anal fissure was unlikely healed with medical treatment and surgery is mandatory after pharmacological treatment failure \textsuperscript{5}. Lateral internal sphincterotomy was first described in 1835, it was the treatment of choice, with high healing rate, but unfortunately risk of incontinence may reach to 30\% based on previous studies\textsuperscript{7,8}. In fact, this increased the need for new surgical procedure that preserve the anal sphincter integrity. Notably, Fissurectomy is the most commonly practiced procedure, however a possible drawback is keyhole defect and guttering that may lead to fecal soiling\textsuperscript{4}. So, Fissurectomy either with pharmacological agent like botulinum or with advancement flap introduced to overcome this complication\textsuperscript{6}. Fissurectomy has been defined as a complete excision of the fissure keeping the internal anal sphincter (IAS) intact, while anal advancement flap (AAF) is the transfer of well vascularized tissue to cover the base of fissure to improve healing. These procedures when combined together give high healing rate with less complication specially incontinence\textsuperscript{9}. Island advancement flap, V-Y flap, rotational flap, sliding skin flap and house advancement flap different in shape but, all induce and accelerate healing process\textsuperscript{9,10}. Other option is Fissurectomy with injection of 25U of botulinum toxin into IAS, to avoid lateral internal sphincterotomy (LIS), this combination had good healing with low incontinence\textsuperscript{11}. Patient who doesn’t heal or has recurrent anal fissure should be evaluated using anoscope and rigid proctosigmoidoscopy to exclude other pathology. Current study aiming to extensively analyze the outcomes of Fissurectomy with advancement flap procedure in the treatment of chronic anal fissure and highlight its advantage in terms of healing rate and complications especially anal incontinence.

**Methods**

From Sep. 1\textsuperscript{st}, 2015 to Aug. 31\textsuperscript{st} 2020, 395 patients (305 males and 90 females) were attending to private clinic in Al-Kut city /Wasit province/ Iraq. They were complaining of variable symptoms mainly: anal pain during and after defecation, passage streak of blood, constipation, itching, and were diagnosed as cases of chronic anal fissure.

During this period of about 5 years only 345 patients; 260 males and 85 females, were included in this study, while the others were excluded on the base of the following criteria:

**Exclusion criteria:**

* Patient’s age less than 20 years and older than 59 years.
* Patients with associated pathological conditions with fissure like piles, anal fistula and anal wart.
* Multiple or lateral fissures.

Following history taking, general and local clinical examination of patients’ perianal and anal region by putting the patient in left lateral position with good light illumination and gentle inspection of the anal region they found to have signs of chronic anal fissure mainly posterior and anterior midline of variable duration which were more than 6 weeks and they were tried conservative medical treatment but, they didn’t respond for this type of medical treatment.

Explanation of the disease process for the patients, decision and consent were taken for surgical interference for those patients. Then preparation by positioning the patient in left lateral position, shaving the operative area, using plastering strap on the both buttocks for separation to get good access, scrub by povidone iodine, draping, administration of local anaesthesia; lidocaine with adrenaline 1:100000, has been performed. After good infiltration we use electro cautery for excision of the fissure edges and its skin tag up to anal papilla, proximal to the chronic anal fissure providing a fresh mucocutanous edges, meticulous hemostasis was done from the base, limited undermining of skin and mucosal side were practiced to achieve tension free flaps on both sides, advancing and suturing the 2 flaps transversely to avoid anal narrowing and decrease jeopardizing the circulation and covering the row area.
by the 2 mucocutaneous flaps. The suturing is done by using 40 vicryl suture ininterrupted manner, dressing the operative area and discharging the patient on antibiotic cover, analgesia, sometimes laxative and special advice regarding personal hygiene; using salty water for washing the area to decrease congestion and pain. In addition, dietary advices concerning high fiber diet, and avoiding pepper and spicy food, then to be seen 10 days later followed by regular visit until complete healing take place. This was done in outpatient clinic. Period of following up range from 6 to 24 months, depending on the presence or absence of postoperative complications. Data analyzing has been performed using SPSS version 13.0, and described in terms of frequency and percentage.

**Results**

About 345 patients out of 395 attending the private clinic were included in this study 75.37% (260) of them were males while, 24.63% (85) were females. Their ages ranged 20-59 year with a mean of 36.06 (St=±0.037), their age distribution is shown in table no. 1, which shows that most of the patients were in thirties, nevertheless most of the females were in twenties (table 1).

<table>
<thead>
<tr>
<th>Table 1, gender* age by categories Cross tabulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
</tr>
<tr>
<td>Age by categories</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Twenties</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Concerning clinical characteristics of CAF, it sites were along the midline, anteriorly in 18.55% and posteriorly in 81.45%, this after the exclusion of patients with multiple fissures and those with fissures outside the midline. (Table 2). Presence of other signs of chronicity like skin tag and papilla were frequent, about 36% of patients had hypertrophied papilla, 48% had skin tag and both signs were present in 15% of patients (Table 3).

<table>
<thead>
<tr>
<th>Table 2: Shows the gender distribution of fissure site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site of fissure</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Anterior</td>
</tr>
<tr>
<td>Posterior</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
Table 3: shows distribution of sign of chronicity between genders

<table>
<thead>
<tr>
<th>Sign of chronicity</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertrophied anal papilla</td>
<td>90</td>
<td>35</td>
<td>125</td>
<td>36.23</td>
</tr>
<tr>
<td>Skin tag</td>
<td>121</td>
<td>45</td>
<td>166</td>
<td>48.12</td>
</tr>
<tr>
<td>Both signs</td>
<td>49</td>
<td>5</td>
<td>54</td>
<td>15.65</td>
</tr>
<tr>
<td>Total</td>
<td>260</td>
<td>85</td>
<td>345</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean time for wound healing was 10 days. Whilst complete fissure healing process took about one month. About 11.9% (41) of the patients take more than one month for complete healing process due to either infection or wound dehiscence. Interestingly, healing rate was 100% after the follow up period of 6-24 months.

Nearly 14.49% (50) of patients underwent surgery have developed complications like infection, partial breakdown or recurrence as shown in (Table 4). Importantly, none of the patients developed incontinence, anal stenosis, key hole deformity, necrosis or urine retention.

Recurrence developed in 7 patients (2%), all of the new fissures developed after one year and in another site. None of them need surgical intervention. Wound dehiscence or partial breakdown in the flap arises in 23 patients comprises 6.67% of the sample, however all patients healed without surgical intervention.

Table 4: Shows the frequency of complication in relation with gender

<table>
<thead>
<tr>
<th>Complication</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>14</td>
<td>6</td>
<td>20</td>
<td>5.79</td>
</tr>
<tr>
<td>Partial breakdown</td>
<td>16</td>
<td>7</td>
<td>23</td>
<td>6.67</td>
</tr>
<tr>
<td>Recurrence</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>2.02</td>
</tr>
<tr>
<td>Incontinence</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anal Stenosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flap Necrosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Urine retention</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Keyhole deformity</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>15</td>
<td>50</td>
<td>14.49</td>
</tr>
</tbody>
</table>
Discussion

In a period of five years 345 patients were included in the current study. Patients were diagnosed as chronic anal fissure (CAF), most of them 75.37% were males and only 24.63% were females. Nevertheless, Mapel et al in their cohort study (2014) calculated more percentage in female which was 55.8%. This controversy may be due to social and religious factors that made females in our society hide their medical problems or seek for a female surgeon. But in both studies the higher number of females was in twenties and for males was in middle aged groups12.

Notably, observations of present study illustrate that Fissurectomy with advancement flap in patients with CAF regardless of internal anal sphincter (IAS) tone has a good outcome with minimum postoperative complications, low recurrence rate and no incontinence.

Wound dehiscence is a known complication of Fissurectomy with advancement flap. In this study partial breakdown of the wound was the major complication 6.67%, that is near to the percent of Patti et.al.(2012), Pateland Aarakiet.al. which were (5.1%), (8%) and (5.9) respectively13. We suppose that this type of complication could be attributed to abuse of topical steroid-containing drugs by the patient him/herself before surgery. However, none of these patients needed repeated surgical intervention. Infection is a minor complication of any wound healing especially after Fissurectomy. We found that infection rate was mild and responded to antibiotic therapy in 5.79% of patients involved in the current study in contrast to Patti et.al study, where infection was developed in 5 per each 48 patients(10.4%) involved in the study6. Patti et al recorded 8% recurrence rate, all of them responded to medical treatment and didn’t need surgery6. Giordano et.al. reported new fissure development in 3 patients (5.9%) one of them need surgical treatment and the others treated medically10. While in this study the recurrence rate was only 2%. All recurrences developed after 12 months and treated medically. Our data demonstrate that the complete healing was achieved in a mean period time of 30 days.

Delayed healing process was observed only in about 12% of the patients, it took about 40-45 days for healing. Based on study done by Patti et. al.2012 and another study in the same year, refer that all their patients healed within 30 days 6,14.

Furthermore, Fissurectomy with advancement flap is known as sphincter saving procedure, since the sphincter remains intact14. Consistently, Fissurectomy alone without flap might raise the risk of keyhole deformity that leads to stool soiling. As mentioned previously in this study we excised fissure completely and apply a mucocutaneous flap to substitute the defective anal canal, this provides good blood nourishment, thus facilitate healing and minimize or prevent complications like fecal incontinence, keyhole deformity, stenosis and flap necrosis. Sahebally et.al. in his review and analysis of 4 studies’ results, compared between AAF and LIS and conclude that AAF had less incontinence than LIS but, same other complications associated with healing process9. It is worth to mention that in addition to low wound complication and nil incontinence rate, the surgery in this study was done under LA, in outpatient clinic and with short resting time.

Conclusion

Fissurectomy with advancement flap in the treatment of CAF has the advantages of:

1. It’s done under LA.
2. Least time-off work
3. Cost effective method.
4. Low complication and nil incontinence since it preserves the IAS.
5. Low recurrence rate.

Source of Funding: Self

Conflict of Interest: Non

Ethical Clearance: The study was performed under the guide lines supervision of Ethical Committee for lab.
References


Evaluation of Leachate Quality at Municipal Solid Waste Landfill Site: Case Study in Sliwung, Situbondo Regency, East Java, Indonesia

Khoiron 1,2, Ari Probandari 3, Wiwik Setyaningsih 4, Heru Subaris Kasjono 5

1Postgraduate Program, Department Of Environmental Science, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia, 2Department of Environmental Health, Public Health Faculty, Universitas Jember, Jember, East Java, Indonesia, 3Department of Public Health, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia, 4Department of Architect, Faculty of Engineering, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia, 5Department of Environmental Health, PoltekkesKemenkes Yogyakarta, Yogyakarta, Indonesia

Abstract

Objective: This study aims to evaluate the quality of landfill leachate that has been treated in a wastewater treatment plant.

Methods: Leachate samples were taken for 12 months (January-December) in 2018. The leachate quality parameters studied were: BOD 5, COD, TSS, total N, and heavy metals (Hg and Cd).

Results: The results showed that the BOD 5 quality met environmental quality standards in May, June, July, September, October and December, COD exceeded environmental quality standards, TSS met environmental quality standards in February, March, August, and December, N total met quality standards environment for 11 months, only December did not meet environmental quality standards, heavy metals (Cd and Hg) were below environmental quality standards.

Conclusion: The Sliwunglandfill to conduct an approach study that can reduce the BOD 5, COD, and TSS values so that they do not exceed the specified quality standards.

Keywords: leachate, coagulation, landfill, municipal solid waste

Introduction

Waste is a on of the world problem and not only in Indonesia, in several developed countries waste is a complex problem that causes environmental pollution. Symptoms of population growth correlate with waste production 1. In Indonesia, plastic waste is a kind of waste that is often found in several areas, especially in big cities. The most of plastic waste is produced by urban areas. The excess waste capacity and the lack of optimal waste management causes the waste to be carried by the river to the ocean.

The government in conducting management and processing efforts, forms a shelter known as a final disposal site (landfill). The garbage that has accumulated and is not immediately handled will produce looking waste which is called leachate. Leachate is liquid waste that contains harmful organic and inorganic materials 2,3. The leachate with high concentrations also has the potential to contain heavy metals Fe, Cr, Hg, Pb, and Cl 1. In Indonesia, the leachate management has been regulated in the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number P.59/Menlhk/Setjen/Kum.1/ 7/2016 concerning the quality standard of leachate. In this regulation, the government can assess and evaluate the management of leachate in each landfill which is spread throughout Indonesia.
The process of leachate formation occurs when large amounts of buried waste are wetted by rainfall, causing sediment to occur in the soil\textsuperscript{2,5}. The leachate leaks will cause dangerous impacts ranging from environmental pollution and health problems\textsuperscript{6,7}. The pathogenic bacteria produced exceeds the ferocity of \textit{Escheria coli}. The contamination of soil and water will cause a decrease in the biodiversity of biota components and a decrease in environmental quality.

Landfill of Sliwung is the final waste management sites in Situbondo Regency, East Java, Indonesia. The purpose of this study is to evaluate the feasibility of a waste management site based on the quality standards set by the government. This assessment can be a recommendation and study in increasing efforts to improve the quality of waste management to maintain environmental sustainability and safety.

\textbf{Methods}

\textbf{Research Sites.} This research was conducted in January - December 2018. The Landfill Sliwung is located in Situbondo Regency, East Java, Indonesia with a coordinate point of 7°45’16.16”S South Latitude and 114° 1’8.51”E East Longitude. Situbondo Regency has an area of 1,638.5 KM\textsuperscript{2}.

\textbf{Kind of Study.} This study was included in quantitative descriptive. The data obtained will be analyzed descriptively using literature references. Scientific studies will be a method of comprehensive discussion analysis by referring to several sources of research.

\textbf{Research procedure.} Leachate samples were taken 12 times each month from January to December 2018. At the time of sampling, the research also recorded whether it was rainy or dry. Sampling is located at a single location. This is because the sampling based on the source of pollution is included in the point sources discharges\textsuperscript{8}. The collected samples were then analyzed in an accredited laboratory, namely PT. JasaTirta I. The parameters observed in this study refer to the provisions of the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number P.59/ Menlhk/Setjen/Kum.1/7/2016. The methods used in the analysis of leachate can be seen in table 1 below.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Nomor & Parameter & Method & Characteristic \\
\hline
1 & BOD5 & APHA. Ed. 20.5210 B, 1998 & Chemical \\
\hline
2 & COD & QI/LKA/19 (Spectrophotometri) & Chemical \\
\hline
3 & TSS & QI/LKA/23 (Spectrophotometri) & Chemical \\
\hline
\hline
5 & Cd & APHA. Ed. 21.3111 B, 2005 & Chemical \\
\hline
6 & Hg & QI/LKA/56 (HVG) & Chemical \\
\hline
7 & Temperature & QI/LKA/12 & Physic \\
\hline
8 & pH & QI/LKA/08 & Physic \\
\hline
9 & Smell & - & Physic \\
\hline
10 & Total Coliform & QI/LKA/53 (Double Tub) & Biological \\
\hline
\end{tabular}
\caption{Parameters observed and the method of analysis}
\end{table}
The existence of the quantity of leachate is influenced by the season in a region. So that environmental conditions affect the concentration level of the leachate produced. The seasons in Indonesia consist of the dry and rainy seasons. So table 2 shows the recording of the sampling process based on season conditions and the date of collection.

**Table 2. Sampling, Analysis Date, and season**

<table>
<thead>
<tr>
<th>No.</th>
<th>Sampling date</th>
<th>Analysis Date</th>
<th>Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26 January 2018</td>
<td>26 January-09 February 2018</td>
<td>Rainy season</td>
</tr>
<tr>
<td>2</td>
<td>20 February 2018</td>
<td>20 February – 6 March 2018</td>
<td>Rainy season</td>
</tr>
<tr>
<td>3</td>
<td>22 March 2018</td>
<td>22 March - 6 April 2018</td>
<td>Rainy season</td>
</tr>
<tr>
<td>4</td>
<td>6 April 2018</td>
<td>6 – 20 April 2018</td>
<td>Drainy season</td>
</tr>
<tr>
<td>5</td>
<td>9 May 2018</td>
<td>9 – 24 May 2018</td>
<td>Drainy season</td>
</tr>
<tr>
<td>6</td>
<td>28 June 2018</td>
<td>28 June – 12 July 2018</td>
<td>Drainy season</td>
</tr>
<tr>
<td>7</td>
<td>24 July 2018</td>
<td>24 July – 7 August 2018</td>
<td>Drainy season</td>
</tr>
<tr>
<td>8</td>
<td>31 July 2018</td>
<td>31 July – 14 August 2018</td>
<td>Drainy season</td>
</tr>
<tr>
<td>9</td>
<td>20 August 2018</td>
<td>20 August – 4 September 2018</td>
<td>Drainy season</td>
</tr>
<tr>
<td>10</td>
<td>12 October 2018</td>
<td>12 – 26 October 2018</td>
<td>Rainy season</td>
</tr>
<tr>
<td>11</td>
<td>09 November 2018</td>
<td>09 – 26 November 2018</td>
<td>Rainy season</td>
</tr>
<tr>
<td>12</td>
<td>6 December 2018</td>
<td>6 - 20 December 2018</td>
<td>Rainy season</td>
</tr>
</tbody>
</table>

**Results and Discussion**

The management of leachate in Indonesia is regulated in accordance with the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number P.59/Menlhk/Setjen/Kum.1/7/2016 concerning leachate quality standards (Table 3). Efforts to manage and treat leachate are very important to minimize the pollution load they cause. Table 3 shows some of the specific observational parameters of leachate. The presence of leachate becomes a pollutant when it exceeds the specified quality standards. The presence of leachate is influenced by several factors such as the type of waste buried, the influence of the microclimate, namely the intensity of rainfall, and the condition of the garbage collection."
Based on primary data from sliwung processing, periodically in each month of the year the amount of waste that enters for processing is recorded (Table 4). The characteristics of the waste produced consist of organic and inorganic waste such as household waste, plastics, and market waste. The amount of waste that comes in every year is getting higher. This is similar to the research results that the composition of the type of waste affects the quality of leachate produced\textsuperscript{10}. Organic waste is the dominant waste produced in Nepal and affects the amount of BOD\textsubscript{5} and COD. Organic waste is easier to break down by microbes than plastic waste.

### Table 4. Amount of waste production in Sliwung sites landfill

<table>
<thead>
<tr>
<th>Monthly</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>731.770</td>
<td>881.140</td>
<td>782.340</td>
<td>986.748</td>
<td>1.094.834</td>
</tr>
<tr>
<td>2</td>
<td>619.310</td>
<td>683.940</td>
<td>802.620</td>
<td>868.560</td>
<td>1.011.560</td>
</tr>
<tr>
<td>3</td>
<td>636.210</td>
<td>811.140</td>
<td>818.660</td>
<td>933.770</td>
<td>1.062.670</td>
</tr>
<tr>
<td>4</td>
<td>593.710</td>
<td>774.020</td>
<td>788.220</td>
<td>911.800</td>
<td>909.760</td>
</tr>
<tr>
<td>5</td>
<td>525.610</td>
<td>737.230</td>
<td>819.540</td>
<td>857.760</td>
<td>883.960</td>
</tr>
<tr>
<td>6</td>
<td>563.120</td>
<td>680.780</td>
<td>840.740</td>
<td>783.110</td>
<td>760.559</td>
</tr>
<tr>
<td>7</td>
<td>576.750</td>
<td>744.230</td>
<td>747.750</td>
<td>847.851</td>
<td>878.700</td>
</tr>
<tr>
<td>8</td>
<td>626.200</td>
<td>648.690</td>
<td>807.640</td>
<td>784.195</td>
<td>920.630</td>
</tr>
<tr>
<td>9</td>
<td>590.720</td>
<td>643.620</td>
<td>758.050</td>
<td>727.740</td>
<td>885.430</td>
</tr>
<tr>
<td>10</td>
<td>621.730</td>
<td>672.900</td>
<td>749.820</td>
<td>858.810</td>
<td>908.840</td>
</tr>
<tr>
<td>11</td>
<td>577.100</td>
<td>749.820</td>
<td>858.810</td>
<td>908.840</td>
<td>994.428</td>
</tr>
<tr>
<td>12</td>
<td>734.390</td>
<td>764.590</td>
<td>958.870</td>
<td>917.000</td>
<td>888.520</td>
</tr>
</tbody>
</table>
Based on Figure 1 shows the results of pH, BOD$_5$, and COD in leachate. Every month the pH value is relatively stable. The pH value of the measurement results was still below the quality standard set by the government in accordance with Table 3. The average pH value every month reaches 7.9. However, in July the pH value reached 4.1 (in Figure 1). Such conditions can be caused by activities from the ecological conditions of the final processing plant. Then it is assumed that there are buried garbage that is difficult to oxidize so that it is confirmed that the waste experiences inhibition of decomposition. In contrast to BOD$_5$, the graph shows that in May to November the BOD$_5$ value was just below the specified quality standard. This is influenced by the intensity of rainfall. In Indonesia, this month enters the dry season. This is similar to research conducted that stated that rainfall has a correlation with the quality of leachate produced$^{11,12}$. The highest BOD$_5$ value occurred in December, this is due to the fact that Indonesia is entering the beginning of the rainy season in this month (Figure 1). Delays in waste processing can increase humidity and temperature in the decomposition process. BOD$_5$ and COD are parameters related to pollutants. The existence of BOD$_5$ and COD is very dynamic. Therefore COD in every month exceeds the quality standard limit set. The highest COD value occurred in December with a value of 3,840 mgL$^{-1}$. There are several ways that can be done to reduce COD in leachate, namely using the coagulation flocculation technique$^{13,12,14}$.

![Figure 1.PH, temperature, BOD$_5$, and COD parameters from leachate in Sliwung.](image)

TSS is one of the leachate quality parameters that can pollute the environment. TSS by definition is part of the solid residue left behind, one of which can produce sludge. TSS can be observed physically because it can increase the turbidity level of leachate. In Figure 3, the TSS value in several months reaches a value that exceeds the quality standard. The quality standard value set by the government is 100 mgL$^{-1}$. However, the graph in Figure 2 shows that the highest TSS value was in June at 343.3 mgL$^{-1}$. TSS value affects the presence of aerobic bacteria so that the respiration process decreases. The oxygen level in waste with high TSS will reduce cellular metabolic processes in aerobic microorganisms. Then it has an effect on the digestibility of the amount of solids
produced. The use of FeCl$_2$ can reduce the amount of TSS by 95%, COD by 95% and the level of turbidity $^{13,15}$ Meanwhile, the parameter N Total per month is still in safe conditions the average value is below 60 mgL$^{-1}$. In December, the total N value was 223.9 mgL$^{-1}$ (Figure 2). The increase in this condition is proportional to the increase in COD values related to the amount of organic matter available in leachate.

![Graph showing TSS and N Total parameters of leachate in landfill Sliwung for one year.](image1)

The heavy metals Hg and Cd are non-essential heavy metals which can have a very dangerous impact if there is contamination. Both metals have toxic properties despite their presence in limited quantities. So based on the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number P.59/Menhik/Setjen/Kum.1/7/2016 concerning the standard of leachate, the amount of heavy metals Cd and Hg is strictly limited to not more than 0.1 mgL$^{-1}$ and 0.005 mgL$^{-1}$. In Figure 3, the parameter of heavy metals, the number of results of the analysis, shows that the waste processing location in Sliwung is still in accordance with the quality standards applied by the government. Based on the analysis results, the Cd and Hg values in leachate reached an average value of 0.00025 mgL$^{-1}$ and 0.0000159 mgL$^{-1}$. The presence of essential heavy metals in waste can cause extraordinary pollution events. The contamination is not only in the environment but can also occur in human health. The essential heavy metals such as Hg and Cd have a very difficult breakdown rate, so an integrated management effort is required.

![Graph showing Hg and Cd parameters from leachate in landfill Sliwung while one year.](image2)
Conclusions

Based on the results of the analysis on several parameters BOD\textsubscript{5}, COD, and TSS still require special handling to meet the environmental quality standards set by the government in accordance with the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number P.59/Menhk/Setjen/Kum./7/2016 concerning the quality standard of leachate. This evaluation aims to assess the feasibility of processing waste to improve its management in maintaining environmental balance. So that the results of this study can be a recommendation for the Sliwung landfill to conduct an approach study that can reduce the BOD\textsubscript{5}, COD, and TSS values so that they do not exceed the specified quality standards.

Acknowledgements: The researcher would like to thank the Indonesian Endowment Fund for Education (LPDP) and the parties involved in the technical implementation, namely the Situbondo Environmental Service.

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A Study to Assess the Effectiveness of Planned Teaching Program on Knowledge Regarding Electro Convulsive Therapy among Patient’s Relatives Undergoing ECT in Selected Hospitals in Vadodara

Kiran Patel¹, Seema Boresa², Mural Christian³
¹Assistant Professor, ²M.sc Nursing Tutor, Dinsha Patel College of Nursing, Nadiad

Abstract

Background of Study: Electroconvulsive therapy (ECT) is a safe and efficacious treatment; there is a widespread negative view of electroconvulsive therapy in public and professional circles. Clinical experience and research have resulted in continued improvements to the efficacy and safety of electroconvulsive therapy. It is necessary to conduct research on this basis to modify the misconceptions and prejudices about the electroconvulsive therapy¹.

Objectives: 1. To assesses the existing level of knowledge regarding electro convulsive therapy among patient’s relatives undergoing ECT.2. To determine the effectiveness of planned teaching on knowledge regarding electro convulsive therapy among patient’s relatives undergoing ECT.3. To find out association between pre test knowledge scores of patients relatives with their selected demographic variables.

Methodology: Quantitative Research Approach was use with 2 Group pre test & Post test Design. The investigator used pre-experimental one group pre-test post-test research design. Purposive sampling technique used for Selecting 30 Samples. The Structural questioners Used Check knowledge of ECT before & after administration of planned teaching program.

Results: Analysis of paired t test is done to assess the effectiveness of planned teaching on knowledge regarding electroconvulsive therapy. Researcher has found t value= 24.853 thus the obtained t value in this study is more than the table value of t test at 0.05 level of significance. Hence the obtained t value is significant. So it reveals that planned teaching on knowledge regarding electroconvulsive therapy is improved in knowledge among patient’s relatives.

Key words: Effectiveness, Planned Teaching Program, Knowledge, Electro Convulsive Therapy

Introduction

Mental illness is shrouded in the gloom of ignorance, superstition, feeling of mystery and fear among the public. Many mental ill patients are taken to different healers and religious places where they usually undergo torturous procedures. Often the family spends most of its income in seeking relief from various other sources before coming to mental hospital; by this time mental illness would have reached an advanced stage and the family members have lost all their hopes. With the emergence of biological psychiatry and the growing knowledge bases in the neurosciences, interest has increased in treatment resistant psychiatric disorders and refinement in treatment techniques have placed greater emphasis on evaluating the indications for and efficacy of somatic therapeutic interventions.

Corresponding author:-
Ms. Seema Boresa, M.sc Nursing Tutor, Dinsha Patel College of Nursing, Nadiad,
Email Id: seemaboresasb@gmail.com
Address:- Dinsha Patel College of Nursing, Behind Hyundai Showroom, College road, Nadiad, District-Kheda, Gujarat-387001.
Electroconvulsive therapy is a type of somatic treatment in which electric current is applied to the brain through the electrodes placed on the temples of the patient. 200-1600mA of electric current is passed for 0.7-1.5 seconds.²

Electroconvulsive treatment (ECT) is a powerful non-pharmacological mediation utilized for treatment in psychiatry. It offers a helpful, safe, and sometimes, life-sparing intercession, during which a minuscule electrical flow is applied to the patient’s cerebrum through terminals. The current delivers a seizure enduring from 30 s to 1 min. The actuated convulsive seizures in neurons in the whole mind mitigate indications of issues, for example, significant discouragement, intense hyper scenes, schizophrenia, or schizoaffective problem.³

Methodology

Research Approach: - Quantitative research approach

Research Design: - Pre-experimental, one group pre-test and post-test.

Sampling method: - Purposive sampling technique

Study population: - Relatives of patients are undergoing ECT in selected mental hospitals in Vadodara.

Study Setting: Selected mental hospital in Vadodara.

Study Size: - The sample size of the study was 30 relatives of the patients undergoing ECT

Results

Association of age in years with the patients relatives score the calculated value of chi-square is 1.45 less than the table value of chi-square 3.18 , at the 3 degree of freedom and P<0.05 level of significance. Therefore, age is non-significant with the score of patient’s relatives.

Association of sex (Gender) the patients relatives score, calculated value of chi-square is 1.152 less than the table value of chi-square 12.71, at the 1 degree of freedom and P<0.05 level of significance. Therefore, gender is non-significant with the patients relatives score.

Association of patients relatives score, calculated value of chi-square is 6.11 more than the table value of chi-square 3.18, at the 3 degree of freedom and P<0.05 level of significance. Therefore, patient’s relatives’ education score is significant.

Association of occupation of the family, calculated value of chi-square is 2.49 less than the table value of chi-square 12.71, at the 1 degree of freedom and P<0.05 level of significance. Therefore, Occupation of family is Non significant with the patients relatives score.

Association of type of family with the patients relatives score, calculated value of chi-square is 0.000 less than the table value of chi-square 12.71, at the 1 degree of freedom and P<0.05 level of significance. Therefore, type of family is no significant with the patients relatives score.

Association of relation with the patients relatives score, calculated value of chi-square is 0.67 less than the table value of chi-square 3.18, at the 3 degree of freedom and P<0.05 level of significance. Therefore, relation with patient non-significant with the patients relatives score.
Table 1: Frequency and percentage distribution of Demographic Data:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Demographic variables</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Age of patients relatives</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) 18-30</td>
<td>08</td>
<td>26.7%</td>
</tr>
<tr>
<td></td>
<td>b) 31-40</td>
<td>06</td>
<td>20.0%</td>
</tr>
<tr>
<td></td>
<td>c) 41-50</td>
<td>09</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>d) Above 50</td>
<td>07</td>
<td>23.3%</td>
</tr>
<tr>
<td>2.</td>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Male</td>
<td>19</td>
<td>63.3%</td>
</tr>
<tr>
<td></td>
<td>b) Female</td>
<td>11</td>
<td>36.7%</td>
</tr>
<tr>
<td>3.</td>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Illiterate</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>b) Primary and secondary</td>
<td>13</td>
<td>43.3%</td>
</tr>
<tr>
<td></td>
<td>c) Graduate</td>
<td>17</td>
<td>56.7%</td>
</tr>
<tr>
<td></td>
<td>d) Post graduate</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>4.</td>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Employment</td>
<td>19</td>
<td>63.03</td>
</tr>
<tr>
<td></td>
<td>b) Unemployment</td>
<td>11</td>
<td>36.07</td>
</tr>
<tr>
<td>5.</td>
<td>Types of family</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Nuclear family</td>
<td>09</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>b) Joint family</td>
<td>21</td>
<td>70%</td>
</tr>
<tr>
<td>6.</td>
<td>Relation with patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Parents</td>
<td>03</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>b) Husband or wife</td>
<td>12</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>c) Family relatives</td>
<td>15</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>d) Neighbor</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>
Table 2: Frequency and percentage distribution in pre-test and post-test level of knowledge among patient’s relatives regarding electroconvulsive therapy.

<table>
<thead>
<tr>
<th>Knowledge of patients relatives</th>
<th>Pre-test</th>
<th>Post test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>f</td>
<td>%</td>
</tr>
<tr>
<td>Inadequate</td>
<td>10</td>
<td>33.3%</td>
</tr>
<tr>
<td>Moderately adequate</td>
<td>20</td>
<td>66.7%</td>
</tr>
<tr>
<td>Adequate</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100%</td>
</tr>
</tbody>
</table>

Pre-test depicts that prior to the knowledge regarding ECT majority 20 (66.7%) patients relatives had moderately adequate level of knowledge (score: 11-20) while 10 (33.3%) patients relatives had inadequate level of knowledge (score: 0-10) and 0 (0%) patients relatives had adequate level of knowledge (score: 21-30) in particular study.

Post-test depicts that prior to the knowledge on ECT majority 26 (86.67%) patients relatives had adequate level of knowledge (score: 21-30) while 04 (13.33%) patients relatives had moderately adequate level of knowledge (score: 11-20) in particular study.

Table 3: Mean standard deviation, mean difference and ‘T’ value of pre-test and post-test scores.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>Mean difference</th>
<th>Std. Deviation</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge of patients relatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-test</td>
<td>11.43</td>
<td>8.57</td>
<td>1.88</td>
<td>24.853 &lt; 2.05</td>
</tr>
<tr>
<td>Post-test</td>
<td>20.00</td>
<td></td>
<td>.0000</td>
<td>Df = 29</td>
</tr>
</tbody>
</table>

Level of knowledge of patients relatives is with the maximum possible score of Mean pre-test was 11.43, SD is 1.88 and, Mean post-test is 20.00, SD is .0000 and comparison between pre and post-test level 8.57 Knowledge of patients relatives score of the sample was shows the paired ‘t’ value as 24.853 (Significant at the p<0.05 level). The mean post-test knowledge score regarding electroconvulsive therapy among patients relatives significant higher than there mean pre-test score.

Conclusion

The present study was done to find out the effectiveness of planned-teaching program on knowledge regarding electroconvulsive therapy among patient’s relatives undergoing ECT in selected hospitals in Vadodara.
Conclusion drawn based on the finding of the study revealed the Pre – test knowledge score of patient’s relatives regarding “electroconvulsive therapy” undergoing ECT was poor. Planned-teaching program by the investigator was found to be effective in improving the knowledge of patient’s relatives.

Planned-teaching program is effective method. There was no significant relationship between pre test knowledge score and selected variable like age, gender, occupation, Types of family, relation with patients there is only significant with education of the patient’s relatives. Therefore it could be concluded that planned-teaching program can help in improving the knowledge of patient’s relatives and could be used in various settings. It can be concluded that there is no significant association between knowledge and selected demographic variable.

The analysis has been organized and presented under various sections like description of demographic variables, description of pre-test knowledge score, comparison of pre-test and post-test knowledge score and association between the post-test knowledge score and selected demographic variables. It is found that post-test knowledge score is higher than the pre-test knowledge score so it indicate planned teaching program was effective.

Conflict of Interest: Nil

Source of Funding: College Management

Ethical Clearance: The study was approved by the research committee, IEC – DPCN/1st IEC/2018-19/09 and a formal written permission was gathered from the hospital, Vadodara.

Statement of Informed consent: Informed consent was acquired from the participants

References
Sensitivity and Specificity of Postmortem CT for Detection of Thoracic Injury

Komet Kosawiwat¹, Rathachai Kaewlai², Pinporn Jenjitranant³, Wisarn Worasuwannarak⁴

¹Resident, ²Associate Professor, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, ³Instructor, Department of Radiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, ⁴Instructor, Department of Diagnostic and Therapeutic Radiology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Abstract

Objective: Postmortem CT (PMCT) can help conventional autopsy in determining the cause of death and finding injury to various organs. Since injuries among several vital organs in the thorax can cause death, this research aims to assess the performance of PMCT in detecting injuries of the thoracic cavity organs.

Materials and Method: A total of 56 dead bodies by unnatural traumatic death who underwent PMCT before autopsy were collected. Thoracic traumatic findings from PMCT were compared with data obtained from conventional autopsy where the autopsy was the reference standard and calculated for sensitivity, specificity, PPV, NPV, and accuracy.

Results: Findings in which PMCT showed high sensitivity included air (100%) and fluid (86.67%). In terms of bone fracture, sensitivity and accuracy were 79.18% and 88.69%, respectively, especially for T-spine fractures (sensitivity 92.31%, accuracy 87.50%) and clavicle fractures (sensitivity 90%, accuracy 89.29%). For rib fractures, PMCT exhibited moderate to high sensitivity (68.18-91.67%) and high accuracy (78.57-98.21%). For soft tissue injuries, PMCT had high specificity (99.21%), but low sensitivity (34.94%).

Conclusion: PMCT is useful in detecting thoracic injuries in conjunction with the conventional autopsy by helping to diagnose bone fractures, abnormal air, and fluid with high sensitivity and specificity.

Keywords: Postmortem CT; Virtual autopsy; Chest injury; Trauma; Forensic Imaging; Forensic Pathology.

Introduction

Imaging is useful for diagnosing pathologies in the body, making it useful in postmortem inquest. This is especially true for postmortem CT (PMCT), which plays a role in enhancing conventional autopsy to determine the cause of death¹⁻⁹, detect organ injury, and some internal organ pathologies. The conventional autopsy had limited access to some internal injuries¹⁰⁻¹² such as facial bone fractures⁷,¹³, spinal fractures⁷,⁹,¹³,¹⁴, etc, and had limitations in its methods to detect pathologies caused by abnormal air, such as pneumothorax and air embolism¹⁵⁻¹⁸. Air generated within the body after death is something that is difficult to prove with conventional autopsy and requires additional techniques. In our experience, there were cases where this could not be proven, or if it was present in small quantities, may not have been possible to verify¹⁹. With these types of injuries, PMCT can be helpful for detection and in the detection of multiple injuries³,¹⁰,¹⁸,²⁰,²¹.
The thoracic region contains many vital organs, such as the heart, lungs, aorta, etc. Injury to the thoracic organs can be a common cause of death. For example, tension pneumothorax had a death rate of 40%\textsuperscript{22}, flail chest had a death rate of 18%\textsuperscript{23}, and cardiac tamponade\textsuperscript{24} can come from injury to the heart or the aorta. These are all possible causes of death. Injury to the chest is the fourth most common cause of death with a death rate of 18.7%\textsuperscript{25}.

PMCT is effective in detecting trauma to the pleura, thoracic bony structures, with high sensitivity and specificity of 100%\textsuperscript{7,26} but it has a lower sensitivity of 50-94% and specificity of 85-94% for soft tissue injuries\textsuperscript{26}. It could be seen that thoracic PMCT showed differences in performance between bone, soft tissue, fluid, and air. In addition, the researcher observed that there were several times when the autopsy revealed injuries that did not match the PMCT results. The researcher, therefore, would like to study the sensitivity and specificity of PMCT in the detection of thoracic injuries.

**Material and Methods**

**Sample**

The sample was comprised of unnaturally dead bodies that were fully autopsied between 2012 and 2020 and underwent PMCT before the autopsy. The exclusion criteria were the dead bodies without a history of injury prior to death, or with signs of decomposition on external examination.

**Data Collection**

Data collected were gender, age, incident history, cause of death, circumstances of death, and the time period from deceased to autopsy (postmortem interval: PMI). The cause of death was divided into 6 groups: 1) head injury, 2) neck injury, 3) chest injury, 4) abdominal injury, 5) multiple injuries (when more than one fatal injury group was involved) and, 6) others.

**Postmortem CT**

PMCT was performed on a 128-slice Aquilion CX scanner (Toshiba Medical Systems Corporation, Tokyo, Japan) or a GE revolution HD (GE Healthcare, Chicago, IL, USA). Scan parameters were as follows: slice thickness 2.0-2.5 mm, rotation time 0.5-0.6 s, tube voltage 120-140kVp, and tube current 200-300mAs without IV contrast. The examination was performed from the head to the halfway point of the upper leg or below, depending on the height of the body. Images were collected for analysis in the Picture Archiving and Communication Systems (PACS) and results were formally reported in the reporting system. The images were interpreted by emergency radiologists who had some experiences in postmortem imaging. The radiologists who interpreted the images knew the trauma history of the corpses but did not know the results of the autopsy.

**Conventional autopsy**

The autopsy was performed by forensic pathologists who were aware of the PMCT results using standard methods. The body was dissected throughout the body with the removal of all internal organs, including the brain, heart, lungs, liver, pancreas, spleen, kidneys, adrenal glands, and uterus (in the case of female corpses) for a thorough examination. The cause of death was recorded in the report.

**Definitions**

The definitions of each type of injury were described below.

1. Bone injuries included rib fracture, sternum fracture, clavicle fracture, and thoracic spine fracture. Rib fractures were described according to each rib separately, as 1\textsuperscript{st}-12\textsuperscript{th} ribs, right and left. The scapular fracture was not included in this study because of technical limitation of the conventional autopsy.

2. Pleura injury included hemothorax or pneumothorax.

3. Lung injury included lung contusion or lung laceration.

4. Heart injury included heart contusion or heart...
laceration.

5. Aorta injury included aortic tear or periaortic hemorrhage.

6. Superior vena cava (SVC) and inferior vena cava (IVC) injuries included hemorrhage or tear of the SVC or IVC.

7. Diaphragm injury included diaphragm contusion, diaphragm laceration, or traumatic diaphragmatic hernia.

8. Thoracic spinal cord injury included spinal cord hemorrhage (epidural hemorrhage or subdural hemorrhage), spinal cord contusion, or spinal cord laceration.

The definitions of the injury groups are divided into four groups of injuries:


2. Soft tissue and organ injury: heart, lung, diaphragm, and thoracic spinal cord injuries.


**Statistical Analysis**

Injury data of chest organs and parts from conventional autopsy and PMCT were compared and calculated to determine sensitivity, specificity, PPV, NPV, and accuracy using conventional autopsy as the reference standard.

**Results**

Data were collected from 2012 - 2020 for corpses that were PMCT tested. From a total of 63 cases, 7 cases were non-trauma and were excluded. Thus, a total of 56 cases were included in the study, 47 were men and 9 were women, ranging from 15-81 years of age, with a median age of 33 years. There was one case with no age identified. For the cause of death, the manner of death, and the postmortem interval (PMI), the data are shown in Table 1.

<table>
<thead>
<tr>
<th>Details</th>
<th>Number (N = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Age*</td>
<td>11-20 y</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>21-30 y</td>
</tr>
<tr>
<td></td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>31-40 y</td>
</tr>
<tr>
<td></td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>41-50 y</td>
</tr>
<tr>
<td></td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>51-60 y</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>61-70 y</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>71-80 y</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt;80 y</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
Table 1: Cause of death, manner of death, and postmortem interval of the included cases.

<table>
<thead>
<tr>
<th>Cause of death</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Head injury</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Neck injury</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Chest injury</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Abdominal injury</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Multiple injuries</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Natural**</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Manner of death</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Homicide</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Suicide</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Accident</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Natural**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Undetermined</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Postmortem interval</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 6 hours</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>6 – 12 hours</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>12 – 18 hours</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>18 – 24 hours</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>&gt; 24 hours</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

* one case of unknown age

** History of trauma before death but the cause of death was myocardial infarction.

The results of PMCT compared to conventional autopsy are shown in Table 2, comparing the number of findings.

Table 2: Comparison of thoracic injuries detected with PMCT and autopsy (n=56)

<table>
<thead>
<tr>
<th>Injury</th>
<th>PMCT + Autopsy +</th>
<th>PMCT – Autopsy -</th>
<th>PMCT + Autopsy -</th>
<th>PMCT - Autopsy +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sternum fracture</td>
<td>8</td>
<td>38</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Rib fracture*</td>
<td>298</td>
<td>898</td>
<td>67</td>
<td>81</td>
</tr>
<tr>
<td>Clavicle fracture</td>
<td>9</td>
<td>41</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>T-spine fracture</td>
<td>12</td>
<td>37</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>5</td>
<td>23</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Hemothorax</td>
<td>26</td>
<td>20</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Lung injuries</td>
<td>24</td>
<td>15</td>
<td>1</td>
<td>16</td>
</tr>
</tbody>
</table>
Lung contusion & 16 & 21 & 1 & 18 \\
Lung laceration & 17 & 27 & 3 & 9 \\
Heart injuries & 0 & 29 & 0 & 27 \\
Heart contusion & 0 & 36 & 0 & 20 \\
Heart laceration & 0 & 46 & 0 & 10 \\
Aorta injury & 0 & 49 & 1 & 6 \\
SVC, IVC injury & 0 & 56 & 0 & 0 \\
Diaphragm injury & 4 & 51 & 0 & 1 \\
Spinal cord injury & 1 & 51 & 0 & 4 \\

*Rib fractures were counted from each rib

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of PMCT when the conventional autopsy was the reference standard, are shown in Table 3. For heart injury and aorta injury, values could not be calculated except for the negative predictive value, because either the PMCT results were all negative or the number of pathologies was very low.

Table 3: Sensitivity, specificity, PPV, NPV, and accuracy of PMCT in the detection of organ injury

<table>
<thead>
<tr>
<th>Injury</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sternum fracture</td>
<td>72.73%</td>
<td>84.44%</td>
<td>53.33%</td>
<td>92.68%</td>
<td>87.50%</td>
</tr>
<tr>
<td>Rib fracture</td>
<td>78.63%</td>
<td>93.06%</td>
<td>81.64%</td>
<td>91.73%</td>
<td>88.99%</td>
</tr>
<tr>
<td>Clavicle fracture</td>
<td>90.00%</td>
<td>89.13%</td>
<td>64.29%</td>
<td>97.62%</td>
<td>89.29%</td>
</tr>
<tr>
<td>T-spine fracture</td>
<td>92.31%</td>
<td>86.05%</td>
<td>66.67%</td>
<td>97.37%</td>
<td>87.50%</td>
</tr>
<tr>
<td>Hemothorax</td>
<td>86.67%</td>
<td>76.92%</td>
<td>81.25%</td>
<td>83.33%</td>
<td>82.14%</td>
</tr>
<tr>
<td>Lung injury</td>
<td>60.00%</td>
<td>93.75%</td>
<td>96.00%</td>
<td>48.39%</td>
<td>69.64%</td>
</tr>
<tr>
<td>Lung contusion</td>
<td>47.06%</td>
<td>95.45%</td>
<td>94.12%</td>
<td>53.85%</td>
<td>66.07%</td>
</tr>
<tr>
<td>Lung laceration</td>
<td>65.38%</td>
<td>90.00%</td>
<td>85.00%</td>
<td>75.00%</td>
<td>78.57%</td>
</tr>
<tr>
<td>Heart injury</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>51.79%</td>
<td>NA</td>
</tr>
<tr>
<td>Heart contusion</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>64.29%</td>
<td>NA</td>
</tr>
<tr>
<td>Heart laceration</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>82.14%</td>
<td>NA</td>
</tr>
<tr>
<td>Aorta injury</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>89.09%</td>
<td>NA</td>
</tr>
<tr>
<td>Diaphragm injury</td>
<td>80.00%</td>
<td>100.00%</td>
<td>100.00%</td>
<td>98.08%</td>
<td>98.21%</td>
</tr>
<tr>
<td>Thoracic cord injury</td>
<td>20.00%</td>
<td>100.00%</td>
<td>100.00%</td>
<td>92.73%</td>
<td>92.86%</td>
</tr>
</tbody>
</table>
For main venous injuries (SVC, IVC injury), PMCT results and autopsy were all negative, so values could not be calculated. The conventional autopsy could not detect pneumothorax in some cases and therefore could not be used as a reference standard.

Thoracic injuries with the highest sensitivity of PMCT were T-spine fractures (92.31%), followed by clavicle fractures (90%), and hemothorax (86.67%). The injuries with the highest specificity included diaphragm injury and thoracic cord injury (100%), followed by lung contusion (95.45%), though the latter two had low sensitivity of 20-47%.

Detecting rib fractures with PMCT resulted in an overall sensitivity of 78.63% and specificity of 93.06%.

Sensitivity ranged from 63.64% to 91.67%, with the highest sensitivity being recorded at the right 10th rib, left 9th rib, left 10th rib, and left 12th rib, respectively. Specificity ranged from 85.29% to 100%, with the highest being at the left 12th rib, left 10th rib, and right 8th rib, respectively. PPV ranged from 55.56% to 100%, with the maximum found at the left 12th rib, right 4th rib, right 3rd rib, respectively. NPV ranged from 79.49% to 97.87%, with the highest being the left 12th rib, left 10th rib, and right 12th rib, and left 9th rib, respectively. Accuracy ranged from 80.36% to 98.21%, with the highest being the left 12th rib, left 10th rib, right 8th rib, and right 10th rib, respectively. The details were shown in Table 4.

<table>
<thead>
<tr>
<th>Injury</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>left 1st rib</td>
<td>78.95%</td>
<td>94.59%</td>
<td>88.24%</td>
<td>89.74%</td>
<td>89.29%</td>
</tr>
<tr>
<td>left 2nd rib</td>
<td>71.43%</td>
<td>88.57%</td>
<td>78.95%</td>
<td>83.78%</td>
<td>82.14%</td>
</tr>
<tr>
<td>left 3rd rib</td>
<td>63.64%</td>
<td>91.18%</td>
<td>82.35%</td>
<td>79.49%</td>
<td>80.36%</td>
</tr>
<tr>
<td>left 4th rib</td>
<td>75.00%</td>
<td>87.50%</td>
<td>81.82%</td>
<td>82.35%</td>
<td>82.14%</td>
</tr>
<tr>
<td>left 5th rib</td>
<td>68.18%</td>
<td>85.29%</td>
<td>75.00%</td>
<td>80.56%</td>
<td>78.57%</td>
</tr>
<tr>
<td>left 6th rib</td>
<td>73.68%</td>
<td>91.89%</td>
<td>82.35%</td>
<td>87.18%</td>
<td>85.71%</td>
</tr>
<tr>
<td>left 7th rib</td>
<td>75.00%</td>
<td>90.00%</td>
<td>75.00%</td>
<td>90.00%</td>
<td>85.71%</td>
</tr>
<tr>
<td>left 8th rib</td>
<td>80.00%</td>
<td>90.24%</td>
<td>75.00%</td>
<td>92.50%</td>
<td>87.50%</td>
</tr>
<tr>
<td>left 9th rib</td>
<td>90.00%</td>
<td>93.48%</td>
<td>75.00%</td>
<td>97.73%</td>
<td>92.86%</td>
</tr>
<tr>
<td>left 10th rib</td>
<td>90.00%</td>
<td>97.83%</td>
<td>90.00%</td>
<td>97.83%</td>
<td>96.43%</td>
</tr>
<tr>
<td>left 11th rib</td>
<td>81.82%</td>
<td>95.56%</td>
<td>81.82%</td>
<td>95.56%</td>
<td>92.86%</td>
</tr>
<tr>
<td>left 12th rib</td>
<td>90.00%</td>
<td>100.00%</td>
<td>100.00%</td>
<td>97.87%</td>
<td>98.21%</td>
</tr>
<tr>
<td>right 1st rib</td>
<td>76.47%</td>
<td>87.18%</td>
<td>72.22%</td>
<td>89.47%</td>
<td>83.93%</td>
</tr>
<tr>
<td>right 2nd rib</td>
<td>75.00%</td>
<td>94.44%</td>
<td>88.24%</td>
<td>87.18%</td>
<td>87.50%</td>
</tr>
<tr>
<td>right 3rd rib</td>
<td>75.00%</td>
<td>97.22%</td>
<td>93.75%</td>
<td>87.50%</td>
<td>89.29%</td>
</tr>
<tr>
<td>right 4th rib</td>
<td>85.00%</td>
<td>97.22%</td>
<td>94.44%</td>
<td>92.11%</td>
<td>92.86%</td>
</tr>
<tr>
<td>right 5th rib</td>
<td>78.95%</td>
<td>91.89%</td>
<td>83.33%</td>
<td>89.47%</td>
<td>87.50%</td>
</tr>
<tr>
<td>right 6th rib</td>
<td>86.67%</td>
<td>92.68%</td>
<td>81.25%</td>
<td>95.00%</td>
<td>91.07%</td>
</tr>
<tr>
<td>right 7th rib</td>
<td>86.67%</td>
<td>92.68%</td>
<td>81.25%</td>
<td>95.00%</td>
<td>91.07%</td>
</tr>
<tr>
<td>right 8th rib</td>
<td>85.71%</td>
<td>97.62%</td>
<td>92.31%</td>
<td>95.35%</td>
<td>94.64%</td>
</tr>
<tr>
<td>right 9th rib</td>
<td>84.62%</td>
<td>93.02%</td>
<td>78.57%</td>
<td>95.24%</td>
<td>91.07%</td>
</tr>
<tr>
<td>right 10th rib</td>
<td>91.67%</td>
<td>95.45%</td>
<td>84.62%</td>
<td>97.67%</td>
<td>94.64%</td>
</tr>
<tr>
<td>right 11th rib</td>
<td>71.43%</td>
<td>91.84%</td>
<td>55.56%</td>
<td>95.74%</td>
<td>89.29%</td>
</tr>
<tr>
<td>right 12th rib</td>
<td>87.50%</td>
<td>91.67%</td>
<td>63.64%</td>
<td>97.78%</td>
<td>91.07%</td>
</tr>
</tbody>
</table>
When the trauma pathology was grouped into four groups to calculate the diagnostic performance of PMCT compared to conventional autopsy (Table 5), PMCT was found to have relatively high accuracy (78.13% - 88.69%), with the highest value indicated for bone injuries. For abnormal air detection, PMCT had the highest sensitivity, detecting NPV at 100%, but its specificity, PPV, and accuracy could not be determined because it could not be detected in conventional autopsy in many cases due to the technical limitations.

### Table 5

<table>
<thead>
<tr>
<th>Group of Injury</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>79.18%</td>
<td>92.27%</td>
<td>79.37%</td>
<td>92.18%</td>
<td>88.69%</td>
</tr>
<tr>
<td>Soft tissue and Organ</td>
<td>34.94%</td>
<td>99.21%</td>
<td>93.55%</td>
<td>82.30%</td>
<td>83.33%</td>
</tr>
<tr>
<td>Fluid</td>
<td>86.67%</td>
<td>76.92%</td>
<td>81.25%</td>
<td>83.33%</td>
<td>82.14%</td>
</tr>
<tr>
<td>Air</td>
<td>100.00%</td>
<td>NA</td>
<td>NA</td>
<td>100.00%</td>
<td>NA</td>
</tr>
</tbody>
</table>

### Discussion

This research shows that PMCT is highly accurate for the diagnosis of fluid and air, as well as injuries to soft tissue and organs in the thoracic cavity, with the highest sensitivity for pneumothorax diagnosis and the highest accuracy for bone fractures (especially T-spine fracture and clavicle fracture). It is also superior to conventional autopsy in the diagnosis of pneumothorax.

**Bone fracture**

This research is consistent with previous research findings proclaiming PMCT to be very accurate for bone injuries, with accuracy levels up to 88.69%. Clavicle fractures display the highest accuracy of 89.29%, as shown in research by Moskala A. et al.\(^{27}\), where PMCT can detect clavicle injuries better than any other bones in the thorax. Overall sensitivity, specificity, and accuracy in this study were lower than that found by Sifaoui I. et al.\(^{26}\), who showed the sensitivity of 100% and specificity of 100%, but similar to a study by Ampanozi G. et al.\(^{28}\), which had a sensitivity of 86%.

For rib fractures, PMCT was found to have a sensitivity of 78.63% and specificity of 93.06%. Research by Schulze C. et al.\(^{29}\) obtained lower sensitivity rates, but slightly higher specificity. In the area of rib fractures, some differences from past research were found. In research by Hamanaka K. et al.\(^{30}\), it was shown that PMCT detected fractures of the 2nd, 5th and 6th ribs very well. As for the Schulze C. et al.\(^{29}\) research, PMCT presented better detection at the 1st rib. In this research, the rib fracture sites where PMCT showed the highest accuracy were the left 10th rib, left 12th rib, right 8th rib, and right 10th rib, which are false ribs, as well as floating ribs.

As for scapular fractures, it was found that PMCT revealed a certain number of positive results, whereas conventional autopsy could not reach the scapula during the examination in many cases because of technical limitation. Therefore, we did not include the scapular fracture in this study.

**Soft tissue and organ injury**

For injuries to soft tissue and organs, PMCT exhibited high specificity (99.21%), comparable with Aghayev E. et al.\(^{31}\), but showed lower sensitivity (34.94%) than the study of Ampanozi G. et al.\(^{28}\).
In lung injuries, PMCT was higher in specificity than Aghayev E. et al.\textsuperscript{31} for both lung contusion and lung lacerations. Diaphragm injury was the best soft tissue and organ injury to be detected by PMCT, with a sensitivity of 80\%, a specificity of 100\%, and an accuracy of 98.21\%. This is similar to research by Sifaoui I. et al.\textsuperscript{26} with 94\% sensitivity, 94\% specificity, and 97\% accuracy. Three-quarters of the four true positive cases were detected for diaphragmatic herniation. These values were higher than those found in research by Aghayev E. et al.\textsuperscript{31} (sensitivity 50\% and specificity 100\%), and that of Christe A. et al.\textsuperscript{32} (sensitivity 0\% and specificity 94\%).

\textit{Pneumothorax}

Pneumothorax was the most common condition, presenting in 28 out of 56 cases (50\%) in PMCT images, but not in the conventional autopsy. Only 5 cases were found in both conventional autopsy and PMCT. No cases were found only in the autopsy but not by PMCT. This is consistent with other studies, as well, showing that PMCT was able to detect pneumothorax\textsuperscript{3,31,33}. This is the unique property of PMCT allowing abnormal air collection to be detected even in small amounts, while a conventional autopsy is not able to confirm pneumothorax in those cases.

\textit{Hemothorax}

In this study, PMCT had a sensitivity of 86.67\% in the diagnosis of hemothorax, which was 76\% higher than that of the research of Ampanozi G. et al.\textsuperscript{28}

\begin{table}[h!]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{Finding} & \textbf{This research} & \textbf{Ampanozi G et al. (2020)}\textsuperscript{28} & \textbf{Sifaoui I et al. (2017)}\textsuperscript{26} & \textbf{Schulze C et al. (2013)}\textsuperscript{29} & \textbf{Christe A et al. (2009)}\textsuperscript{32} & \textbf{Aghayev E et al. (2008)}\textsuperscript{31}\textsuperscript{*} \\
\hline
Bone fracture & Sen 79.18\% Spec 92.27\% & Sen 86\% & Sen 100\% Spec 100\% & - & - & - \\
\hline
Rib fracture & Sen 78.63\% Spec 93.06\% & - & - & Sen 63\% Spec 97\% & - & - \\
\hline
Soft tissue & organ & Sen 34.94\% Spec 99.21\% & Sen 65\% & - & - & - \\
\hline
Lung injury & Sen 60\% Spec 93.75\% & - & Sen 97.5\% Spec 85\% & - & - & - \\
\hline
Lung contusion & Sen 47.06\% Spec 94.45\% & - & - & - & - & Sen 95-100\% Spec 60\% \\
\hline
Lung laceration & Sen 65.38\% Spec 90\% & - & - & - & - & Sen 90-100\% Spec 57-70\% \\
\hline
Diaphragm injury & Sen 80\% Spec 100\% & - & Sen 94\% Spec 100\% & - & Sen 0\% Spec 94\% & Sen 50\% Spec 60-100\% \\
\hline
Fluid (hemothorax) & Sen 86.67\% Spec 76.92\% & Sen 76\% & - & - & - & Sen 100\% Spec 50-75\% \\
\hline
Air (pneumothorax) & Sen 100\% & - & - & - & - & Sen 100\% Spec 50\% \\
\hline
\end{tabular}
\caption{Comparison of sensitivity and specificity with previous studies}
\end{table}

* Calculated from data provided within the article
Limitations

The limitations of this research are its retrospective nature with a relatively small number of cases, and some of the findings were infrequent that the statistical values could not be calculated. Our results were obtained from PMCT results without re-reviewing images. Although this reflects a real clinical practice it does not allow an evaluation of the most optimal performance of PMCT. The study period was quite long and may influence the experience of the radiologists who interpreted the results. The PMCT technique did not use a contrast agent injection, making it limited in the diagnosis of vascular injury. The conventional autopsy had limitations, as well, as some areas were inaccessible, difficult to reach, or some techniques were difficult to perform, making it impossible to use as a reference standard for certain pathologies.

Conclusion

PMCT is highly accurate in the diagnosis of bone fractures, abnormal fluid, and air in the thoracic cavity, and can be used as a complement to autopsy, particularly in the diagnosis of pneumothorax and some fractures. This test has a high specificity for diaphragm injury, but is not very good on other soft tissue injuries.

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Funding: None.

Ethical approval: This project has been reviewed and approved by the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University, based on the Declaration of Helsinki (MURA2019/1106).

References


Medical Coercive Measures Under the Albanian Legislation and their Application to Criminally Irresponsible Individuals

Kreshnik Myftari¹, Gëzim Myftari ², Sevada Guço³

¹ PHD, Full Time Lecturer of Criminal Law, Department of Criminal Law, Law Faculty, University of Tirana, ² Forensic Psychiatrist, ³ Master of Laws, Scientific Researcher

Abstract

Individuals, who are characterized by mental disorders and manifest them in a criminal behavior, cannot be subjects to the same punitive regime provided for other subjects of criminal offenses. Neither penitentiary institutions nor civilian psychiatric hospitals are suitable for these individuals; the former, because they are conceived only for that category of subjects who culpably violate criminal norms; second, because, since psychiatric hospitals have therapeutic purposes, they are not suitable for controlling the social risk of irresponsible subjects.

Any decision of irresponsibility due to mental state brings a certain consequence, namely the dismissal of the case and the imposition of coercive measure. The defendant, due to his social danger, may undergo outpatient treatment or compulsory treatment in a medical institution.

The following manuscript analyzes the importance of medical coercive measures provided by the criminal legislation and at the same time, their importance in the treatment of irresponsible persons, perpetrators of criminal offenses. It tries to give an overview of the different orientations that characterize the issue in question, to underline the conclusions reached by jurisprudence and at the same time to reason not only on the basis of applicable norms, but also on the basis of perspective and opportunity for reform, seeking to develop points of reflection and avoid unreasonable discussions.

Keywords: medical coercive measures, compulsory outpatient treatment, compulsory treatment in a medical institution, psycho-social care, psychiatric hospital.

Introduction

The problem of social risk, rather than an indisputable problem related to the category of socially dangerous individuals, essentially constitutes a problem that has to do with guaranteeing social protection, specifically for the treatment of these individuals.¹

Through coercive measures, the socially dangerous subject is prohibited or prevented from committing criminal offenses. It is necessary to distinguish the coercive measures that are applied after the commission of a criminal offense from an irresponsible person, in the sense of article 46 of the Albanian Criminal Code and article 239 of the Albanian Code of Criminal Procedure from involuntary treatment given pursuant to law no. 44/2012 “On mental health”, in order to prevent persons suffering from a mental illness to have aggressive behavior before they commit a criminal offense.

Medical measures are different from punishments² because they are not a consequence of a punitive trial, but a consequence of a judgment on dangerousness, not on criminal responsibility but on the probability of recidivism in the future.³ Since they provide for a

Corresponding author:
LLM Sevada Guço
Rr. “Nikolla Tupe”, pallati 7, hyrja 3, Tirana Albania, E-mail: seviguco@yahoo.com
Mob. No.: 00355 676072248

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reduction of the rights or personal freedom of the subject, their implementation is not conceived in the function of punishment, but it is an inalienable consequence of a measure with another purpose. Consequently, if the punishment is stipulated and placed in proportion to the fact that occurred, the coercive measure is logically indefinite, but in proportion to the social danger, as such it ends when the social danger ends.

Compulsory outpatient treatment as a medical coercive measure

Compulsory outpatient treatment intends to leave the person free under the care of the family or the respective guardian, who undertakes to treat him/her in an ambulatory or hospital condition. This type of measure is given mainly for criminal misdemeanor or for those criminal offenses that are not characterized by social danger, or when the latter has disappeared.

Compulsory outpatient treatment should always be based on the current state of mind and as long as no other more severe measures are needed.\(^4\)

In imposing the measure of compulsory outpatient treatment, the court must be very careful, so that the treatment is not neglected, since in this case the person is not deprived from liberty and his treatment is carried out in a free state.

In other words, the person to whom has been given such a measure must be assigned a family caregiver in order to follow him continuously and make sure that the subject undergoes medical therapy correctly. In any case the appointment of compulsory outpatient treatment cannot be given if the person does not have proper care from a family member. Other circumstances that the court must take into account in addition to the appointment of a guardian, are both economic and physical opportunity, proven and guaranteed before the court. In the absence of family members,\(^5\) as a guardian to follow at the same time the person himself and the treatment, the court cannot decide otherwise than the hospitalization in a psychiatric hospital, especially in cases of a temporary stabilized condition. Thus the main difference between outpatient treatment and hospitalization in a psychiatric hospital lies in the fact that the mental state of the irresponsible individual is permanently stabilized. In this way there is no reason why a person should be “deprived from his liberty”, but it is sufficient only the existence of treatment and its non-discontinuation.

In revoking and changing the medical measure of compulsory treatment in a medical institution to the measure of compulsory outpatient treatment, the courts often reject the request of the person on the grounds that in some cases he is not an integral part of the family tree. From this point of view the law has not made any provision for the appointment of a guardian, who will supervise the person who undergoes treatment as determined by psychiatric experts. Some courts also accept persons outside the family tree as guardians. In other decisions, the court requires the existence of economic opportunity to be proven and not only declared by the family members, but without determining the concrete evidence that the court needs to consider the request.

It is a mistake to think that in order to impose the most appropriate medical measure, the court should suffice with the act of expertise, but it should also take into consideration other evidence which sheds light on mental health, which is reflected in the behavior of the person in the community where he lives.\(^6\) An exception is made only in the case when the act of expertise stands as the only evidence, a case in which the court must accept it without any doubt.

The decision of the court to change the medical measure from hospitalization in a medical institution to compulsory outpatient treatment must in any case be justified, with the relevant arguments, why the conditions of treatment in a medical institution have ended and the person must be treated in outpatient treatment. The court should also refer to the act of expertise and in no case should decide differently from what the psychiatrists have prescribed.\(^7\)
The medical measure should be revoked in any case when the conditions and the need for safety are absent, being replaced by the measure of compulsory outpatient treatment. These conditions are always based on the assessments and statements of experts, specifically on improving the mental state of the person.8

Outpatient treatment, as a coercive measure is executed in the residence of the irresponsible person due to mental state who has committed the criminal offense. When the relevant specialists or a relevant institution are missing near the place of residence, he is accompanied in any case to the institutions of the nearest district, according to article 45 of law no. 8331, dated 21.04.1998 “On the execution of criminal decisions”, as amended. When there are obstacles in the execution of the compulsory outpatient medical measure, the prosecutor orders the compulsory execution by the state police and, if there’s the case, submits a request to the court to change the outpatient medical measure to a more severe measure, hospitalization in a medical institution.

Compulsory treatment in a medical institution as a coercive measure

This medical coercive measure is given by the court for persons declared irresponsible, against whom the criminal prosecution has not started or the trial has been terminated under article 387 of the Albanian Code of Criminal Procedure. It is thought that this measure does not apply to persons who have lost the ability to understand and control actions as a result of a transient illness, because at the moment of recovery from this disorder, the subject regains mental abilities and no longer poses a risk to society.

Compulsory treatment in a medical institution is applied to persons who due to their mental condition have not been aware of the importance of the criminal offense they have committed and have not been able to control their actions or omissions.9 The implementation of these measures by the court is an important tool of the Criminal Code and aims to prevent the commission of other criminal offenses by the mentally ill.

In case of dismissal of the litigation because of the irresponsibility due to mental condition, it is always given the medical measure of compulsory hospitalization in a psychiatric hospital for a period of not less than 2 years according to the Italian Criminal Code. According to this code, the minimum duration of hospitalization in a medical institution is not less than 10 years for offenses punishable by life imprisonment.10

On one hand, compulsory treatment in a medical institution, is considered as an opportunity to keep irresponsible and dangerous persons under control, and on the other hand, there is a discussion regarding the real possibility of imposing this measure.11 The Code defines the treatment of a completely irresponsible perpetrator in the form of compulsory isolation in a specialized institute in accordance with the vision that the irresponsible person is seen as a necessarily dangerous subject and that the only medical treatment is isolation from the rest of society.12

The Albanian judicial practice, has encountered a problem regarding the conditions of imposing medical measures, specifically that of compulsory treatment in a psychiatric hospital. The persons declared by the court as irresponsible in the absence of medical institutions, were sent for treatment to the prison hospital. Taking into account law no. 8331, dated 21.04.1998 “On the execution of criminal decisions” and the order of the Minister of Justice no. 329, dated 15.01.2009 “On the categorization of institutions for the execution of criminal decisions”, as amended, the prison hospital is considered a security prison and not a medical institution.

Therefore, the Albanian High Court13 has ruled that the law has been wrongly applied and the person has been unjustly deprived of his liberty, living in the conditions of a prisoner even though he is not such. In the same decision, the court expresses the opinion of the expert for imposing the most appropriate medical measure, emphasizing that according to article 46 of the Criminal Code, the only condition for the application of the medical measure is the irresponsibility of the person who committed a criminal act. The article in question
has not made any determination whether these measures are given to persons who are of great social danger or for any kind of offense.

Courts go beyond the content of the article when placing the emphasis on social danger, which is not required by article 46 of the Criminal Code. The court on itself does not have the means to determine the mental state of the person and necessarily seeks the help of psychiatric experts, who in addition to the mental state determine the need for treatment or isolation. The opinions of the expert often constitute a decisive evidence for the resolution of the case, but like all other evidence they are subject to judicial review, especially in cases where they must be analyzed within all of the evidence administered by the court. The court cannot take over and cannot play the role of the expert nor give it a predetermined value.

The medical measure of treatment in a medical institution must be reviewed by the court in any case after the expiration of the one-year period, as per article 46, paragraph 2 of the Criminal Code. In the review session, the court, with the help of experts, once again re-evaluates the mental state of the person, the progress of the disease, but above all the conditions of the existence of social danger. This reassessment is done for the sole reason that if the situation is improved and the person no longer shows danger, the need of this measure no longer exists and therefore the court is obliged, in accordance with the law, to apply another more lenient measure. The re-evaluation is done on the basis of the appeal of the party, but also mainly by the court where the acts are found.

The execution of the medical measure is done upon the request of the prosecutor. In case the person is locked up in a penitentiary institution, the execution of the medical measure is done by the body where the person is convicted. While in cases when the person is free the execution is carried out by the state police.14

**Psycho-social type custody measures**

An institution that needs special attention and that in many countries of the world is in itself a coercive measure imposed by the court in the full sense of the word, is the placement in custody in psycho-social centers.15

In itself this measure has all the effects of the coercive measure and is imposed by the court in the following cases:

1. as an initial measure for persons who have committed not very serious criminal offenses that present a controllable social danger without the need for enclosure in a medical institution;

2. to persons who have stayed for a certain period of time in a closed structure, when the social danger enters the limits defined in point 1, while waiting for the extension or revocation of the measure.16

The implementation of the psycho-social custody measure must be supervised by the court and as such can be no other than temporary. In this type of measure, the personality of the irresponsible person is monitored with special care, despite the importance of the criminal offense and the fact that the perpetrator is free. Here we are faced with the fact that these irresponsible sick subjects need appropriate therapy and a preventive measure for not committing other acts in the future. Psycho-social type of custody is not a coercive measure, but a complexity of mandatory psychotherapeutic interventions of a preventive nature.

Supervision of the irresponsible subject is closely related to a disease condition that necessarily requires medical intervention. Otherwise stands the supervision of the mentally responsible author on parole, who is free and mostly agrees to cooperate with the guardian. Irresponsible persons in custody are difficult persons who undergo treatment and in this sense it is not only necessary the need of a guardian, but also the existence of a medical observation.17

Another model of the Nordic countries is that of placing these persons in private institutions authorized and controlled by the state.18
Actually psycho-social custody measures are not provided in our Criminal Code, but referring to the practice of other countries, they have resulted to be quite effective in dealing with the above-mentioned subjects. Currently, these subjects, i.e. those who have committed a criminal offense under the conditions of intoxication or use of narcotic and psychotropic substances, are not subject to any kind of rehabilitative measures, but only to imprisonment, the same as other subjects. In these types of persons, quite vulnerable, manifestations of mental disorders have been observed during the execution of the sentence, precisely because of the lack of specialized treatment.

**Reasons for the closure of the judicial psychiatric hospitals in western countries**

In Italy and other countries of Western Europe, the psychiatric hospitals, where mentally ill individuals who have committed crimes, but are legally irresponsible for their actions, are kept under medical measures, or individuals with lower mental balance who need medical treatment, are moving towards closure. In order to have a clear picture on the reasons why these institutions are closing, we should list a number of weak points of the application of this system in practice. Anyway, what results to be the main reason of closure has to do with the fact that this particular type of treatment of this category of subjects, in itself, lost its remedial and rehabilitative function.

First of all, the forced closure in a psychiatric hospital is contrary to constitutional principles, such as the right to health and the respect for human dignity.19

Secondly, the forced closure in a psychiatric hospital is linked with the principle of flexibility that leads the restrictive measures, so that a certain measure can be appropriate for the therapeutic needs of the subject, in other words to the medical treatment which the latter must undergo.

Also, the forced closure in a psychiatric hospital does not respect the principle of proportionality, considering the specific circumstances of each case in respect of freedoms and human rights. The importance of this principle has been emphasized many times by the European Court of Human rights, in the interpretation of the provisions of the European Convention on Human Rights.

This measure in the same time violates the principle of distinctness between the sentence as a punishment and restrictive measures.

This enumeration can’t be claimed to be exhaustive. It is limited in the appearance of the main indicators and rules to which any reform of criminal law have to undergo.

Year 2015 marked in most European countries the end of psychiatric hospitals.20 The completion of this stage should be accompanied with the construction of suitable structures to hold this category of subjects, who were at the stage of execution of these measures. In principle, it was estimated that these new structures should totally be under medical management.

This category of subjects should be admitted to these institutions under the criterion of territoriality, in other words, the institutions closer to the place of residence or stay.

Another condition regarding these new structures was the inclusion of the professional staff made up of psychiatrists, psychologists, nurses and educators. On the other hand, persons who are not characterized by social dangerousness would receive mandatory outpatient treatment, while being nearby their families.

**Conclusions**

The Albanian penitentiary system needs a profound reform and restructuring of institutions that house irresponsible individuals. Currently in Albania there is no institution of the type of forensic psychiatric hospital, leaving the treatment of this category in the prison hospital or in the hospital of Kruja, based on social risk. These two institutions are conceived as penitentiary institutions and accommodation of these subjects constitutes another constitutional violation.
These subjects are not considered convicted and in the conditions of the impossibility of the state to provide service according to the provisions of the law, they have the right to request outpatient treatment. Practically, this cannot happen, as many of them have committed extremely serious crimes such as those against life, health or sexual freedom.

We are of the opinion that it is the time to provide in the Criminal Code a new coercive measure, in addition to compulsory outpatient treatment and compulsory treatment in a medical institution, that of psycho-social type of care. The provision of this measure would be particularly effective in treating those subjects who have committed a criminal offense under the conditions of intoxication or use of narcotic and psychotropic substances, as has resulted from the practice of other countries.

Currently, these subjects, i.e. those who have committed a criminal offense under the conditions of intoxication or use of narcotic and psychotropic substances, are not subject to any kind of rehabilitative measures, but only to imprisonment, the same as other subjects. In these types of persons, very vulnerable, manifestations of mental disorders have been observed during the execution of the sentence, precisely because of the lack of specialized treatment.

**Recommendations**

The definitive closure of psychiatric hospitals will set lawyers and politicians facing each other, in order to overpass the complexity of criminal system and of medical intervention in full respect of human rights and fundamental freedoms.

For a full analysis of this issue associated to irresponsibility because of mental state, it’s important to examine the penalties to which undergo the irresponsible individuals.

Although at the time of their creation, medical measures were presented as a model of progress and openness to human sciences, in a short time they showed their authoritative nature and resulted in a failure.

**This happened for many different reasons.**

First of all, in legislator’s point of view, while the punishment should have a retributive and preventive function, the medical measures were intended to have a specific preventive function (at least theoretically), as they were finalized by the treatment and rehabilitation of a socially dangerous subject. Medical measures, given to irresponsible individuals, or half responsible, but socially dangerous, were applied individually or uphold the sentence, giving rise to a dual sanctioning system. The doctrine of criminal law considers medical measures more severe than the authentic imprisonment sentence.

Medical measures are contrary to the principle of lawfulness in defense of the personal freedom, because of the fact that they are indeterminate in term, being on time for as long as lasts the social dangerousness, as well as of the fact that they are exposed to a great degree of uncertainty.

In practical terms, it is important to note that medical measures cannot be simply implemented because of the current lack of specialized institutions, such as the psychiatric hospital of Elbasan, but also because of the fact that outpatient treatment in many districts is almost impossible.

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**Conflict of Interest:** Nil

**Ethical Clearance:** The manuscript is in compliance with the rules and regulations on ethics in research and publishing activity approved from the University of Tirana, Albania

3. Muci S. E drejta penale, pjesa e pergjithsme


7. Court AH. Decision no.18. 2010 May 12.


The Role of IFN gamma and IL-10 in Breast Cancer

Lale Maulin Prihatina1,2, Willy Sandhika3,4, Grace Ariani3,4
1 Resident, Departement of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, 2 Resident, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, 3 Lecturer, Departement of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, 4 Lecturer, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

Abstract

Inflammation in the tumor microenvironment is an essential aspect of tumor biological activity. Interferon γ and IL-10 are pro-inflammatory and anti-inflammatory cytokines that play a crucial role in regulating the host’s immune response to cancer cells. IFN-γ and IL-10 expression are associated with poor prognosis and low survival rate in breast carcinoma patients. This cross-sectional study was performed on the 60 paraffin-embedded samples of radical mastectomy during January 2016-December 2019 at Anatomical Pathology Laboratory of Dr. Soetomo General Academic Hospital Surabaya. The samples were divided based on tumor size into four groups (T1, T2, T3, T4). The analysis was using Kruskal Wallistest. Immunohistochemical staining was performed to detect the expression of IFN-γ and IL-10. There was a significant difference in IFN-γ expression in the four groups (p=0.005) and no significant difference in IL-10 expression in the four groups (p=0.191). Interferon gamma and IL-10 were important in determining prognosis and targeted therapy in breast cancer patients. These results may contribute to the development of breast cancer research.

Keywords: Breast cancer, IFN-γ, IL-10, T stage.

Introduction

Inflammation in the tumor microenvironment is an essential aspect of tumor biological activity because it is associated with tumor initiation, tumor development, response to therapy, and prognosis. Most cancers arise associated with inflammation that occurs continuously.1,2

The incidence of breast cancer increases with age; 80% of breast cancers appear in women over 50 years. Breast cancer is rare in young women, but it becomes more aggressive, with 5-years survival rates reaching 81% at less than 45 years. Early detection with immunoregulatory cytokines including interferon-alpha, beta, gamma, interleukins 2, 6, and 10, and alpha tumor necrosis factor (TNF), which is often associated with breast cancer and can help determine the patient’s prognosis.3

Interferon-gamma and interleukin 10 are pro-inflammatory and anti-inflammatory cytokines that regulate immune responses and inhibit pro-inflammatory function to antigen-presenting cells (APCs) through the expression of antagonist molecules. Interferon-gamma and interleukin expression are associated with poor prognosis and low survival rates4. IFN-γ has an antitumor function, and a reduced amount of IFN-gamma in the tumor environment is associated with a worse prognosis in breast cancer patients.3

IL-10 overexpression triggers a pro-inflammatory effect by increasing IFN-γ, IL-10, and other IFN-γ-induced releases of monokines. Therefore, giving IL-10 antagonists is expected to be a more effective targeted therapy.5
This research aimed to analyze the expression of IFN-γ and IL-10 and investigate differences in expression at various stages of breast cancer.

**Materials and Methods**

This study was analytic observational research with a cross-sectional approach performed on the 60 paraffin-embedded samples of radical mastectomy during January 2016-December 2019 at Anatomical Pathology Laboratory of Dr. Soetomo General Hospital Surabaya. The samples were grouped based on tumor size into four groups (T1, T2, T3, and T4); each group was 15 samples.

Immunohistochemistry staining was performed to detect the expression of IFN-γ and IL-10. The tissues were cut into four mm sections, deparaffinized three times with xylol for five minutes each, and rehydrated through graded alcohol. Antigen retrieval was achieved by microwave treatment in sodium citrate buffer (pH 6.0) for ten minutes. The tissue sections were then incubated with monoclonal antibodies for IFN-γ (LLO6Z: sc-74108; dilution 1:200; Santa Cruz Biotechnology) and IL-10 (GT5111; dilution 1:100; GeneTex) overnight, followed by the secondary antibody for 10 minutes at room temperature. Sections were then counterstained with hematoxylin and dehydrated with alcohol.

Cytoplasmic staining for IFN-γ and IL-10 were evaluated on tumor cells. IFN-γ and IL-10 are considered positive if expressed in tumor cells’ cytoplasm.5

Two pathologists evaluated all samples in a blinded fashion. Any discordant was solved by interobserver agreement. The expression and comparison of IFN-γ and IL-10 expression in any T stages of breast carcinoma was tested using Kruskal- Wallis and Mann-Whitney U test.

**Results and Discussion**

Kruskal-Wallis test showed difference IFN-γ expression in any T stage breast carcinoma \( (p=0.005) \) (Figure 1), and the Mann-Whitney U test found a significant difference in IFN-γ expression between T1 - T2 and T2 - T3 \( (p=0.03; p=0.04) \) meanwhile there is no considerable difference IFN-γ expression in T3 - T4 \( (p=0.2) \) or other T stages. Interferon-gamma and IL-10 expressed at cytoplasm (Figure 2 and 3). Kruskal-Wallis test showed no difference in IL-10 expression in any T stages breast carcinoma \( (p=0.191) \) (Figure 3). Interleukin-10 is expressed in the cytoplasm (Figure 1).
Interferon-γ (IFNγ) has a vital role in activating cellular immunity and stimulation of the antitumor immune response. Based on its cytostatic, proapoptotic, and antiproliferative functions, IFNγ is considered potential adjuvant immunotherapy for various cancer types. IFNγ may inhibit angiogenesis in tumor tissue, induce regulatory T-cell apoptosis, and stimulate pro-inflammatory M1 macrophage activity to support tumor cells proliferation.6

The statistical analysis results showed a significant difference between IFNγ expression in any T stages breast carcinoma ($p = 0.005$). The most significant
difference between each group on the IFNγ expression was performed using the Mann-Whitney U statistical test. This test shows that the T1 and T2 groups are the groups that have the most significant difference \((p = 0.03)\), and the T2 and T3 groups \((p = 0.04)\) while the T3 and T4 groups did not have a substantial difference with \(p = 0.203\) as well as other groups.

High IFNγ expression was found at stage T1, then decreased at stage T2 and increased again at T3 and T4. In T1 tumors, IFNγ will be secreted in large quantities by pro-inflammatory cytokines to support tumor cell proliferation; at this early stage, the body is still able to adapt to inhibit tumor cell proliferation so that IFNγ levels can be suppressed in T2 tumors, hence inflammatory agents are not eliminated (tumor). The inflammatory process continues, causing an increase in pro-inflammatory cytokines and an accelerated proliferation of tumor cells; as a result, the body cannot keep up with the speed of tumor cell proliferation and loses its homeostasis ability, the tumor size increases, and IFNγ levels increase again at stages T3 and T4.\(^{2,7,8}\)

These results were consistent with the study by Tuñón et al., who found differences in IFNγ expression at various T stages of breast carcinoma, and the highest expression was found at stages T1 and T2.\(^9\) He et al. stated that continuous exposure to IFN-γ had been shown to increase the growth of hepatoma tumors, breast tumors, adenocarcinoma, and melanoma.\(^{10}\) These results indicate that the higher levels of IFN-γ in the tumor microenvironment can increase tumor cell growth and proliferation.

Interleukin-10 is known to have an inhibitory effect on T-cell function and proliferation. Interleukin-10 has also been shown to inhibit antigen presentation by macrophages and Langerhan cells and manifest tumor-related antigens by tumor cells. IL-10 production is associated with the induction of anergy in T lymphocytes so that the tumor utilizes IL-10 production in the tumor microenvironment to escape from the immune system.\(^{11}\)

The statistical analysis results showed no difference in IL-10 expression at various stages of T breast carcinoma with a \(p\)-value = 0.191. Expression of IL-10 tends to be high in all T stages, and the highest is found in staging T4 of breast carcinoma. This can occur because tumor cells naturally produce large amounts of IL-10 themselves. Many human cancer cell lines have been shown to secrete IL-10 into their supernatants. Breast tumor cells have been shown to express high levels of IL-10 mRNA. This elevated expression causes high levels of IL-10 to be expressed in the tumor microenvironment and breast tumor cells.\(^2\) These results are in line with Bhattacharjee et al., which stated that there was no correlation between IL-10 expression and patient age, tumor size (T), and ER status, PR.\(^5\)

Gonzalez-Garza et al. Examined samples from breast cancer patients and normal peritumoral breast tissue. Found a correlation between IL-10 expression and a worse prognosis in breast carcinoma patients, and no IL-10 was expressed in normal breast tissue samples. The determination of IL-10 expression in breast cancer patients suggests that IL-10 can be used as a biological marker to differentiate between advanced cancer.\(^{13}\)

Lianes-Fernandez et al. obtained strong IL-10 expression in 23 of 27 breast cancer patients based on immunohistochemical examination. In another study of 105 samples of breast cancer patients and 13 samples of healthy breast tissue, IL10 expression was only seen in breast cancer tissue and no normal healthy tissue.\(^{11,14}\)

The high concentration of IL-10 in the serum of cancer patients does not appear to be related only to the expression of this protein by the immune system. Evidence suggests that cancer cells can synthesize themselves, thereby causing an imbalance in the homeostasis of the immune system and causing tumors to escape.\(^{13}\)

**Conclusion**

There were differences of IFNγ expression in any T stages of breast carcinoma, and there was no difference of IL-10 expression in any T stages of breast carcinoma.

**Conflict of Interest**: The authors declare that they have no conflict of interest.
Source of Funding : This study is supported by the Ministry of Education and Culture of the Republic of Indonesia.

Acknowledgments : We thank Dr. Budi Utomo, Department of Public Health and Preventive Medicine, Universitas Airlangga, for statistical analysis.

Ethical Approval : This study had been approved by the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia 0337/LOE/301.4.2/II/2021.

References
Thyroid Storm in Post-Partum in Bangkalan Hospital: A Case Report

Lambu Henderika Dacosta1, Ferdy Royland. Marpaung2

1Resident, Clinical Pathology Specialization Programme, Department of Clinical Pathology, Faculty of Medicine, Airlangga University - Dr. Soetomo General Hospital, Surabaya, Indonesia, 2Lecturer, Department of Clinical Pathology, Faculty of Medicine, Airlangga University - Dr. Soetomo General Hospital, Surabaya, Indonesia

Abstract

Thyroid crisis is a rare complication of hyperthyroidism, with a greater risk about 10 times during pregnancy, also clinical manifestations and significant increasing of level of thyroxine (T4) and tri-iodothyronine (T3). Thyroid crisis could cause mortality in >10%. A 58-year-old female was referred from Bangkalan Hospital, presented with palpitation, shortness of breath, diarrhea 2-3×/day, vaginal bleeding, fever for 1 day before admittance. Hyperthyroid for 3 years ago and routinely consumed thyrozol (1×2tab) in 2 years but did not take the medicine for 1 year ago. Physical examination: BP 147/100 mmHg, pulse rate 124×/minute, temperature 38.4 ºC, RR 30/minute, conjunctiva anemia, thyroid palpable, exopthalmus. ECG results: presence of sinus tachycardia, Chest X-Ray: cardiomegaly. Burch Wartofsky Score obtained a total score of 45, (temperature 38.5 C=15, diarrhea=10, tachycardia=15, CHF=5). Laboratory results: TSH <0.004 IU/mL, FT4 5.31 ng/dl. Thyroid crisis is a rare case of hyperthyroidism, a greater risk during pregnancy which could lead to death. Many symptoms arise in hyperthyroidism which could lead to thyroid crisis. The criteria which used to assess thyroid analysis is the Burch Wartofsky Score, FT4 levels increased 5.31 ng/dl. This patient was diagnosed as thyroid storm in post-partum, based on Burch Wartofsky Score, low TSH level and an elevated FT4 level and not taking medicine for 1 year ago.

Keywords: Thyroid storm, maternal hyperthyroidism, thyroid function, hyperthyroid.

Introduction

Thyroid crisis is a rare complication of hyperthyroidism, with a 10 times greater risk of occurring during pregnancy1. The term “storm” describes the intensity of clinical manifestations and the significant increase in levels of thyroxine (T4) and tri-iodothyronine (T3)2,3. The incidence of hyperthyroidism during pregnancy is relatively rare and usually treated medically with the drug thioamide. One of the most dreaded symptoms of hyperthyroidism is thyroid crisis/thyroid storm, with a mortality rate of >10%1,2,4,5. In the United States survey, the incidence of thyroid storm ranged from 0.57 to 0.76 cases per 100,000 per year in the normal population. According to the Japanese National Survey, the incidence of thyroid storm is 0.2 per 100,000 population per year, approximately 0.22% of all thyrotoxicosis patients3. Poor control of hyperthyroidism during pregnancy is associated with number of problems, including Intrauterine Fetal Death (IUFD), hypertension in pregnancy, preterm birth, low birth weight, intrauterine growth restriction and maternal congestive heart failure6. Diagnosing a thyroid crisis is not easy, therefore an approach is needed to expedite a thyroid crisis diagnose by using the Burch Wartofsky Point Scale with a value of ≥45 to diagnose a thyroid crisis. The principles of management of thyroid crisis in pregnancy reduce the synthesis and secretion of thyroid hormones, decrease the peripheral effects of thyroid hormones, inhibit the conversion of T4 to T3, therapy to prevent systemic decompensation, therapy of precipitating diseases, pregnancy management and supportive therapy7,8,9,10.
Case

Patient Mrs. H, 25 years, referral from Bangkalan Hospital, 7 hours post-partum, came with complaints of palpitations, shortness of breath (+) and diarrhea 2-3 times/day and fever for 1 day before admittance to the hospital, as well as vaginal bleeding (+). The patient suffered from hyperthyroidism for 3 years, had routine treatment for 2 years, and the last 1 year did not take PTU drugs. Physical examination: Blood pressure: 147/100, pulse 124×/m, temperature 38.5°C, RR 30×/m, anemia, palpable enlargement of the thyroid, exophthalmus with a stell wag’s sign (+). ECG results: sinus tachycardia, chest X-ray results: cardiomegaly. The Burch Wartofsky Score got a total score of 45, (temperature 38.5 °C=15, diarrhea=10, tachycardia=15, CHF=5). Laboratory results TSH values <0.004uIU/mL, FT4 5.31 ng/dl.

Table 1. Results of Clinical Chemistry Laboratory Tests.

<table>
<thead>
<tr>
<th></th>
<th>25/12/19</th>
<th>Normal Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (mmol/L)</td>
<td>4.3</td>
<td>3.5-5.1 mmol/L</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>141</td>
<td>136-145 mmol/L</td>
</tr>
<tr>
<td>Cl (mmol/L)</td>
<td>110</td>
<td>98-107 mmol/L</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>10</td>
<td>mg/dL</td>
</tr>
<tr>
<td>SCr (mg/dL)</td>
<td>0.83</td>
<td>mg/dL</td>
</tr>
<tr>
<td>Alb (g/dL)</td>
<td>2.1</td>
<td>g/dL</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>62</td>
<td>U/L</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>18</td>
<td>U/L</td>
</tr>
<tr>
<td>Calcium</td>
<td>6.8</td>
<td>mg/dL</td>
</tr>
</tbody>
</table>

Table 2. Blood Gas Analysis Table.

<table>
<thead>
<tr>
<th></th>
<th>25/12/19</th>
<th>26/12/19</th>
<th>Normal Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.35</td>
<td>7.29</td>
<td>7.35-7.45</td>
</tr>
<tr>
<td>pCO2 (mmHg)</td>
<td>16.0</td>
<td>14</td>
<td>35-45</td>
</tr>
<tr>
<td>pO2</td>
<td>134</td>
<td>123</td>
<td>80-100</td>
</tr>
<tr>
<td>HCO3(mmol/L)</td>
<td>8.8</td>
<td>6.7</td>
<td>22-26.</td>
</tr>
<tr>
<td>BaseExcess (mmol/L)</td>
<td>-16.8</td>
<td>-19.9</td>
<td>Mmol/L</td>
</tr>
<tr>
<td>SO2%</td>
<td>99</td>
<td>98</td>
<td>%</td>
</tr>
<tr>
<td>Aado2</td>
<td>-4</td>
<td>123</td>
<td>mmHhg</td>
</tr>
</tbody>
</table>
### Table 3. Immunological Results.

<table>
<thead>
<tr>
<th></th>
<th>25/12/19</th>
<th>Normal Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT4</td>
<td>5.31</td>
<td>0.9-1.76 ng/dl</td>
</tr>
<tr>
<td>T3 Total</td>
<td>2.05</td>
<td>0.6-1.81 ng/dl</td>
</tr>
<tr>
<td>TSH</td>
<td>&lt;0.004</td>
<td>&lt;2&lt;12th: 0.64-6.27 μIU/mL 12-&lt;18th: 5.1-4.94 &gt;18th: 0.55-4.78</td>
</tr>
<tr>
<td>TIBC</td>
<td>380</td>
<td>250-450 ug/dl</td>
</tr>
<tr>
<td>Serum Iron</td>
<td>29</td>
<td>35-150 ug/dl</td>
</tr>
</tbody>
</table>

### Table 4. Results of the Laboratory of Hematology at Dr. Soetomo General Hospital, Surabaya.

<table>
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<th></th>
<th>25/12/19</th>
<th>26/12/19</th>
<th>Normal Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>7.4</td>
<td>6.9</td>
<td>13.3-16.6</td>
</tr>
<tr>
<td>RBC (10⁶/µl)</td>
<td>2.94</td>
<td>2.71</td>
<td>3.69-5.46</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>22.2</td>
<td>21.3</td>
<td>41.3-52.1</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>75.5</td>
<td>78.6</td>
<td>86.7-102.3</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>25.2</td>
<td>25.5</td>
<td>27.1-32.4</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>33.3</td>
<td>32.4</td>
<td>29.7-33.1</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>16.7</td>
<td>16.6</td>
<td>12.2-14.8</td>
</tr>
<tr>
<td>WBC (10³/µl)</td>
<td>13.05</td>
<td>17.03</td>
<td>3.37-10</td>
</tr>
<tr>
<td>% Eo</td>
<td>0.2</td>
<td>0.0</td>
<td>0.6-5.4</td>
</tr>
<tr>
<td>% Ba</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3-1.4</td>
</tr>
<tr>
<td>% Neu</td>
<td>81.9</td>
<td>84.3</td>
<td>39.8-70.5</td>
</tr>
<tr>
<td>% Ly</td>
<td>15.6</td>
<td>13.4</td>
<td>23.1-49.9</td>
</tr>
<tr>
<td>% Mo</td>
<td>2.1</td>
<td>2.2</td>
<td>4.3-10</td>
</tr>
<tr>
<td>Plt (10³/µl)</td>
<td>90</td>
<td>121</td>
<td>150-450</td>
</tr>
</tbody>
</table>
**Table 5. Results of Urinalysis.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Date</th>
<th>Value</th>
<th>Normal Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>25/12/19</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>2.05</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Keton</td>
<td>&lt; 0.004</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>1.010</td>
<td>1.030-1.030/dl</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1+</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>5.50</td>
<td>4.5-8.0</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>1+</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>0.2</td>
<td>&lt; 0.1 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Nitrit</td>
<td>+/-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Colour</td>
<td>Yellow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarity</td>
<td>Clear</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1. A. AP chest X-ray results. B. Exophthalmus.**

Cor: Big and enlarged impression shape.
Pulmo: invisible infiltrate.
Trachea in the middle.
The right and left phenicocostalis sinus is sharp.
Right and left hemidiaphragm looks good.
The bones look good.
There was no visible soft tissue abnormality.

Impression: Cardiomegaly.

Pulmo did not appear abnormal.

**Discussion**

Thyroid crisis is a rare complication of hyperthyroidism, with a 10 times greater risk of occurring during pregnancy. Patients with hyperthyroidism during pregnancy with poor control could precipitate a thyroid crisis.\(^1\) In this case, there was poor control during pregnancy where patient stopped taking the drug for a year. The patient was referred from Bangkalan Hospital, 7 hours after post-partum, with complaints of palpitations, shortness of breath, and diarrhea 2-3 times a day for 1 day before admittance to the hospital, vaginal bleeding and fever was also complained of. Theoretically, pregnancy will cause an increase in Thyroid Binding Globulin (TBG) levels which result in an increase in total T4 and T3.\(^5\) Pregnancy could worsen the state of hypertiroidism, resulting in various symptoms. Clinical symptoms which arise such as fever, tachycardia, heart failure, decreased consciousness, seizures, gastrointestinal disorders.\(^2,4,6\)

The patient had a 3-year history of taking the drug and stopped taking the drug for the last 1 year. There were clinical symptoms of fever, diarrhea, tachycardia, rapid breathing and shortness of breath, therefore the patient was referred to the hospital. Cardiomegaly was found on the chest X-ray and to diagnose a thyroid crisis is not easy using the Burch-Wartofsky Point Scale (Table 6).

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Points</th>
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<tbody>
<tr>
<td>Thermoregulation disorders</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
</tr>
<tr>
<td>37.2–37.7</td>
<td>5</td>
</tr>
<tr>
<td>37.8–38.3</td>
<td>10</td>
</tr>
<tr>
<td>38.4–38.8</td>
<td>15</td>
</tr>
<tr>
<td>38.9–39.3</td>
<td>20</td>
</tr>
<tr>
<td>39.4–39.9</td>
<td>25</td>
</tr>
<tr>
<td>≥ 40.0</td>
<td>30</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td></td>
</tr>
<tr>
<td>Tachycardia per minute</td>
<td></td>
</tr>
<tr>
<td>90–109</td>
<td>5</td>
</tr>
<tr>
<td>110–119</td>
<td>10</td>
</tr>
<tr>
<td>120–129</td>
<td>15</td>
</tr>
<tr>
<td>130–139</td>
<td>20</td>
</tr>
<tr>
<td>≥ 140</td>
<td>25</td>
</tr>
<tr>
<td>Atrial Fibrillation</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>10</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td>5</td>
</tr>
<tr>
<td>Moderate</td>
<td>10</td>
</tr>
<tr>
<td>Severe</td>
<td>15</td>
</tr>
<tr>
<td>Gastrointestinal-hepatic disorders Symptoms</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Moderate (diarrhea, abdominal pain, nausea/vomiting)</td>
<td>10</td>
</tr>
<tr>
<td>Severe (jaundice)</td>
<td>20</td>
</tr>
<tr>
<td>------------------</td>
<td>----</td>
</tr>
<tr>
<td>Central nervous system disturbance</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Mild (agitation)</td>
<td>10</td>
</tr>
<tr>
<td>Moderate (delirium, psychosis, extreme lethargy)</td>
<td>20</td>
</tr>
<tr>
<td>Severe (seizure, coma)</td>
<td>30</td>
</tr>
<tr>
<td>Precipitating event</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>present</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total score</strong></td>
<td></td>
</tr>
<tr>
<td>≥ 45</td>
<td>Thyroid storm</td>
</tr>
<tr>
<td>25–44</td>
<td>Impending storm</td>
</tr>
<tr>
<td>&lt; 25</td>
<td>Storm unlikely</td>
</tr>
</tbody>
</table>

Through this patient’s case was found a Burch-Wartofsky Score 45, fever with a temperature of 38.50°C=15, diarrhea=10, tachycardia=15, CHF=5, where with a score of ≥45 a thyroid crisis was concluded. Patients who are suspected to have a thyroid crisis based on criteria should be treated intensively. The flow of thyroid crisis management is according to the Japan thyroid storm guidelines (Figure 1). Patients with a history of medication and stopping medication are more likely to develop a thyroid crisis. Patients were evaluated for A, B, C, D, E, treatment and referred to the thyroid crisis diagnosis algorithm. Laboratory results TSH value <0.004uIU/mL, FT4 5.31 ng/dl, anemia, also low albumin in the presence of laboratory results which support a thyroid crisis2,4,5,6. When patients are diagnosed by thyroid crisis should be suggested with PTU 600 mg/day4,7,8.

**Conclusion**

Thyroid crisis is an emergency characterized by acute hypermetabolic rapid deterioration of a life-threatening condition. Early suspicion, prompt to diagnosis and intensive treatment which will improve survival in patients with thyroid crisis. The diagnosis criteria for thyroid crisis using the Burch Wartofsky Score, this patient obtained a score of ≥45 and with a history of stopping taking PTU drugs for 1 year.

**Conflict of Interest:** The author declare that they have no conflict of interest.

**Source of Funding:** None.

**Acknowledgements:** We thank Arif Nur Muhammad Ansori for editing the manuscript.

**Ethical Approval**

This study approved by the Faculty of Medicine, Airlangga University - Dr. Soetomo General Hospital, Surabaya, Indonesia.

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Role of Serum Levels of Thymidine Kinase 1 in Diagnosis and Differentiating of Prostatic Tumor

Lara Balasim Al-Dahy1, Basil O. Mohammed2, Saad Dakhil F. Daraji3, Mazin M. Farhan4

1Scholar Researcher, Msc, Clinical Biochemistry, Nahrain University College of Medicine, Iraq, 2Scholar Researcher, PH.D Clinical Chemistry, Department of Biochemistry, College of Medicine, University of Baghdad, Iraq, 3Scholar Researcher, Prof.Dr (Consultant) Urologist, Department of Urology, College of Medicine, University of Baghdad, Iraq, 4Scholar Researcher, Consultant Urologist, Center of Urology, Al-Yarmouk hospital, Iraq

Abstract

Background: Both benign prostatic (BPH) and prostate (Pca) hyperplasia include prostate enlargement. The distinction between benign prostatic hyperplasia and prostate cancer is a major challenge, since the prostatic specific antigen (PSA) cannot be considered a reliable predictor of prostate cancer. Aims: Efficiency of serum thymidine kinase 1 and PSA-related biomarkers in prostate tumor, BPH and PCa diagnosis and differentiation, especially when serum PSA is in the gray (4-10 ng/ml) region and in the pronostic of these patients after surgical therapy. Subjects and Methods: A case control and cross-sectional review. There were 110 elderly patients (45-81 years) and 45 controle. Serum experiments involved the use of ELISA technologies to measure tPSA, fPSA, and TK1. Result: In comparison to both of the BPH and controls, the mean (±S D) of serum tPSA and fPSA in Pca were significantly improved (all p =0,001) while the mean value of f PCa in comparison with BPH and control was dramatically decreased (p = 0,001) for fPSA/tPSA. There was no difference between BPH and controls in these parameters. In both of the Pca and Controls (P<0.001) the mean TC1 was slightly higher (P<0.001). This serum TK1 has the most receptive and specific diagnostic and differentiating potency in the gray zone of tPSA (4-10 ng/ml), which has tPSA in the gray zone with AUC=1 in the 924 pg/ml cut-off zone.

Conclusion: Serum level of TK1 was superior of tPSA in diagnosis of prostate tumor and differentiating between BPH and PCa.

Keywords: Serum, Thymidine Kinase 1, prostatic tumor

Introduction

Prostatic Cancer (PCa) is a complex and heterogeneous disease and the most common malignancy in males worldwide, and the second-leading cause of cancer-associated mortality. While the prevalence of PCa in Arab countries is lower than that in Western countries (1). The majority of PCa cases are indolent and localized at diagnosis, localized tumors can develop into aggressive tumors in the long term (2). A major clinical challenge in prostate cancer clinical management is posed by the inability of current diagnostic tests, such as serum PSA testing, digital rectal examination, and histopathologic grading of tissues, to discern between indolent and aggressive disease (3). Prostate enlargement, is a condition that affects the prostate gland in men. The incidence of occurrence of BPH has recently been estimated at 42% in males aged 51–60, 70% in those aged 61–70 and 90% in males between 81–90 years (4). A homo dimer with an underunit size of 25 kDa is the essential intracellular shape of TK1. In the event of DNA disruption in advance of a new synthesis, intracellular TK1 dimers may be easily transformed into active tetramer (5). TK1 is released from cell death through proliferation; an indicator of cell disruption is the concentration of TK1 in extracellular fluid. Regular cells rarely disintegrate during proliferation and normally, like malignancies, only occur during rapid or non-regulated proliferation. Following its release, TK1 forms
complexes of various molecular weights and enzymes, which is an important element in developing tests (6). TK1 moves the phosphate from ATP and transforms deoxythymidine (dT) to deoxythymidine (dTMP). Monophosphate is exposed to additional phosphorylation of DNA-incorporated deoxidation thymidin triphosphate (dTTP). dTTP self-regulates TK1’s negative feedback DNA precursor synthesis (7). TK1 activity is regulated by the cell cycle and has a distinct pattern of activity compared with tumor cells in normal proliferating cells (2,8).

**Material and Method**

This case-control study took place at the University of Baghdad, the Department of Biochemistry, the University and the Ghazi Al-Hariri Hospital for Specialized Surgery/Medical City in September 2019 to March 2020. During this period the study was conducted. The range of patients (45-81 years) included 110 patients; BPH (n=55) and prostatic cancer (n=55) and controls were 45. The consultant Urologist and Oncology Group was able to obtain diagnosis of BPH and PCa. Each individual obtained formal consent. The Scientific Committee of the department of Biochemistry, University of Baghdad (Iraq), accepted our ethics in this regard. Exclusion criteria is any other conditions such as prostatic intraepithelial neoplasia and other non-malignant diseases of the prostate are excluded in this study and Chronic disease cases like renal failure. The peripheral vein venipuncture of each patient and healthy control woman was extracted by five milliliters of blood, transferred to the simple tube and allowed to coagulate over 15-30 minutes and the serum had been isolated by centrifugation over 10 minutes at 2500-3000 rpm, and stored at 20°C, until the day of the assessment of the TPSA, PSA, and Thymidine (TK1). TK1 kits were provided from My BioSource, Inc., USA.

**Result**

The mean (±SD) values of serum TK1, TPSA, FPSA and fPSA/tPSA ratios of the groups studied are shown in Table 1. Compared to BPH and controls (for all; P<0.001), and Pca serum TK1 levels was significantly higher. The mean value of BPH serum TK1 was significantly higher than that of the controls (P<0.001). Compared to BPH and controls (both P=0.001), the mean (±SD) value of Pca serum tPSA levels was significantly higher. After all, there was no significant difference in tPSA between BPH and controls. In Pca the mean (±SD) value of serum fPSA was slightly higher than in BPH (P=0.001) and controls (P=0.001), respectively. Variance between BPH and controls is also not significant. The mean (±SD) of the fPSA/tPSA ratio of BPH (P=0.001) is also seen in the same table and the controls (P=0.003) is slightly higher than the Pca ratio with no other significant difference.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BPH (n=55)</th>
<th>Control (n=45)</th>
<th>Pca (n=55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tPSA (ng/ml)</td>
<td>3.06±0.07</td>
<td>6.78±3.95</td>
<td>31.41±17.96*</td>
</tr>
<tr>
<td>fPSA (ng/ml)</td>
<td>0.89±0.44</td>
<td>2.25±1.99</td>
<td>6.07±3.32*</td>
</tr>
<tr>
<td>fPSA/tPSA ratio</td>
<td>31.28±17.77</td>
<td>36.01±18.8</td>
<td>20.96±7.12**</td>
</tr>
<tr>
<td>TK1 (pg/ml)</td>
<td>570.09±150.40</td>
<td>1221.20±122.87</td>
<td>205.06±80.80***</td>
</tr>
</tbody>
</table>

*ANOVA & t-test revealed  ● Significant increase in Pca concentrations (tPSA, fPSA) relative to each BPH and control (for both, p<0.001). ●● Significant increase in BPH and control (fPSA/tPSA ratio) levels compared with Pca, (p<0.001). ●●●● Significant differences between Pca and each of the BPH and Controls (P<0.001) in TK1 levels. NS: non-significant differences in levels between BPH and controls (tPSA, fPSA, fPSA/tPSA ratio)
Table (2) In the difference between Pca and normal subjects, the cutoff value of the serum tPSA level was 4.30 ng/ml with a ROC of 1.00. The serum TK1 level was 1.00 with a cutoff value of 924 pg/ml and ROC and 100 percent sensitivity and 100 percent accuracy for such distinction. The fPSA cutoff value was 2.10 ng/ml with a ROC value of 0.94 and the fPSA/PSA ratio was 28.72 with a ROC value of 0.67. Differentiation by serum tPSA level at a cutoff value of 4.2 ng/ml and ROC of 1.00 between BPH and normal individuals. The ROC values for TK1 was a cutoff value of 924 pg/ml with a ROC of 1.00 for the differentiation between BPH and Pca. Although the fPSA ratio was 1.30 ng/ml and the ROC was 0.92 and the fPSA/tPSA ratio was 83.3 with ROC 0.43. The serum tPSA level at the cutoff value of 10.1 ng/ml has a ROC of 0.83 in the distinction between Pca and BPH. The cutoff value of the fPSA standard was 5.10 ng/ml and the ROC was 0.79.

Table 2: The receiver operator curve (ROC) for (TPSA, FPSA, FPSA/TPSA ratio, TK1) in studied groups

<table>
<thead>
<tr>
<th>Marker</th>
<th>Diagnostic criteria</th>
<th>Pca vs Control</th>
<th>Pca vs BPH</th>
<th>BPH vs Control</th>
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<tbody>
<tr>
<td></td>
<td>SE</td>
<td>98%</td>
<td>71%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>98%</td>
<td>100%</td>
<td>96%</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td>98%</td>
<td>100%</td>
<td>98%</td>
</tr>
<tr>
<td></td>
<td>NPV</td>
<td>98%</td>
<td>77%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Cut Point</td>
<td>4.30</td>
<td>10.1</td>
<td>4.20</td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td>1.00</td>
<td>0.85</td>
<td>1.00</td>
</tr>
<tr>
<td>TPSA (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>FPSA (ng/ml)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>78%</td>
<td>65%</td>
<td>87%</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>100%</td>
<td>100%</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td>100%</td>
<td>100%</td>
<td>84%</td>
</tr>
<tr>
<td></td>
<td>NPV</td>
<td>79%</td>
<td>74%</td>
<td>84%</td>
</tr>
<tr>
<td></td>
<td>Cut point</td>
<td>2.10</td>
<td>5.10</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td>0.94</td>
<td>0.79</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>89%</td>
<td>89%</td>
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<td>SP</td>
<td>49%</td>
<td>62%</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td>68%</td>
<td>70%</td>
<td>56%</td>
</tr>
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<td></td>
<td>NPV</td>
<td>79%</td>
<td>85%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Cut point</td>
<td>28.72</td>
<td>28.72</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td>0.67</td>
<td>0.75</td>
<td>0.43</td>
</tr>
<tr>
<td>FPSA/TPSA ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>100%</td>
<td>100%</td>
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<tr>
<td></td>
<td>SP</td>
<td>100%</td>
<td>98%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td>100%</td>
<td>98%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>NPV</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Cut point</td>
<td>924</td>
<td>924</td>
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<td></td>
<td>AUC</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>
In the gray zone, tPSA was 69 patients (4-10 ng/ml). Fifty-three (73 percent) of them were with PBH and 16 (22 percent) were with Pca, indicating that those with BHT are the largest patients with tPSA gray zone. In PBH and Pca patients in Gray Zone, Table (3) indicates the mean value (±SD) of tPSA, fPSA, fPSA/tPSA, and TK1. The mean value of serum tPSA between Pca and BPH was not substantially different. In Pca, the mean value of serum fPSA in comparison with BPH was significantly lower (P=0.01). The mean fPSA/tPSA ratio value was also considerably lower at Pca than at the BPH stage (P=0.01). In Pca patients, the mean value of serum TK1 was slightly higher than in BPH patients (P<0.0001).

Table (3) Mean (±SD) values of the Age and the measured Serum Biomarker (TPSA, FPSA, FPSA/TPSA ratio, TK1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PCa (n=16)</th>
<th>PBH (n=53)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>tPSA (ng/ml)</td>
<td>6.76+1.82</td>
<td>6.66+1.85</td>
<td>0.86</td>
</tr>
<tr>
<td>fPSA (ng/ml)</td>
<td>1.58+0.73</td>
<td>2.27+1.00</td>
<td>0.01●</td>
</tr>
<tr>
<td>fPSA/tPSA ratio</td>
<td>23.11+8.45</td>
<td>36.73+18.59</td>
<td>0.01●</td>
</tr>
<tr>
<td>TK1 (pg/ml)</td>
<td>1078+91.85 ●●</td>
<td>571.30+151.66 ●●</td>
<td>&lt;0.0001 ●●</td>
</tr>
</tbody>
</table>

Test revealed ▪significant decrease of (fPSA, fPSA/tPSA ratio) levels in Pca compared to BPH (for p=0.01), ●● significant increase of (TK1) levels in Pca compared with BPH, (p=<0.0001).

The cutoff value for fPSA/tPSA ratio was found to be 28.72 in gray zone differentiation between BPH-Paca patients and 0.73 for tPSA. The low ROC values of 0.51 and 0.71 were achieved by both tPSA and fPSA at cutoff values 7.6 NG/ml and fPSA at cutoff value 0.90 NG/ml; respectively The ROC was 1.00, and was the excellent biochemical marker for measured differentiations between patients with Pca and BPH with tPSA in the gray zone, Table of Disposition for Patients with TK1 in gray zone at cutoff value 924pg/ml, Table (4)

Table (4): The receiver operator curve (ROC) for (TPSA, FPSA, FPSA/TPSA ratio and TK1) between Pca and BPH groups.

<table>
<thead>
<tr>
<th>Marker</th>
<th>AUE</th>
<th>Cut-point</th>
<th>SE%</th>
<th>SP%</th>
<th>PPV%</th>
<th>NPV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPSA (ng/ml)</td>
<td>0.51</td>
<td>7.6</td>
<td>44%</td>
<td>67%</td>
<td>26%</td>
<td>79%</td>
</tr>
<tr>
<td>FPSA (ng/ml)</td>
<td>0.70</td>
<td>0.90</td>
<td>38%</td>
<td>92%</td>
<td>60%</td>
<td>83%</td>
</tr>
<tr>
<td>FPSA/TPSA ratio</td>
<td>0.73</td>
<td>28.72</td>
<td>88%</td>
<td>64%</td>
<td>42%</td>
<td>94%</td>
</tr>
<tr>
<td>TK1 (Pg/ml)</td>
<td>1.00</td>
<td>924</td>
<td>100%</td>
<td>100%</td>
<td>94%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Discussion

The findings of this study showed that tPSA and Pca values in Pca were substantially higher in average values than patients with BPH and stable controls (Table 1) (1; 9;10). Furthermore, the present research finding that the mean fPSA/tPSA ratio in Pca patients has been considerably reduced to those of BPH that is consistent in the fPSA/tPSA ratio (11,12) finds that fPSA/tPSA is significantly lower in Pca than in BPH.

The current study has shown that the best cutoff of tPSA was 10.1 ng/ml for the differentiation of Pca from BPH patients, with 100%, 71% for sensitivity, and AUC 0.85, with 98% for specificity 4.30 ng/ml for Pca and for healthy individuals. In patients with BPH and healthy persons, the optimal cut for tPSA was 4.20 ng/mL with AUC=1.00, respectively (table 2), indicating that tPSA level of 4.25 ng/ml is useful in discriminating prostate diseases from healthy individuals. Razzaghi et al. observed in the evaluation of Cancer of the Prostate Strategic Urologic Research (CAPSURE) cohort in the United States that median of tPSA at diagnosis in the higher screened Swedish counties was 10.80 ng/ml (13).

Erdogan et al. found that PCa was diagnosed in 35.1% of their patients and clinically significant variations in f/t PSA were found in patients with and without PCa (14). The current study bought a significant increase in the mean value of TK1 serum levels in the Pca group relative to the BPH group and healthy controls and in the BPH patients compared to experiments that had previous studies agreed to (15;6). The results showed that the serum TK1 evaluation was an excellent biochemical marker for differentiating Pca patients from healthy individuals. Razzaghi et al. observed in the evaluation of Cancer of the Prostate Strategic Urologic Research (CAPSURE) cohort in the United States that median of tPSA at diagnosis in the higher screened Swedish counties was 10.80 ng/ml (13).

The current study bought a significant increase in the mean value of TK1 serum levels in the Pca group relative to the BPH group and healthy controls and in the BPH patients compared to experiments that had previous studies agreed to (15;6). The results showed that the serum TK1 evaluation was an excellent biochemical marker for differentiating Pca patients from healthy individuals with a cut-off of 924 pg/ml (AUC=1.00). Serum TK1 is also outstanding for differentiating BPH from healthy controls at 412 pg/ml (AUC =1) (table 2).

TK1 levels are high in all prostate cancer patients and even higher in patients with severe prostate cancer (16). It is indicated that serum TK1 can be used in the screening of BPH or PC patients and that tPSA is a less reliable prostate screening method compared to STK1.

The gray zone The Bewildering outcome is characterized by serum tPSA level between 4-10 ng/ml; whether BPH or PCa is the condition, resulting in unnecessary prostate biopsies. Out of a total of 235 patients with tPSA level, Psa2 Liu et al. found that the results of the gray zone biopsy were negative for 179 (76.2%) patients (non-PCa group) and positive for 56 (23.8%) patients (PCa group) (14). The result found that the serum TK1 level at a cutoff value of 924 pg/ml (AUC=1, Table 4) was the most sensitive and specific biochemical marker for differentiating between PCa and BPH patients with gray-zone tPSA. However, the tPSA, fPSA, fPSA/tPSA ratio serum measurements were poor biochemical markers for differentiating prostatic origin in the gray zone (table 3). It was concluded that serum TK1 concentrations in patients with BPH and PCa were considerably higher relative to healthy people, suggesting that the serum TK1 concentration could be used to monitor for prostate complications. The individual serum TK1 values between BPH patients and PCa patients, it can be impractical to differentiate between these two categories by measuring STK1 concentration alone. BPH patients with elevated STK1 concentrations are expected to have an increased chance of progression of malignancy (15). Hanousková et al. observed that TK-1 serum levels were dramatically elevated in prostate cancer patients relative to healthy individuals and concluded that the determination of TK-1 serum concentration may be a valuable measure also for prostate cancer risk screening in individuals (16). TPSA levels above 4 ng/mL and below 10 ng/mL have a ~25 percent risk of PCa occurrence, according to the American Cancer Society, and tPSA levels above 10 ng/mL increase the probability of PCa occurrence by more than 50 percent (17).

Conclusion

In the diagnosis of prostate tumor and differentiating between BPH and PCa, the serum level of TK1 was superior to tPSA, particularly when tPSA was present in the gray zone, which could prevent the need for invasive
prostatic biopsy in such a distinction.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

References


Stigma and Mental Health during COVID-19 New Normal Transition in Indonesia

Leny Latifah¹, Yusi Dwi Nurcahyani¹, Diah Yunitawati¹, Marizka Khairunnisa¹, Cati Martiyana¹
¹Magelang Health Research and Development Center, Ministry of Health, Indonesia

Abstract

Background: Social isolation and economic catastrophic became the main characters of the current pandemic. Historically, the strongest stigmas related to diseases were characterized as highly transmittable, potentially lethal, and without remedy. All represented COVID-19 during the early transition into the new normal. We aimed to analyzed stigma and economic stressors concerning mental health. Method: We conducted a cross-sectional online survey of 1375 participants in Java, Indonesia. Mental health status evaluated with DASS-21. The presence of social and self-stigma related to Covid-19 assessed with a questionnaire developed based on seven domains of public stigma. Demographics and other psychosocial stressors were assessed with an online questionnaire. Descriptive analyses and logistic regression had carried out. Result: A very high percentage of perceived and anticipated self-stigma and social stigma revealed. Controlling demographics factors, clear self-stigma increased the risk for depression (2.323 (1.241-4.346), p<0.05), anxiety (2.134 (1.205-3.777), p<0.05) and stress 3.931 (1.779-8.685), P<0.001). Clear social stigma increased the risk for anxiety (2.000 (1.066-3.756), p<0.05) but not on depression and stress. Conclusion: Ensuring basic needs fulfilment and eliminating stigma is critical for supporting mental health in the Covid-19 pandemic. Further research into the stigma-related risks is necessary because it represents an important need for intervention in public health.

Keywords: stigma, mental health, COVID-19

Introduction

Awareness, anxiety, and distress due to rapid changes and uncertainty, according to the World Health Organization (WHO) are natural psychological responses¹. But beyond natural responses, COVID-19 stated as the perfect vector for psychological distress pandemic. Mitigation measures of COVID-19 forced people to isolate themselves from one another when social support and togetherness were most needed²,³. Rising concern on the importance of understanding mental health problems during the COVID-19 pandemic proved by extended researches on the general population and/or health workers’ mental health condition during the COVID-19 pandemic⁴. Every phase of a pandemic had its characteristics, therefore understanding mental health dynamics in each unique phase is important.

WHO declared the crisis of COVID-19 global outbreak as a pandemic on 11th March 2020⁵. Indonesia announces first and second patients with positive COVID-19 virus on 1st March 2020⁶. To contain COVID-19 transmission, mass social and physical distancing was declared. If it is essential to go outside their home, face masks wearing, avoid gathering, and maintain physical distance is emphasized⁷. After almost three months of mass social distancing, on 1st June 2020, the Indonesian government declared the new phase of “transition into new normal” which later changed the term into “new habit adaptation”⁸. New normal phase marked by gradually lifting stay-at-home measures and
reviving the economic activities. Indonesia enters the new normal phase in the still steep rising confirmatory person contracting COVID-19 situation, and the effective medicine and vaccine to inhibit COVID-19 infection had not been available. It leads to a heightened risk of infection and possibly psychological distress.

Historically and nowadays, the strongest stigmas related to diseases are characterized as highly transmittable, potentially lethal, and without an acknowledged remedy, all could find in COVID-19 during the transition into the new normal. Studies relating to stigma and mental health in COVID-19 mostly came in a conceptual study or qualitative research. There still limited study relating mental health to stigma COVID-19 in quantitative research with a representative sample, and no scientific manuscript found in an Indonesian context. Research on Vietnamese health workers and the Canadian general population found stigma worsen mental health condition. To fill the gap, this research aimed to assess health workers and general population stigma and mental health during the transition into the new normal. Finding from this research could potentially support policymakers in formulating comprehensive interventions.

**Material and Methods**

**Participants**

We adopted a cross-sectional survey design by using an online questionnaire. A snowball sampling strategy focused on recruiting the general population and health workers living in 5 provinces of Java Island (Central Java, East Java, West Java, Special Capital Region of Jakarta, Special Region of Yogyakarta, and Banten), Indonesia was utilized. Participants were recruited by sending the survey through various social network channels. The final sample obtained with the snowball method was 1,385 people. Inclusion criteria were a participant aged 15-64 years to ensure the understanding of questions based on their knowledge and attitudes.

**Variables and Instruments**

Stigma about COVID-19 was measured using seven-domain public stigma from Pescosolido and Martin. Stigma domains measured including social distance, traditional prejudice, exclusionary sentiments, negative affect, dangerousness perceptions, also carryover in treatment and disclosure. A social and self-stigma scale was developed for this research. Social stigma included peoples’ beliefs and negative behavior related to COVID-19. Self-stigma covered perceived and anticipated stigma, covering personal concerns about negative judgment and behavior when diagnosed as COVID-19 positive, and actual and anticipated stigma in participants with the experience of COVID-19 positive diagnosed. The stigma scale uses a 4-point Likert scale (1: strongly agree; 2: agree; 3: disagree; 4: strongly disagree) to measure agreement with statements about each domain. The score was calculated by adding responses from items and dividing by the total number of items in the domain. A mean score above the midpoint (2.0) indicated a stigmatizing attitude, with higher scores indicating more severe stigma. In this research the score further divided into: 0-2 non stigma; >2 - <3: suggestive for stigma; >3 clear stigma. The reliability of social and self-stigma was 0.795 and 0.800 (Alpha Cronbach). Social and self-stigma had 14 and 10 items. The self and social-stigma items are carefully structured with several tryouts to ensure the balancing concern of COVID-19 precautionary measures with stigma mitigation.

Mental health status was measured using the Depression, Anxiety, and Stress Scale (DASS-21). The DASS-21 is a self-reported tool containing 21 items (7 per scale) that assess three constructs: depression, anxiety, and stress. Participants read statements about the constructs and picked their answers using a 4-point Likert scale ranging from 0 (Did not apply to me at all) to 3 (Applied to me very much or most of the time). Items comprising the scales are summed and doubled to be equivalent to the longer DASS-42 version. The cut-off was using normative DASS-42 data, divided into normal, mild, moderate, severe, and very severe.
Several studies of DASS-21 showed good psychometric results. Demographic data had collected on age, gender, education level, employment status, monthly income, and marital status.

**Statistical Analysis**

Descriptive statistics had used for continuous and categorical variables. Logistic regression analysis had done to determine the relationship between stigma and mental health status. All the analyzes had conducted using IBM SPSS Statistics v.21. The level of significance had set at 5%.

The study has approval from The National Institute of Health Research and Development Ethics Committee, Indonesia (LB.02.01/KE.386/2020). Research information is given in the first section of the online questionnaire. Respondents stated informed consent before starting to fill the data.

**Result**

**Tabel 1. Distribution of depression, anxiety and stress across sociodemographic variables**

<table>
<thead>
<tr>
<th></th>
<th>N=1385</th>
<th>Depression</th>
<th>Anxiety</th>
<th>Stress</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>% cases</td>
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<td>% cases</td>
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<tr>
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<td>Male (N=360)</td>
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<td>28.6</td>
<td>0.837 (0.643-1.089)</td>
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<tr>
<td>Female (N=1025) (ref)</td>
<td>22.1</td>
<td>32.4</td>
<td>9.7</td>
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<tr>
<td><strong>Age (years)</strong></td>
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<tr>
<td>&lt; 25 (N=234)</td>
<td>33.3</td>
<td>2.426 (1.601-3.674)***</td>
<td>50.0</td>
<td>3.661 (2.490-5.383)***</td>
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<tr>
<td>26 – 45 (N=876)</td>
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<td>1.263 (0.896-1.800)</td>
<td>29.6</td>
<td>1.537 (1.113-2.121)*</td>
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<tr>
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<tr>
<td>High school (N=191)</td>
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<td>1.871 (1.340-2.613)***</td>
<td>46.1</td>
<td>2.085 (1.528-2.845)***</td>
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<tr>
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<tr>
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<td>31.4</td>
<td>2.052 (1.575-2.674)***</td>
<td>43.9</td>
<td>2.201 (1.728-2.804)***</td>
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<tr>
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<td>7.1</td>
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<tr>
<td><strong>Occupation</strong></td>
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<tr>
<td>General population(N=911)</td>
<td>23.6</td>
<td>1.300 (0.988-1.712)</td>
<td>33.8</td>
<td>1.396 (1.092-1.784)*</td>
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<tr>
<td>Health workers (N=474) (ref)</td>
<td>19.2</td>
<td>26.8</td>
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*p< 0.05
Participant characteristics are described in Table 1. The sample (N = 1385) were mostly women (74%), with university studies (86.2%), married status (70.5%), and non-health workers (65.8%). The average age was 36.8, reflecting a majority of persons aged between 26-45 years old (63.2%). Being younger (OR 2.426 95% CI 1.601-3.674, p<0.000; OR 3.661 95% CI 2.490-5.383, p<0.000; OR 3.814 95% CI 2.123-6.852, p<0.000), lower education (OR 1.871 95% CI 1.340-2.613, p<0.000; OR 2.085 95% CI 1.528-2.845, p<0.000; OR 2.454 95% CI 1.613-3.736, p<0.000), and no married status (OR 2.052 95% CI 1.575-2.674, p<0.000; OR 2.201 95% CI 1.728-2.804, p<0.000; OR 2.540 95% CI 1.772-3.639, p<0.000) correlated with higher risk of depression, anxiety, and stress. Being health workers lower the risks for anxiety (OR 1.396 95% CI 1.092-1.784, p < 0.05).

Self-stigma (perceived and anticipated stigma) and social stigma were reported by 84.1% and 93.4% of the participants. Clear self-stigma (score > 3) increased the risk for depression (2.323 (1.241-4.346), p<0.05), anxiety (1.512 (1.081-2.117), p<0.05) and stress (OR 2.717 (1.515-4.873)**, p<0.05). Higher mental health problems reported among participants who had a clear social stigma (32.0% vs 16.8% in depression, 43.5% vs 22.1% in anxiety, 14.3% vs 8.4% in stress). Participants suggestive for social stigma could lead to

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<th>Variables (N=1.385)</th>
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<th>Stress</th>
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<td>OR (95% CI) adjusted</td>
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<tr>
<td>Suggestive (N=1075)</td>
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<td>1.819 (1.213-2.728)*</td>
<td>1.077 (0.600-1.931)*</td>
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<td>Non (222) (reff)</td>
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<tr>
<td>Clear (N=147)</td>
<td>32.0</td>
<td>2.321 (1.224-4.398)*</td>
<td>1.618 (0.820-3.193)</td>
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<td>Suggestive (N=1143)</td>
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<td>Non (95) (reff)</td>
<td>16.8</td>
<td>22.1</td>
<td>8.4</td>
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</tbody>
</table>

* p<0.0
statistically significant results on anxiety (OR 2.000 95% CI 1.066-3.756, p<0.05), but not on depression (OR 1.618 95% CI 0.820-3.193, p>0.05) and stress (OR 1.052 95% CI 0.417-2.655, p>0.05).

**Discussion**

Our research revealed that 22% of respondents depressive, 31.3% anxiety, and 9.6% stress symptoms, 14.2%, 18.8%, and 4.4% of the respondents revealed moderate to severe degrees of depression, anxiety, and stress. Compared to the general population with the same measure of DASS-21 in the earlier phase of the pandemic\textsuperscript{20,21}, our result shows a lower prevalence of depression, anxiety, and stress. Even lower when compared to research in a specific population of psychiatric patients\textsuperscript{22}, doctors\textsuperscript{23}, and health workers in Indonesia in the earlier phase of pandemic\textsuperscript{24}. The general population had a higher risk of anxiety compared to health workers in transition into the new normal. It contradicts research in Saudi Arabia and China that showed medical field workers had higher scores on sleep deprivation, stress, and depression\textsuperscript{21,25}. Better access to medical and health facilities of health workers also may serve as protecting factors.

While there is a tendency towards a lower prevalence of psychosocial distress in the general population and health workers in this research compare to the previous phase, a very high prevalence of stigma was found. Stigma towards self and others both increased the risk of depression, anxiety, and stress. This study, encompassing health workers and the general population, strengthen several previous research on COVID-19 related stigma in a more specific group. Internalized stigma, shame, and guilty feeling in hospitalized COVID-19 patients triggered by the fear of virus infections, and worry about community acceptance\textsuperscript{26}. Research on health care providers in Lebanon had found symptoms of stress, frustration, fear to contract the virus, and stigma of being infected\textsuperscript{13}. Historically and nowadays, the strongest stigmas related to diseases are characterized as highly transmittable, potentially lethal, and no acknowledged remedy\textsuperscript{12}, all could find in COVID-19 during the transition into the new normal. It could explain the very high percentage of participants considered having self and social stigma. Accurate discernment is necessary between optimizing the effort to contain the risk of infection and reducing the fear, stigma, and blaming others\textsuperscript{27}. Educating the public about COVID-19, reasons for isolation, and providing adequate health information can contribute to reducing stigmatization in the community. It serves as early identification and treatment to reduce the development of mental health problems\textsuperscript{28}. Training is needed for health workers and professionals to improve the mental health and knowledge of COVID-19 of the community\textsuperscript{29}. The role of the government and community leaders in COVID-19 education to the public can contribute to increase health literacy at the community level to prevent stigma related to COVID-19.

The current study presents several limitations. Firstly, we used online convenience sampling that may not represent the Javanese population. The use of online tools has limitations and challenges. The digital divide (people who do not access the internet, such as the lower education are lower in scope compared to participants with high education, with these groups being underrepresented) and validity concern (for example whether the participant is as they say). Our protocol strives to carry out rigorous validity checks to reduce some of these inherent problems.

**Conclusion**

The very high prevalence of stigma during early transition into new normal in Indonesia became important note. Once a pandemic with unknown cure could cause widespread stigma. Clear self and social stigma related to worsening mental health condition in early transition into new normal. Several demographic characters as young people (<25 years old), having non-marital status, and lower education related to worsening mental health condition. Healthcare workers had better mental health status than the general population. This research provides several directions for future research. Eliminating stigma is critical for supporting mental health in the Covid-19
pandemic. The anxiety towards higher risk of infection due to new normal transition conceivably manifested in stigma towards self and others. Intervention to enhance mental health conditions should also be considered a vulnerable group identified like young people and lower education group.

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References


Lintang Puspita Prabarini, Kumboyono Kumboyono, Laily Yuliatun

1Graduate Student at Master of Nursing Program, School of Nursing, Faculty of Medicine, 2Lecture and Researcher at Department of Community Health Nursing, School of Nursing, Faculty of Medicine, 3Lecture and Researcher at Department of Maternity, School of Nursing, Faculty of Medicine, Brawijaya University, Malang, Indonesia

Abstract

Background: WHO declared COVID-19 as a global pandemic due to its rapid spread and has been confirmed worldwide. Preventive efforts are made to minimize the virus spread among the community. Sociodemographic factors play an integral role in determining the community’s knowledge, perception, attitudes, and practice towards COVID-19 prevention protocols.

Methods: This review was conducted on various journal articles to see the sociodemographic factors that influence the levels of knowledge, perceptions, attitudes, and practices of the community towards COVID-19 prevention protocols. Journal articles were selected under the PRISMA guidelines, from which 28 articles were found to meet the predetermined criteria.

Result: Several sociodemographic factors found to affect the levels of knowledge, attitudes, practices, and perceptions of respondents towards the COVID-19 prevention protocols were age, gender, education, marital status, occupation, socio economic status, area of residence, and nationality.

Conclusion: Education and socio economic status/monthly income were found to be the main factors that influenced the respondents’ level of knowledge. Individuals with higher education tended to take health problems more seriously, as shown by their better scores of compliance with the COVID-19 prevention protocols, compared to the scores shown by other groups.

Keywords: Sociodemographic factors, knowledge, perception, attitudes, and practice, COVID-19

Introduction

World Health Organization declared COVID-19 as a global pandemic due to its rapid spread and has been confirmed worldwide. Preventive efforts are made by many countries to minimize the virus spread among the community. The effectiveness of these mitigation steps highly relies upon the cooperation and compliance of all community members. Knowledge, perceptions, attitudes, and practices towards COVID-19 play an integral role in determining the community’s readiness to accept behavioral change and health authority actions. Some research works indicate that public knowledge is important in dealing with the pandemic.

People around the world have varied levels of knowledge, perceptions, attitudes, and practices towards the COVID-19 pandemic. They are influenced...
by various factors to form new habits as encouraged by policymakers. In the concept of health behavior, socio-demographic factors have a strong influence on the levels of knowledge, attitudes, and practices. Assessing various socio-demographic factors inherent in society and influencing public awareness and knowledge about COVID-19 can provide deeper insights into community perceptions and practices in implementing the established prevention protocols.

**Research Objectives**

The aim of this scoping review was to critically synthesize scientific proofs of sociodemographic factors that affect the level of knowledge, perceptions, attitudes and practice of community towards COVID-19 prevention protocols. During the pandemic, information related to socio-demographic factors that affect knowledge, perceptions, attitudes, and practices is necessary. It is related to efforts to understand matters that affect the level of public awareness towards COVID-19 and the health protocols established by the authorities.

**Methods and Analysis**

**Search Strategy**

A systematic search following the PRISMA guidelines (Fig. 1). The authors conducted a scoping review of journal articles with a focus on community knowledge, perceptions, attitudes, and practices towards COVID-19 prevention protocols. This study was conducted to determine the extent to which the factors influence the level of public knowledge, perceptions, attitudes, and practices towards the COVID-19 prevention protocol. Articles were derived from four electronic bibliography databases—PubMed, Science Direct, ProQuest, Scopus, EBSCO, and DOAJ—using the keywords “knowledge” OR “attitude” OR “practice”, “public perception”, AND “COVID 19”. The authors also searched extra articles with search engine Google Scholar by manually checking out the references of the review articles identified. Literature search was commenced in July 2020 and ended in October 2020. There was no limit as to the dates and years of publications given that the COVID-19 pandemic was first confirmed at the end of 2019.

**Articles Selection and Restrictive Criteria**

The title and abstract of every publication were to be screened to obtain a relevant article. The restrictive criteria in this scoping review consisted of inclusion and exclusion criteria. The inclusion and exclusion criteria were developed based on the PICOS (Population, Intervention, Comparison, Outcome, and Study Design) standard to include the following: 1) the populations and samples extracted were community groups in general during the COVID-19 pandemic; 2) the publications’ interventions took the form of questionnaires related to community knowledge, attitudes, practices, and perceptions on COVID-19, include sociodemography characteristics of the respondent; 3) the results were in the form of measurements articles on sociodemographic factors that affect the variables of knowledge, attitude, practice, and perception to the pandemic; and 4) the studies used the observational research design with a cross-sectional approach. Articles would not be considered meeting the criteria if they used focused on a specific group of populations (e.g., health service providers, health personnel, students, age groups, or patients with particular comorbidities).

**Screening and Data Extraction**

The manuscripts filtering and extraction to retrieve information covered the following six domains: 1) Lead authors; 2) Country/region. 3.) Methodological characteristics (study design, study objectives, research questions or hypotheses, sample characteristics, sampling method, and statistical analysis); 4) Sample size;5) Main findings, relationships between variables (e.g., the ratio at 95% confidence interval and p<0.05); 6) Conclusion. The data from all journal articles were summarized by category and conceptual framework. This process involved synthesis, analysis, and interpretation of the data from the studies to understand the mechanism of identified sociodemographic factors.
Measurement Instruments

The majority of the studies measured sociodemographic factors and the level of knowledge, perceptions, attitudes, and practices on COVID-19 using online questionnaires. The questionnaires used Google Forms, Survey Monkey, Alphabet Inc, and Wenjuanxing links. The questionnaire links were spread via social media platforms such as Facebook, WhatsApp, Twitter, and WeChat app. Two articles distributed questionnaires directly to willing respondents. The research was conducted by spreading questionnaires to pedestrians and from door to door or at gathering points. Questionnaires were given to people who were willing to participate.7,8

Results

Articles Selection

Initial search gained a total of 945 studies from 4 databases, namely: PubMed, ProQuest, ScienceDirect, and DOAJ. The authors also searched via search engine Google Scholar and found 10 additional articles. Before conducting a review on the articles, a duplication extraction process was completed. The selected duplication-free papers review process was performed in three stages: a review of the title, abstract, and the entire article. Out of 945 papers, 481 were excluded due to duplication. Upon complete screening, a total of 28 articles were found to meet the criteria for review.

Research Characteristics

Study characteristics from 28 articles are presented in Table 1. The 28 journal articles averaged the sample sizes at 201, with the highest number of respondents being 6,910. The total number of participants in all articles was 48,338. The participants’ average age was 12 years to 65 years old. Female participants (n: 26,994) outnumbered male participants (n: 21,344). The 28 articles were based in 18 different countries, namely Egypt (1 article), Pakistan (1 article), Saudi Arabia (3 articles), Nepal (2 articles), Malaysia (1 article), Ecuador (1 article), Lebanon (1 article), Jordan (1 article), Bangladesh (3 articles), India (3 articles), Sudan (1 article), Iran (1 article), China (3 articles), Cameroon (1 article), Vietnam (1 article), Nigeria (1 article), Indonesia (1 article), and Ghana (1 article).

Sociodemographic factors in journal articles focus on age, gender, education, occupation, socio-economic status/monthly income, marital status, and region/area of residence. Some journals involving more than 1 country, or involving foreigner in data collection, also study the nationality factor of the respondents.

Measurement Instrument

The majority of the studies measured using online questionnaires. The questionnaires were shared via Google Forms, Survey Monkey, Alphabet Inc, and Wenjuanxing links. The questionnaire links were spread via social media platforms such as Facebook, WhatsApp, Twitter, and WeChat. Two articles distributed questionnaires directly to willing respondents. Questionnaires were given to people who were willing to participate while still obeying the health protocols.7,8

Sociodemographic Factors

Age

Age can be a determining factor in taking health measures. Younger age is associated with higher access to social media, as a source of information, than older age.9,10 Respondents from the older adult group who were over 50 years old had a lower level of knowledge, compared to the other age groups.8,11 However, other studies found that those aged 30-50 years had a higher level of knowledge, compared to those in the 18-29 years age group.3,6,12,13 The higher the age, the better the attitude shown towards the COVID-19.13 This was in line with the practice shown by respondents aged over 30 years who tended to show higher prevention practices, compared to those in younger age groups.8,9,13

Gender

In some articles, there was no significant difference in the level of knowledge possessed by men and women.7,9,12 However, other research stated that women had a better level of knowledge and attitudes than men.
The majority of female respondents, compared to the male ones, paid more attention when filling out the questionnaire. This condition might affect their COVID-19-related attitude scores. Female respondents also tended to have a positive attitude 1.9 times higher than that of the men. This was related to the habit of women to be responsible for maintaining family hygiene and teaching their children. Men had a significant relationship to dangerous practices in dealing with COVID-19.

**Education**

Respondents’ education level is affected their levels of knowledge. The study found that respondents with a bachelor’s degree or higher education had a significantly higher level of knowledge, compared to those with less education. The knowledge of respondents, towards COVID-19, with high school education was at least 4.7 times higher than that of those who did not have formal education. Educational status was the strongest predictor of the levels of knowledge and perceptions of participants.

**Marital Status**

Respondents who were not married had a better level of knowledge than those who were married. This could be related to their education level. Unmarried respondents tended to be young and have a higher educational background. In other studies, it was found that married respondents showed better prevention practices than those unmarried. Young and unmarried men showed lower prevention practices, compared to the other groups. An optimistic attitude regarding the handling of COVID-19 was also shown by married respondents.

**Occupation**

Respondents with a scientific background or working in the health sector showed a better level of knowledge, perceptions, and attitudes than those without a background in the health sector. There were surprising results regarding the occupation and the level of attitude shown. The attitude score for respondents who worked as government staff was low, compared to that of domestic workers.

**Socio Economic Status/Monthly Income**

The respondents’ levels of knowledge increased along with their income levels. Likewise, an increase in positive attitudes and practices occurred in line with the increasing level of income.

**Region/Area of Residence**

Several articles in this review examined the area of residence as one of the sociodemographic aspects of the respondents. The area where the respondent lived had a significant effect on knowledge, perceptions, and attitudes towards the risk factors for COVID-19. People who lived in urban areas tended to have a higher level of knowledge than those who lived in villages. Low levels of knowledge and perceptions occurred in the general public, especially those in rural areas and without internet access. The compliance with the instruction to wear masks was lower by residents in areas with less serious cases of COVID-19, compared to residents in areas with high case rates. Residents living in areas with a low incidence of COVID-19 believed that they were at lower risk of contracting the COVID-19 virus. The low incidence of transmission in the area at the time of the survey affected the level of community compliance with the COVID-19 prevention protocols.

**Nationality**

Studies involving two countries that also included respondents with other nationalities considered nationality as one of the sociodemographic aspects of respondents that can affect the levels of knowledge, perceptions, attitudes, and practices of society towards COVID-19. In a study involving 2 countries, namely Nigeria and Egypt, it was found that the Nigerian population had a positive attitude which was 11x higher than that of the Egyptian population. In a study that included respondents with foreign citizenship status, it was found that those who were native to that country...
had more positive levels of knowledge and attitudes, compared to foreigners 5.

**Table 1: Study Characteristic**

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**Discussion**

*Sociodemographic Factors towards Knowledge*

Several sociodemographic factors also influenced the level of public knowledge about COVID-19. Several articles showed that women were more knowledgeable and aware than male participants. This condition was linked to their innate concern for the condition of their children and their families, which prompted female participants to read and learn more about COVID-1930.

Low socioeconomic status was closely related to an individual’s level of education. Individuals lacking formal education were more likely to be unemployed, which resulted in reduced income, resulting in poor health quality 31. One of the articles reviewed revealed that the level of individual knowledge increased with their high income. Low scores of participants were known to come from those with low income, and the highest scores were obtained from those categorized obtaining high income 6. The percentage of the high level of knowledge was also influenced by the level of education of the respondents; those who were university graduates and aged over 30 years had a good level of knowledge towards COVID-19 16.

Low knowledge scores are associated with older respondents, who live in rural areas, with low levels of education and monthly income 16. Most of the studies showed that the level of respondents’ knowledge of COVID-19 was related to their access to social media as a source of information at a younger age 30. A high level of knowledge also shows a positive relationship between respondents and their works, as a health worker, with high social status23. Respondents with non-working backgrounds have low scores on knowledge related to COVID-19 23.

*Sociodemographic Factorstowards Perception*

The education level and nationality of respondents influenced their perceptions of global and community responses to reduce the transmission of COVID-19 through various policies. Respondents with higher education and medical background showed positive perceptions of global responses 10. Correct perceptions towards COVID-19 transmission, treatment, and prevention measures were shown by those with higher education. Besides, older age groups were associated with better perceptions 13. Regarding the perception of prevention, women indicated that they were more committed to looking after their families through preventive measures 9. Male respondents had perceptions related to higher barriers in implementing compliance with the COVID-19 protocols established by the government9. This might be related to men doing works outside the home.

*Sociodemographic Factors towards Attitudes*

Several articles also examined respondents’ attitudes to government actions to deal with the COVID-19 pandemic that affected their confidence that this pandemic could be passed successfully 20. Confidence in
the success of COVID-19 control increases with the age of respondents. The group with a medical background also showed a high attitude of trust compared to the non-medical group. It was related to a better level of knowledge about COVID-19.

A positive attitude towards the COVID-19 prevention protocols was shown by women. Respondents who were married also showed better attitudes, compared to other groups. Women and married individuals felt more responsible for maintaining family hygiene and teaching their children. Respondents assumed that measures to prevent COVID-19 were a shared responsibility and commitment. A better sense of responsibility was found in older adult respondents.

Sociodemographic Factors towards Practices

Practices are defined as individual behavior, which is influenced by individual attitudes and knowledge. Knowledge of COVID-19 relates to preventive practices carried out by respondents, such as reducing the intensity of going out and wearing masks. However, in some articles observed, there was a gap in translating individual attitudes and knowledge into the practice of preventing COVID-19. This might be due to the economic issues experienced by those who need daily wages.

Practices against COVID-19 prevention were also linked to the area where respondents lived. The inability of various states and local governments to implement strict preventive measures including lockdowns, banning public gatherings, physical distancing, and wearing masks is a risky practice against COVID-19. Visiting the crowd, which is risky, was recorded as being carried out by respondents below 40 years of age. It was related to the work done by the respondents. Most of the young people had to work outside the home for their daily survival even during the pandemic.

Married individuals and those with higher education tended to engage in high-risk practices with the potential to contract COVID-19. Men were more likely to underestimate the severity of the virus’s potential harm to them.

The higher the education level of the respondents, the better the results of the practice carried out on the prevention of COVID-19. Basic educational skills, including knowledge, reasoning skills, emotional self-regulation, and interaction skills, are essential components of health practice. Education is an essential component of health, and a major determinant of long-term health.

Limitations of the Study

The main limitation in writing this review was related to the scope of the discussion. Given the very wide range of factors that may affect the level of knowledge, perceptions, attitudes, and especially the practices of preventing COVID-19, the author only took merely sociodemographic factors. In this review, the author could not include other aspects that might be relevant to the participants’ concepts of knowledge, perceptions, attitudes, and practices such as framework (regulation and policies, health care facilities, health promotion), interpersonal influence (social norms, source of information), public health (respondent or family comorbidity), and self-efficacy.

Conclusion

This review revealed that the education level of respondents is the most influential sociodemographic factor in all articles addressing the concept of knowledge, perceptions, attitudes, and community practices. It was found that individuals with higher education tended to take health problems more seriously than those with less education; individuals with higher education had higher scores in practicing the COVID-19 protocols, compared to other groups. The results of this study are expected to be used in making appropriate interventions to reduce the COVID-19 transmission and face health problems in the future.
Acknowledgement

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Declaration of Interest: All authors declare that we have no conflict of interest.

Ethical Clearance

Due to the characteristic of this research design is a systematic review (use of secondary data) the ethical evaluation was not required. However, statement of research ethics in the journal articles would serve as a consideration of the use of the publication for this review.

References


Comparative Analysis of Acute Physiological Responses between Short and Middle Distance Swimming Athletes with Physical Loading Test

M. Ali Machfud¹, Muchammad Rif’at Fawaid As’ad², HariSetijono³, Lilik Herawati⁴

¹Postgraduate Student, ²Ex Postgraduate Student, Sports Health Science Master Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, ³Professor, Faculty of Sports Science, Surabaya State University, Surabaya, Indonesia, ⁴Senior Lecturer, Department of Physiology, Sports Health Sciences Master Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

Indonesia’s swimming achievements are still far from that of other countries. Among other things, at the 2018 Asian Games, Indonesia did not succeed in getting a single medal. If not improved, Indonesia’s swimming performance will get worse. One of the innovations is to identify the physical abilities and physiological responses of an athlete through a physical loading test. This study aims to analyze the differences in acute physiological responses between short and middle distance swimming athletes through continuous and interval high intensity physical test. The sample of males aged 14-18 years consisted of the short distance (n=12) and middle distance (n=12) swimmers. Participants did a high intensity physical test with an ergocycle for 21 minutes with a 7-minute warm-up and 3-minute cool down, intervally, and without resting for continuous test. The variables studied included resting pulse rate, blood lactate, leg and shoulder muscle strength, arm and leg power. Data collected inbefore (pre) and after(post) physical test. The results showed that the short distance swimmers had greater increase in lactate level and pulse rate in both physical tests. The leg power decreased significantly in the short distance swimmer when performed continue physical test, whereas the middle distance swimmer when performed interval physical test. The conclusion of this study was to differ the leg power as acute physiology response for swimmers is more appropriate using continuous or interval high intensity physical test. Based on these results, training program for swimmer type can be developed. However, further research is still needed to enhance the swimmers performance.

Keywords: Swimming; Athletes; Pulse; Lactate; Power; Health

Introduction

The achievements of Indonesian swimming athletes are still far from that of other countries (¹). It was proven at the 2018 Asian Games, Indonesia failed to get a single medal (²). In fact, some Indonesian swimmers are predicted to win medals because they managed to be the best in the qualifying rounds several times. Swimming sports tend to use high intensity and good physicality is needed to win. Sports activity is one of the physical stressors that affect the homeostatic system, because it has an impact on physiological responses which are marked by an increase in stimulation of the body’s physical performance in the cardiorespiratory system (³).

The swimming race from 50 m to 1,500 m is divided into 3, namely short distance 50-100 m, middle distance 200-400 m, and long distance 800-1,500 m (⁴). To win in swimming, it takes practice, anthropometry, and strong physical abilities (⁵).

Various attempts have been made to improve the performance of swimming athletes, one of which is the right training program that can improve the performance of swimming athletes. However, the efforts that have been made are still not getting maximum results, to
achieve glorious swimming sports. One of the reasons is that there is no known identification of physical ability which is a physiological response in short and middle distance swimming athletes. Therefore, this study was conducted with the aim of analyzing the differences in physiological responses between short and middle distance swimming athletes through continuous and interval high intensity physical loading tests. Physiological responses measured were variables which included lactate, leg muscle strength, shoulder muscle strength, arm power, leg power, and resting pulse rate.

**Materials and Methods**

This study has received approval from the Research Ethics Commission from the Faculty of Medicine, Airlangga University (No.90/EC/KEPI/T’KUA/2020), and was declared ethical in accordance with 7 WHO 2011 standards which refer to the 2016 CIOMS guidelines.

**Research subject**

This study was analytic observational design and cross sectional. The subjects in this study were athletes of short and middle distance swimming aged 14-18 years. The selected subjects were determined according to their specialization and swimming competition numbers which were grouped into the middle and short distance swimming groups. The middle distance swimming group was 12 person and the short distance swimming group was 12 person.

**Physical Loading Test Protocol**

All groups of short and middle-distance swimmers performed a continuous and interval high-intensity test in 31-minute using an ergocycle. The pattern of interval was a 7-minute warm-up, 21-minute core and 3-minute cool down.

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the high intensity loading test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kontinu</td>
</tr>
<tr>
<td>Work Ratio</td>
</tr>
<tr>
<td>Warm up (minutes)</td>
</tr>
<tr>
<td>Working Duration (seconds)</td>
</tr>
<tr>
<td>Recovery intensity</td>
</tr>
<tr>
<td>Recovery duration (seconds)</td>
</tr>
<tr>
<td>Repetition</td>
</tr>
<tr>
<td>Cooling down</td>
</tr>
<tr>
<td>Total Duration (Minutes)</td>
</tr>
</tbody>
</table>
All athletes in short and middle distance swim was fasting for ± 8-10 hours (drinking water was still allowed), before the physical test was carried out, resting pulse rate, blood lactate, leg muscle strength, shoulder muscle strength, arm power, and leg power were measured, and Leg Power as a pre-test. Then, the subject were given a sweet solution and rest for 30 minutes. Subsequently, continuous and interval physical tests were accomplished. Afterward, blood lactate, leg muscle strength, shoulder muscle strength, arm power, and leg power, and resting pulse rate were measured as a post-test.

Lactic acid measurement

Lactic acid measurements were carried out on capillary blood through a transcutaneous puncture on the medial side of the tip of the middle finger\(^6\). Measurement of lactic acid was carried out before the loading test as a pre-test. And immediately after the loading intervention at 32 and 36 minutes. Measurement of lactic acid using the Accutrend plus, Roche, Germany. Lactic acid analysis results were recorded in units (mmol/L).

Measurement of Shoulder and Leg Muscle Strength

Measuring the strength of the shoulder muscles was measured by pulling the expending dynamometer with both hands and the results will appear in Kg.

The measurement of leg muscle strength was measured by pulling the leg dynamometer with both hands using the leg muscle strength and the results appeared in Kg units.

Arm and Leg Power Measurement

Measuring arm power was done with a 3 kg medicine ball, which was measured by sitting on a chair with the chest part held with a rope to stabilize, then a medicine ball was pushed forward horizontally with both hands. The unit was watts.

The measurement of foot power was done with the DF jump tool which was measured by standing on the mattress then jumping as high as possible. The unit was watts.

Pulse Rate

Pulse rate measurement was in resting condition and using a polar connected to the iPad-4 which is measured before (pre-test) in beat per minute (Bpm).

Data analysis

The Statistical Package for the Social Sciences
(SPSS) software was used for data analysis. Data was presented in tables. The paired T test or Wilcoxon Signed Ranks Test was to examine the difference of before and after physical test. The independent T test or Mann Whitney was to evaluate the difference between physical loading test (continue and interval high intensity) and between swimmers (short and middle-distance).

**Results**

The analyzed data were compared between before and after continuous physical loading test, and compared between the short-distance and middle-distance swimmers (tab. 2). The same was done for the interval loading test (tab. 3).

**Table 2. The averages of variables before & after continuous high intensity exercise test**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre</th>
<th>Post</th>
<th>Delta</th>
<th>sig. between group (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate (mmol/L)</td>
<td>3.3±1.4</td>
<td>3.45±1.13</td>
<td>0.796</td>
<td></td>
</tr>
<tr>
<td>Leg muscle strength (Kg)</td>
<td>123.4±33.0</td>
<td>113.1±45.4</td>
<td>0.567</td>
<td></td>
</tr>
<tr>
<td>Shoulder muscle Pull strength (Kg)</td>
<td>25.6±6.8</td>
<td>22.0±9.5</td>
<td>0.342</td>
<td></td>
</tr>
<tr>
<td>Arm Power (Watt)</td>
<td>163.2±36.9</td>
<td>168.2±44.8</td>
<td>0.791</td>
<td></td>
</tr>
<tr>
<td>Leg Power (Watt)</td>
<td>522.9±114.7</td>
<td>456.7±130.2</td>
<td>0.243</td>
<td></td>
</tr>
</tbody>
</table>
The results of the different test with a significance level (p < 0.05) in Table 2, it can be concluded that there was a significant differences before and after continuous loading on middle and short distance swimming athletes on the variables of oxygen saturation, blood lactate, and pulse rate. In leg power, after continuous loading, significant differences between pre and post-test were only found in short-distance swimming athletes.

Table 3. The averages of variables before & after high intensity interval exercise test

<table>
<thead>
<tr>
<th>Variable</th>
<th>short-distance</th>
<th>middle-distance</th>
<th>sig. between group (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean±SD)</td>
<td>(mean±SD)</td>
<td></td>
</tr>
<tr>
<td>Pulse rate (Bpm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>81,7±7,4</td>
<td>84,4±10,5</td>
<td>0,515</td>
</tr>
<tr>
<td>Post</td>
<td>172,6±10,1</td>
<td>165,3±6,7</td>
<td>0,702</td>
</tr>
<tr>
<td>Delta</td>
<td>90,9±7,8</td>
<td>80,9±12,9</td>
<td>0,052</td>
</tr>
<tr>
<td>sig. between pre &amp; post in each group (p)</td>
<td>0,000*</td>
<td>0,000*</td>
<td></td>
</tr>
</tbody>
</table>

*signifikan (p <0,05) antara pre dan postest

** signifikan (p <0,05) antara kelompok jarak pendek dan jarak menengah
In Table 3, the results of different tests with a significance level (p <0.05), were found at before and after loading intervals for middle and short distance swim athletes, on variables of oxygen saturation, blood lactate, and pulse rate. The significant difference after the interval loading test in leg power was only in the middle-distance group.

**Tabel 4. The averages of variables on short-disctance swimmers**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Continue</th>
<th>Interval</th>
<th>sig. between group (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean±SD)</td>
<td>(mean±SD)</td>
<td></td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>3.3±1.4</td>
<td>6.0±1.6</td>
<td>0.002**</td>
</tr>
<tr>
<td>Post</td>
<td>5.3±1.8</td>
<td>16.1±2.8</td>
<td>0.000**</td>
</tr>
<tr>
<td>sig. between pre &amp; post in each group (p)</td>
<td>0.001*</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td>Delta</td>
<td>2±1.2</td>
<td>10.1±3.24</td>
<td>0.000**</td>
</tr>
<tr>
<td>Leg muscle strength (Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>123.4±33.0</td>
<td>118.0±24.0</td>
<td>0.705</td>
</tr>
<tr>
<td>Post</td>
<td>108.9±27.4</td>
<td>123.6±31.8</td>
<td>0.307</td>
</tr>
<tr>
<td>sig. between pre &amp; post in each group (p)</td>
<td>0.053</td>
<td>0.511</td>
<td></td>
</tr>
<tr>
<td>delta</td>
<td>-14.5±20.6</td>
<td>5.6±22.9</td>
<td>0.068</td>
</tr>
<tr>
<td>Shoulder muscle Pull strength (Kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>25.6±6.8</td>
<td>20.±3.1</td>
<td>0.052</td>
</tr>
<tr>
<td>Post</td>
<td>23.3±7.1</td>
<td>20.3±4.4</td>
<td>0.304</td>
</tr>
<tr>
<td>sig. between pre &amp; post in each group (p)</td>
<td>0.181</td>
<td>0.882</td>
<td></td>
</tr>
<tr>
<td>Delta</td>
<td>-2.3±5.0</td>
<td>0.2±2.3</td>
<td>0.225</td>
</tr>
<tr>
<td>Arm Power (Watt)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>163.2±36.9</td>
<td>148.4±18.6</td>
<td>0.317</td>
</tr>
<tr>
<td>Post</td>
<td>154.2±35.3</td>
<td>149.6±30.8</td>
<td>0.773</td>
</tr>
<tr>
<td>sig. between pre &amp; post in each group (p)</td>
<td>0.150</td>
<td>0.875</td>
<td></td>
</tr>
<tr>
<td>Delta</td>
<td>-9±18.0</td>
<td>1.2±21.0</td>
<td>0.285</td>
</tr>
</tbody>
</table>
The response difference of short distance swimmers between continuous and interval physical test can be seen in Table 4. Lactate levels and pulse rates were less increasing and the differences between before and after physical test were also lower for continuous physical test than interval physical test. The decrease in leg power after continuous physical test showed a significant more decreasing, whereas after the interval physical test, the leg power decrease was not significant.

Tabel 4. The averages of variables on short-distance swimmers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Physical testing</th>
<th>sig. between group (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Continue</td>
<td>Interval</td>
</tr>
<tr>
<td></td>
<td>(mean±SD)</td>
<td>(mean±SD)</td>
</tr>
<tr>
<td>Leg Power (Watt)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>522.9±114.7</td>
<td>470.2±108.5</td>
</tr>
<tr>
<td>Post</td>
<td>489.0±112.7</td>
<td>459.7±112.8</td>
</tr>
<tr>
<td>sig. between pre &amp; post in each group (p)</td>
<td>0.015*</td>
<td>0.430</td>
</tr>
<tr>
<td>Delta</td>
<td>-33.9±35.8</td>
<td>-10.5±35.6</td>
</tr>
<tr>
<td>Pulse rate (Bpm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>81.7±7.4</td>
<td>80.5±9.9</td>
</tr>
<tr>
<td>Post</td>
<td>172.6±10.1</td>
<td>181.6±6.0</td>
</tr>
<tr>
<td>sig. between pre &amp; post in each group (p)</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>Delta</td>
<td>90.9±7.8</td>
<td>101.1±6.3</td>
</tr>
</tbody>
</table>

*significant (p <0.05) between pre and postest

**significant (p <0.05) between the short-distance and middle-distance groups

Tabel 5. The averages of variables on middle-distance swimmers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Physical testing</th>
<th>sig. between group (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Continue</td>
<td>Interval</td>
</tr>
<tr>
<td></td>
<td>(mean±SD)</td>
<td>(mean±SD)</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>3.45±1.13</td>
<td>5.9±1.6</td>
</tr>
<tr>
<td>Post</td>
<td>5.01±1.47</td>
<td>12.8±2.4</td>
</tr>
<tr>
<td>sig. between pre &amp; post in each group (p)</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>Delta</td>
<td>1.6±0.76</td>
<td>6.9±2.9</td>
</tr>
<tr>
<td>Leg muscle strength (Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>113.1±45.4</td>
<td>96.4±31.5</td>
</tr>
<tr>
<td>Post</td>
<td>113.5±41.2</td>
<td>95.0±27.9</td>
</tr>
<tr>
<td>sig. between pre &amp; post in each group (p)</td>
<td>0.933</td>
<td>0.627</td>
</tr>
<tr>
<td>delta</td>
<td>0.45±16.4</td>
<td>-1.4±7.7</td>
</tr>
<tr>
<td>Shoulder muscle Pull strength (Kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>22.0±9.5</td>
<td>20.1±7.3</td>
</tr>
<tr>
<td>Post</td>
<td>20.0±9.3</td>
<td>18.1±6.4</td>
</tr>
<tr>
<td>sig. between pre &amp; post in each group (p)</td>
<td>0.109</td>
<td>0.150</td>
</tr>
<tr>
<td>Delta</td>
<td>-2±3.56</td>
<td>-2±2.2</td>
</tr>
<tr>
<td>Arm Power (Watt)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>168.1±44.8</td>
<td>144.0±22.8</td>
</tr>
<tr>
<td>Post</td>
<td>160.4±48.4</td>
<td>133.9±24.2</td>
</tr>
<tr>
<td>sig. between pre &amp; post in each group (p)</td>
<td>0.357</td>
<td>0.127</td>
</tr>
<tr>
<td>Delta</td>
<td>-7.8±25.2</td>
<td>-10.2±16.7</td>
</tr>
</tbody>
</table>
Middle distance swimming athletes responded to continuous and intervals physical test can be seen in table 5. Lactate levels and pulse rate presented less increased and the difference was also lower between before and after continuous test compared to interval physical test. The leg power variable had a significant greater decrease between the pre- and post-interval physical test in middle-distance swimmers (fig. 2).

**Figure 2. The three variables with the significant difference in each groups after physical loading test.**

*a & b: sig. diff. between pre & post within group (p<0.05)

*: sig. diff compared to the continue physical test in the same group (p<0.05)
Discussion

The results of the study of athletes in moderate and short distance swimming showed significant differences in pre-post oxygen saturation variables. This is because during exercise the sympathetic and parasympathetic nervous systems have an effect on an increase in heart rate which affects oxygen transport and the strength of heart contraction, thereby increasing peripheral vasodilation to the blood flow to active skeletal muscles so that during exercise there will be an integrated control of blood pressure which is controlled by reflex through the autonomic nervous system\(^{(7)}\). Another physiological response that occurs is an increase in oxygen diffusion capacity several times between resting and maximum work. Previously blood flow through the pulmonary capillaries flows very slowly and even stops in a resting state. So when the body performs maximum work activities continuously or at intervals it will increase blood flow through the lungs which causes the capillaries to diffuse maximally, so that athletes need more oxygen which is higher\(^{(8)}\).

Blood lactate was found to be a significant difference in pre-post between the two groups of athletes in middle and short distance swimming because when doing stressful activities the body experiences a lack of oxygen to break down glucose in the blood especially at high intensity it can reach 15-25 mM \(^{(7,9)}\). This is because the production of lactic acid is higher than its destruction (cleaning) when given high intensity loading \(^{(10)}\). So that a person experiences fatigue after exercise and will cause an increase in lactic acid up to 10 times during exercise \(^{(11)}\).

Continuous and interval loading on leg muscle strength did not produce a significant difference between pre-post because athletes who have good physical abilities and are trained must have the capacity and strength ability to manage their energy sources properly\(^{(12)}\). The interval loading test resulted in a significant reduction in leg strength in the middle distance swimmer group. The middle-distance swimmer group who had the dominant aerobic muscle ability was unable to quickly restore ATP energy during interval loading. The dominance of red muscle cells which need oxygen to reproduce ATP cannot work optimally due to the lack of oxygen supply at the interval. Meanwhile, the short distance swimmer group who had the ability of anaerobic muscles was used to it and was able to work optimally at intervals. This is what causes a greater decrease in leg power in the middle distance swimmer group than the short distance swimmer during the interval loading test \(^{(13)}\) proved that middle distance swimmers experienced a decrease in strength after the interval compared to continuous training.

The continuous loading test resulted in a significant reduction in leg strength in the short swimmer group. The group of short distance swimmers who have dominant anaerobic muscle ability, are not able to consistently produce ATP energy during continuous loading. The dominance of white muscle cells which are only able to work for a short time and are unable to produce ATP is due to the low number of red muscle cells so that the muscles cannot work optimally. Meanwhile, the middle-distance swimmer group who has the ability of aerobic muscles (red muscles) is accustomed and able to work optimally when continuous \(^{(14)}\). This is what causes a greater decrease in leg power in the short distance swimmer group than in the middle distance swimmer during the continuous loading test.

The variables of shoulder muscle strength and arm power did not show a significant difference in the pre-post that when doing continuous physical loading tests and intervals using ergocycle, this was because the athlete did not experience significant muscle fatigue during loading\(^{(12)}\), so that he did not give the effect of fatigue, especially on the shoulder muscles.

Leg power shows a significant difference in pre-post short distance swimming athletes because short distance swimmers are more dominant anaerobic and have less aerobic capacity which tends to exercise for a short time \(^{(15)}\). So that when given a long or continuous workload, there is an increase in muscle blood flow during strenuous physical exercise which results in increased muscle...
workload\(^8\)). Intermediate distance swimmers have red muscle that tends to be aerobic, which is more dominant in moderate sports for a relatively long time\(^{15}\). So that when given a load, the activity tends to be high. There is an increase in muscle blood flow during heavy physical exercise and results in increased muscle workload\(^8\). In continuous and short distance athletes, it does not make a significant difference because intermediate distance swimmers tend to have high aerobic metabolism and a cardiorespiratory system that can maintain moderate power for a relatively long time\(^{15}\). So that when given continuous loading the physiological response in the body is not so excessive. Short-distance swimming tends to produce much greater instantaneous strength for a short period of time, sufficient to complete a 50 or 100 meter race\(^{15}\). So that when given the physiological response in the body is not so excessive.

The middle distance swimmer group enjoyed and found it easier to load than the short distance swimmer group. This can be due to rest intervals which aid in energy recovery. The middle distance swimmer group had better energy recovery abilities during rest, because it had red muscle cells that were more dominant than the short distance swimmer group\(^{16}\).

On the pulse rate variable in the two groups of athletes in the middle and short distance swimming athletes showed a significant difference in pre test and post test. Due to an increase in muscle blood flow during physical work, the body responds with an increase in oxygen demand as indicated by a much higher pulse rate frequency than at rest. Oxygen consumption and cardiac output also increase during exercise. Also, it is known that exercise will stimulate the sympathetic nerves and the result is an increase in pulse rate\(^8\).

The continuous physical loading test had a greater increasing lactate levels and pulse rate, and more decreasing leg power in short distance than middle distance swimmers. The short distance swimmers, when compared between types physical loading test (continuous and interval), it was found that lactate levels and pulse rates increased more after doing interval physical loading. This had a similar pattern to middle distance swimmers, where the leg power of middle distance swimmers had a greater decrease than short distance swimmers after doing interval physical loading test (fig. 2).

**Conclusion**

The continuous and interval high intensity physical loading tests with ergocycle in short and middle distance swimmers increase blood lactate and pulse rate. The short distance swimmers have greater increase of blood lactate level and pulse rate in both physical test. The leg power examination for short distance swimmers is more appropriate to continue high intensity physical loading test, whereas for middle distance swimmers is interval high intensity physical loading test. Based on these results, training program for swimmer type can be developed. However, further research is still needed to enhance the swimmers performance.

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**Conflict of Interests:** The authors declare no conflicts of interest related to the manuscript.

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Utility of Percutaneous Endoscopic Gastrostomy for Nutritional Support in Patients with Oropharyngeal Dysphagia

Madhavan Iyengar¹, Niket Attarde², Arpan Shah³, Pooja Shah⁴, Poornima. V⁵

¹Professor, Department of General Surgery, Parul Institute of Medical Science and Research, Parul University, Limda, Vadodara, Gujarat, India, ²Associate Professor, Department of General Surgery, Padmashree Dr DY Patil Medical College, Padmashree Dr DY Patil University, Nerul, Navi Mumbai, Maharashtra, India, ³Associate Professor, Department of General Surgery, Parul Institute of Medical Science and Research, Parul University, Limda, Vadodara, Gujarat, India, ⁴Assistant Professor, Department Of Anaesthesiology, S.B.K.S.M.I.R.C, Piparia, Vadodara, Gujarat, India, ⁵Professor and Head Department of Dentistry, Parul Institute Of Medical Science and Research, Parul University, Limda, Gujarat, India

Abstract

Background & Objectives: Maintenance of nutrition status of a patient is of paramount importance to clinicians treating medical or surgical diseases. Enteral nutrition is preferred over parental nutrition in patients who are unable to swallow. Percutaneous endoscopic gastrostomy (PEG) is the preferred route of nutritional support in patients with a functional gastrointestinal system who require long-term enteral nutrition and are unable to maintain oral intake. PEG offers superior access to the gastrointestinal system over surgical methods.

We aim to see utility of percutaneous endoscopic gastrostomy (PEG) for nutritional support in patients with oropharyngeal dysphagia secondary to neurological deficits and its effect on the quality of life of these patients.

Methodology: we studied retrospectively 25 patients with neurological oropharyngeal dysphagia in last 2 years needing PEG insertion. We reviewed data of the patients for 3 months post insertion of PEG. Study was based on body weight, haemoglobin and serum albumin level prior to and after PEG. All complications including sepsis, perforation, hemorrhage and death were recorded.

Results: Our study showed significant improvement in levels of hemoglobin, albumin and weight gain after Percutaneous endoscopic gastrostomy. There were no major complication.

Conclusion: Percutaneous Endoscopic Gastrostomy is an easy procedure that can be done bedside without complications in patients with neurological deficit. It improves nutritional status of the patient and also prevents aspiration pneumonia in patients with good gastric motility.

Keywords: Percutaneous endoscopic gastrostomy, nutritional support, Oropharyngeal dysphagia.

Introduction

The primary indication for enteral and parenteral feeding is the provision of nutritional support to meet metabolic requirements for patients with inadequate oral intake¹.
Enteral feeding is easy, safe, inexpensive and practical to maintain. It also helps to maintain local gastrointestinal defence and integrity thus decreasing translocation of bacteria. Parenteral nutrition is associated with a higher degree of side effects. It is costly, cumbersome and needs special care for maintenance.

Several methods such as nasogastric tube (NGT), percutaneous endoscopic gastrostomy (PEG), percutaneous fluoroscopic gastrostomy (PFG) and surgical endoscopic gastrostomy are available for enteral feeding.

Oropharyngeal dysphagia has been identified as a serious risk factor for patients developing aspiration pneumonia.

Prolonged nutrition through nasogastric tube is difficult. It has been known to cause severe aspiration pneumonia, esophageal stricture and traumatic bleed from gastric mucosa.

Gastrostomy is a well-established mode of enteral nutrition. It can be either open surgical or percutaneous endoscopic gastrostomy.

PEG needs only local anaesthesia and expertise is needed to perform the procedure. It can be done bedside without needing to shift ventilated patients to the operation room.

**Aims and Objective of the Study**

The Aim of the study was to evaluate the effectiveness of percutaneous endoscopic gastrostomy for nutritional status in the patients with oropharyngeal dysphagia secondary to neurological deficits and its effect on the quality of life of these patients.

**Objective of the study**

1. To assess the nutritional status of the patient
2. To assess the rate of post procedure aspiration pneumonia in patients with oropharyngeal dysphagia
3. To assess any other complications associated with PEG.

**Material and Methods**

This retrospective study was done to include cases with neurological oropharyngeal dysphagia in last 2 years needing PEG insertion. Patients with limited life expectancy, having chest injury, contusion of lung, haemothorax or pneumothorax were excluded. Patients with deranged bleeding and coagulation profile were corrected prior to insertion.

We reviewed data of the patients for 3 months post insertion of PEG.

All cases were with a proven diagnosis and were already on nasogastric tube feeding for at least 2 weeks.

Study was based on body weight, haemoglobin and serum albumin level prior to and after PEG. Body weight was calculated with bed and then subtracting dry weight of bed in patients who could not stand.

Short-term complications (symptoms or signs within 30 days of PEG), and long-term complications (symptoms or signs more 30 days after PEG tube insertion) were obtained as the details available on patient’s record sheets. Major complications were defined as per ESPEN guidelines and included sepsis, perforation, and haemorrhage and death.

All statistics was done using JASP (JASP Team (2020). JASP (Version 0.14.1)) Values of \( p < 0.05 \) were considered statistically significant using student t test.

Our study includes 12 females and 13 male patients, youngest was 8 years of age, while the oldest was 92 years of age. 8 patients were having complex head injuries, 8 had neurological deficits post cerebral vascular events, 5 had deficits post tumour excisions, 3 patients had primary metabolic diseases with altered mentation while 1 had deficit post tubercular meningitis.

All PEG insertions were done by the same team of endoscopists. 5 were done bedside while the rest in the endoscopic suite. The average time taken for the procedure was 18.87 minutes. The minimum time was of 10 minutes and the maximum time taken was 35
All the procedures were done under local anaesthesia with sedation when necessary. The procedure was done with patients supine and head high to prevent aspiration during procedure. Patients were given a test feed after 1 hour of insertion and regular feeding started thereafter. All patients tolerated the procedure and the test feed and went on regular feeding.

There was a rise in pulse rate of the patient by about 10 beats per minute above baseline in all the patients during procedure which returned to normal baseline in about 4-7 hours.

Haemoglobin level, weight of the patient and albumin level were compared based on the information taken from patient’s records just before the PEG insertion and after 3 months follow up.

Figure 1: showing improvement of Hb(gm%) after PEG
There is significant improvement of Haemoglobin(Fig 1), weight(Fig 2) and albumin level(Fig 3) of the patient after PEG insertion.
One patient had accidental decannulation on 27th day, tube was reinserted without any problems. 7 patients could start oral intake and they were decannulated after 2 weeks of regular oral intake.

There was no procedure related death in the study. 1 patient died due to severity of underlying disease. There was minor wound infection in 2 patients which was controlled with dressings for less than 7 days. 2 patients had one episode of tube blockage after 2 months of insertion.

**Discussion**

Percutaneous endoscopic gastrostomy is an easy and safe procedure that can also be safely done bedside.

In 1980 Gauderer et al. described inserting a percutaneous gastrostomy tube under local anaesthetic using endoscopy.

We had a delayed insertion of PEG tube as when patients have had a neurological insult, some tend to develop delayed gastric emptying (gastroparesis) even without a prior history of gastric motility disorder especially following severe head injury. Problem faced post PEG insertion related to gastric motility can be avoided if inserted after 15 days of primary insult. The lower esophageal sphincteric pressure and intracranial pressure have an inverse relationship. This combined with delayed gastric emptying and diminished gag reflex lead to higher incidences of aspiration. In critically ill patients on ventilator with or without a tracheostomy microaspirations are regular. This is increased with under inflation of tracheal cuff, Zero positive end expiratory pressure, low peak inspiratory pressure, tracheal suctioning, nasogastric tube and enteral nutrition. A smaller size of the nasogastric tube does not decrease chances of the aspiration.

Enteral nutrition decreases the translocation of gut bacteria and decreases chances of Multiorgan dysfunction syndrome and endotoxemia. Beneficial in maintaining nutrition due better assimilation of nutrients.

PEG helps in decreasing amount of micro-aspiration as evidenced by improving Chest X-rays and TLC of patients with some aspiration before PEG insertion. This correlates to other studies. This aspiration was further decreased by using semi solid feed instead of liquid feeds and by continuous feeding than bolus feeds.

Our study showed improvement in levels of haemoglobin, albumin and weight gain which correlates with other studies showing increase in albumin levels.

PEG can be done safely with minimal complications even in moribund patients. Patient not fit for anaesthesia can also undergo the procedure. The learning curve is not very steep, and reasonable expertise can be gained with few cases.

**Conclusion**

Percutaneous Endoscopic Gastrostomy is an easy procedure that can be done bedside without complications in patients with neurological deficit. Immediate procedure related complications are negligible. It improves nutritional status of the patient and also prevents aspiration pneumonia in patients with good gastric motility. Overall mortality depends on the primary disease process than on PEG insertion.

**Conflict of Interest:** The authors have no conflicts of interest.

**Source of Funding:** This is a retrospective study. We have used institutional funds for research.

**Ethical Clearance:** This is a retrospective study. There is no direct involvement of the patients. Hence ethical clearance is not needed.

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Effect of Health Literacy Intervention on Medication Adherence among Older Adults with Chronic Diseases

Magda M. Mohsen1, Bahiga Galal Abd El-Aal1, Sally S. Hassan2, Amal A. El-Abbassy1

1Community Health Nursing, Faculty of Nursing/ Menoufia University, Egypt, 2Geriatric Health Nursing, Faculty of Nursing/ Menoufia University, Egypt

Abstract

Background: Medication adherence is essential for controlling chronic conditions, treating temporary conditions, and overall health and well-being. Aim: Examine the effect of health literacy intervention on medication adherence among older adults with chronic diseases. Design: A quasi-experimental (study and control design). Settings: Family health unit in Dragel village, Menoufia Governorate, Egypt. Sample: a purposive sample of 140 older adults. Tools: 1) Interviewing questionnaire that includes; the socio-demographic data, medical history, medication knowledge, factor affecting medication adherence and physiological measurement 2) The Medication Adherence Scale. 3) Self-Efficacy for appropriate medication use scale. Results: There was remarkable improved medication adherence and self-efficacy in medication use in the study group compared to pre-intervention and the control group. Also, there was improved knowledge and practice regarding medication use. Also, there was significant control in random blood glucose level and blood pressure in the study group compared to pre-intervention and the control group. Conclusion: The health literacy intervention was effective in enhancing medication adherence, successful in improving self-efficacy in medication use, and significantly controlling random blood glucose and blood pressure among older adults. Recommendation: Health care providers especially nurses should provide regular follow up for older adults with chronic diseases regarding medication adherence.

Keywords: Chronic diseases- Health literacy - Medication adherence - Older Adults

Introduction

Chronic diseases are long-lasting in their effects and require ongoing medical attention or limit activities of daily living[1]. Chronic diseases require long-term treatment and an increase in the demand for long-term healthcare services; that lead to decrease elderly people’s quality of life[2]. Aging is often accompanied by a larger burden of comorbid conditions and the seriousness of illness[3].

Pharmacologic therapy is an essential component in chronic disease management. Most elderly people with chronic diseases do not adhere to prescribed medication which usually leads to poor clinical outcomes [4]. Therefore, it is essential to enhance medication adherence in patients with chronic diseases to decrease premature deaths and social burden[5]. Patient adherence to medication is the degree to which patients follow treatment recommendations recommended as prescribed by their clinician[6].

Health literacy is a patients’ ability to obtain, process, communicate and understand basic health information and services needed to make effective health-related decisions. So that, the patients should adequately be informed and understand the health-related instructions[6]. In maintaining medication
adherence for their patients by establishing therapeutic communication with patients before they receive their medication regimen; to be able to determine the common adherence barriers before adherence becomes a problem for the patient[6-7]. The nurses provide information or guide the patient to engage in the teach-back method that helps patients understand their instructions[8-9].

**Aim of the Study**

Examine the effect of health literacy intervention on medication adherence among older adults with chronic diseases (diabetes and hypertension).

**Research Hypothesis**

1. Older adults who will receive health literacy intervention will have improved medication adherence than older adults who will not receive health literacy intervention.

2. Older adults who will receive health literacy intervention will have controlled random blood glucose levels and blood pressure than older adults who will not receive health literacy intervention.

3. Older adults who will receive health literacy intervention will have improved self-efficacy than older adults who will not receive health literacy intervention.

**Methods**

**Design:** A quasi-experimental (study and control design) was utilized

**Settings:** Family health unit in Dragel village, Menoufia Governorate, Egypt that was selected using multi-stage random selection.

**Sample:** A purposive sample of 140 older adults was divided randomly into two equal-matched groups (study and control group) by using simple random selection.

**Inclusion criteria:-**

- Older adults 60 years old and older.
- Older adults with chronic diseases (diabetes mellitus and/or hypertension).

**Sample Size and Power of The study:**

The sample size was calculated based on power analysis performed by Kelsey, Fleiss, and Fleiss, (2010) which indicated that 140 older adults would yield sufficient power of 80% to detect the effect of health literacy intervention on older adults with chronic diseases, based on the following assumptions effect size= 0.5, and alpha=0.05, the power of 80%, and the ratio of exposed to risk factors to those who were not exposed =1:1.

**Instruments:**

I: **Interviewing questionnaire that includes:**

**Part 1:** Socio-demographic data including name, age, sex, marital status, etc.

**Part 2:** Medical history including the type of chronic disease and medication information.

**Part 3:** Medication knowledge.

**Part 4:** A physiological measurement that includes:

- Random blood glucose
- Blood pressure

II: **The Medication Adherence Scale:** The scale was developed by[10]. The total score of the scale was ranged from 0-8 and categorized into: >2 = low adherence, 1 or 2 = medium adherence, 0 = high adherence

III: **Self-Efficacy for Appropriate Medication Use Scale:** This scale was developed by[11]. The total score of the scale was ranged from 16 to 48 and categorized into: 1-16 low self-efficacies, 17- 32 moderate self-efficacy, 33- 48 high self-efficacy.

**Validity of the instruments:**

The study instruments were tested for validity by a jury of five experts in the field of Community Health Nursing and Geriatric Nursing, to ascertain the relevance, completeness, and simplicity of each component in the instruments.
Reliability: The Cronbach alpha coefficient of the Medication Adherence Scale was 0.81 which indicates that the scale is reliable. Whereas the Cronbach alpha coefficient for self-efficacy for medication use scale was 0.85 which indicates that the scale is reliable.

Pilot Study: was carried out to assess the clarity, feasibility, applicability of the study tools, and the time needed to fill each tool. The pilot study was not included in the study sample.

Ethical Consideration:
· An official letter was obtained from the Dean Faculty of Nursing, Menoufia University to the director of the family health unit at Dragel to collect data and gain their help during the study period.
· Verbal informed consent from each participant was taken after explaining the purpose and duration of the study as well as they assured that their data will be used for research purpose only.

Procedure for Data Collection:
· Data were collected during the period from the beginning of January 2020 to the end of October 2020.
· The researcher interviewed the participants in the waiting room at the family health unit at Dragel two or three times every week.
· Pretest data of the study was filled by the researcher after introducing herself to participants and explaining the aim of the study to gain their cooperation.
· The filling of the instruments took about 20-30 minutes for each participant then the measurements of blood glucose and blood pressure were taken.
· The sample of the study was categorized into two groups (study and control group) using simple random selection. The Control group was received routine care and the study group was received health literacy intervention.
· The health literacy intervention was developed by the researcher and carried out in three sessions. Each session was taken 20-30 minutes. The intervention was given in a small group each group contains from 2-5 participants.
   ü The first session included information about diabetes mellitus and hypertension disease.
   ü The second session related to diet and exercise regimen for diabetes mellitus and hypertension.
   ü The third session related to medication adherence and factor affecting it.
· By the end of each session the researcher provides a summary of essential points as well as at the end of the sessions then, the participants of the study group received an educational intervention booklet.
· Posttest was administered for the study and control group at 3 months post-intervention.
· Physiological measures (random blood glucose and blood pressure) were taken once monthly for three months for the study group and control group.
· Participants of the control group have received a copy of the booklet at the end of the study.

Statistical analysis:
The collected data were coded and entered into the computer and statistically analyzed using SPSS version 22 for categorical variable the number and percent were calculated. The relations between the studied variables were tested. The level of significance was adopted at p<0.05.

Results
Population characteristics: The total sample size of 140 older adults were included in the study. 72.9% of the study group was in the age group 60-70 years, 64.3% were females, 58.6% were married, 37.1% do not read or write, 75.7% were not working and 57.1% with not enough income. While 67.1% of the control group was in the age group 60-70 years, 55.9% were females, 62.8% were married, 37.1% do not read or write, 64.3% were not working and 61.4% with not enough income;
there were no significant differences between the study and control group regarding all sociodemographic characteristics.

**Table (1):** Reveals that, post-intervention knowledge of the study group was significantly improved compared to their pre-intervention knowledge and knowledge of the control group.

**Fig. (1):** Shows that, in post-intervention, the high medication adherence degree among the study group increased to 45.8% than the control group 10% also medium medication adherence degree among the study group increased to 47.1% than the control group 24.3% while low medication adherence degree among the control group increased to 65.7% than the study group 7.1%.

**Table (2):** Shows that, mean random blood glucose showed consequently decreased on three occasions post-intervention in the study group compared to the mean random blood glucose pre-intervention and control group. Also, mean systolic BP showed consequently decreased on three occasions post-intervention in the study group compared to mean systolic BP pre-intervention and control group. Also, mean diastolic BP showed consequently decreased on three occasions post-intervention in the study group compared to the mean diastolic BP pre-intervention and control group.

**Table (3):** demonstrates that the third post-intervention mean random blood glucose was significantly controlled in older adults with high and moderate medication adherence among the study group compared to their pre-intervention and the control groups

**Table (4):** Demonstrates that, post-intervention degree and mean total score of self-efficacy in medication use among the study group was significantly improved compared to their pre-intervention and control group.

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**Answering research hypothesis number 1**

**Table 1: Degree of knowledge about medication among the study and control groups pre and post-intervention**

<table>
<thead>
<tr>
<th>Degree of knowledge about medication</th>
<th>Pre-intervention</th>
<th>Post-intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study group</td>
<td>Control Group</td>
</tr>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Poor knowledge (3-5)</td>
<td>51  72.9</td>
<td>45  64.3</td>
</tr>
<tr>
<td>Good knowledge (6-7)</td>
<td>19  27.1</td>
<td>25  35.7</td>
</tr>
<tr>
<td>Mean total score of medication knowledge</td>
<td>5.2±1.4</td>
<td>4.8±1.2</td>
</tr>
</tbody>
</table>

*LR = The likelihood-ratio*
Fig.1: Post intervention medication adherence degree among the study and the control groups.

Answering research hypothesis number 2

Table 2: Comparison of post-intervention physiological measurements follow up among study and control groups, pre, post1, post2, and post3 intervention

<table>
<thead>
<tr>
<th></th>
<th>Pre-intervention</th>
<th>Post 1 one month post-intervention</th>
<th>Post 2 two months post-intervention</th>
<th>Post 3 three months post-intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean random blood glucose</td>
<td>174.7 ±43.9</td>
<td>169.5 ±54.3</td>
<td>168.3 ±33.6</td>
<td>161.7 ±32.7</td>
</tr>
<tr>
<td>Test of significant</td>
<td>t=0.62, P=0.53 NS</td>
<td>t=1.2, P=0.2 NS</td>
<td>t=-2.94, P=0.004</td>
<td>t=-7.61, P&lt;0.0001</td>
</tr>
<tr>
<td>Mean systolic BP</td>
<td>136.1±17.1</td>
<td>124.6±17.1</td>
<td>129.9±10.9</td>
<td>125.1±10.9</td>
</tr>
<tr>
<td>Test of significant</td>
<td>t=3.9, P=0.001</td>
<td>t=2.5, P=0.01</td>
<td>t=-2.6, P=0.01</td>
<td>t=-6.3, P&lt;0.0001</td>
</tr>
<tr>
<td>Mean diastolic BP</td>
<td>87.4±9.7</td>
<td>81.7±10.5</td>
<td>84.9±7.1</td>
<td>81.6±7.5</td>
</tr>
<tr>
<td>Test of significant</td>
<td>t=3.3, P &lt;0.001</td>
<td>t=2.6, P&lt;0.009</td>
<td>t=-302, P=0.002</td>
<td>t=-7.22, P&lt;0.0001</td>
</tr>
</tbody>
</table>

*gr = group
Table (3): Relation between medication adherence degree and mean blood glucose in third post-intervention among the study and control groups.

<table>
<thead>
<tr>
<th>Post-intervention medication adherence degree</th>
<th>Study</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean± SD</td>
<td>N</td>
</tr>
<tr>
<td>High adherence (0)</td>
<td>32</td>
<td>145.6±20.8</td>
<td>7</td>
</tr>
<tr>
<td>Moderate adherence (1 or 2)</td>
<td>33</td>
<td>141.7±24.4</td>
<td>17</td>
</tr>
<tr>
<td>Low adherence (&gt; 2)</td>
<td>5</td>
<td>187.2±50.2</td>
<td>46</td>
</tr>
</tbody>
</table>

Answering research hypothesis number 3

Table (4): Degree of self-efficacy among the study and control groups pre and post-intervention

<table>
<thead>
<tr>
<th>Self-efficacy degree</th>
<th>Pre-intervention</th>
<th>Post-intervention</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study group</td>
<td>Control group</td>
<td>No</td>
</tr>
<tr>
<td>Low self-efficacy (1-16)</td>
<td>1</td>
<td>1.4</td>
<td>1</td>
</tr>
<tr>
<td>Moderate self-efficacy (17-32)</td>
<td>64</td>
<td>91.5</td>
<td>67</td>
</tr>
<tr>
<td>High self-efficacy (33-48)</td>
<td>5</td>
<td>7.1</td>
<td>2</td>
</tr>
<tr>
<td>Mean total score of self-efficacy</td>
<td>25.9±4.9</td>
<td>27.1±4.2</td>
<td>t=1.5, P=0.12</td>
</tr>
</tbody>
</table>

*LR = The likelihood-ratio

Discussion

The consequences of medication non-adherence to long-term therapies are poor health outcomes, increased health care costs, and frequent hospitalization of older adults\[12-13\]. The purpose of the present study aimed to examine the effect of health literacy intervention on medication adherence among older adults with chronic diseases (diabetes and hypertension). The first hypothesis suggested that older adults who will receive health literacy intervention will have improved medication adherence than older adults who will not receive health literacy intervention. Support for this hypothesis was found in the present study that revealed a highly significant improvement in the different levels of medication adherence among the study group than the control. This finding was supported by\[14\], who reported that a health literacy intervention may be a
viable mechanism for improving cardiovascular-related medication adherence and outcomes. Besides, the present study result came on the same line with\cite{15} who showed that a greater percentage of intervention patients were adherent to medication compared with usual care patients and there was a statistically significant greater percentage of intervention patients were classified as an adherent for medication.

The second hypothesis suggested that older adults who will receive health literacy intervention will have controlled blood pressure and blood glucose level than older adults who will not receive health literacy intervention. Support for this hypothesis was found in the present study that revealed a significant difference was observed between study and control groups regarding the control of random blood glucose. There was a significant difference was observed between the study and control groups regarding the control of blood pressure. A similar finding was recorded by\cite{16} they showed that a significant improvement in pre and post-intervention was detected in the intervention group’s HbA1c levels and the intervention helped improve HbA1c levels. Also, a similar finding was reported by\cite{14}; they found that at six months post health literacy intervention average systolic blood pressure was decreased 0.5 mmHg and diastolic blood pressure was decreased 1.5 mmHg compared to pre-intervention among American patients’ at primary care clinics.

The third hypothesis suggested that older adults who will receive health literacy intervention will have improved self-efficacy than older adults who will not receive health literacy intervention. Support for this hypothesis was found in the present study that revealed a significant improvement in the different levels of self-efficacy among the study group than the control in post-intervention. The level of high self-efficacy increased among the study group, compared to the control group post-intervention. These findings came in agreement with\cite{17} they reported that older adults in the program intervention group showed significantly higher levels of self-efficacy at follow-up among Korean older adults with chronic disease. Participants with low health literacy had greater benefits from the intervention than those with high health literacy. Also, the present study results were in accordance with the study carried out by\cite{18} who reported that the intervention group had significantly better self-efficacy and diabetes knowledge than the control group.

**Conclusion**

The health literacy intervention was effective in enhancing medication adherence, successful in improving self-efficacy in medication use, and significantly controlling random blood glucose and blood pressure among older adults.

**Recommendation:**

Health care providers especially nurses should provide regular follow up for older adults with chronic diseases regarding medication adherence.

**Conflict of Interest:** The researchers declare that they have no conflict of interest within this research and publication

**Source of Funding:** Self-funding

**Ethical Clearance:** Taken from the Ethical Research Committee of the Faculty of Nursing, Menoufia University.

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Self-management of Elderly Patients with Osteoarthritic Knee on Recovery Outcomes

Magda M. Mohsen¹, Nabila E. Sabola¹, Nagwa I. El-khayat², Entsar A. Abd El-Salam³
¹Prof. of Family and Community Health Nursing, ²Assist. Lecturer of Geriatric Nursing, ³Assist. Prof. of Geriatric Nursing, ⁴Faculty of Nursing, Menoufia University-Egypt

Abstract

Background: Knee osteoarthritis accounts for almost four fifths of the burden of worldwide. It is the leading cause of mobility impairment, disability and loss of function in older adults. This study aimed to examine the effectiveness of self-management of elderly patients with osteoarthritic knee on recovery outcomes.

Material and Methods: A quasi- experimental design was utilized to conduct this study. The study was carried out at outpatient clinic of Shebin-Elkom University and Educational Hospital, and then they were following up at their homes. 100 elderly patients were selected who met inclusion and exclusion criteria. A constructed interviewing questionnaire, arthritis self-efficacy scale, and Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index were used to collect the data.

Results: there was increase in the mean total pain self-efficacy score, and other symptoms self-efficacy score in study group than control group. There was decrease in the mean total physical function WOMAC score in study group than control group after intervention.

Conclusion: Implementation of self-management for elderly patients was effective in management symptoms of knee osteoarthritis among study group compared to control group.

Keywords: Elderly, Knee osteoarthritis, Self-management, recovery outcomes.

Introduction

Knee osteoarthritis (KOA), known as degenerative joint disease, is typically the result of wear and tear and progressive loss of articular cartilage. It is most common in elderly women and men. Knee osteoarthritis can be classified as, primary and secondary. Primary osteoarthritis is articular degeneration without any apparent underlying reason. Secondary osteoarthritis is the consequence of either an abnormal concentration of force across the joint as with post-traumatic causes or abnormal articular cartilage, such as rheumatoid arthritis (RA). Osteoarthritis is typically a progressive disease that may eventually lead to disability(1).

Globally, 18% of women and 9.6% of men aged over 60 years have symptomatic OA, with a quarter of these individuals unable to perform routine daily activities. By 2050, a projected 130 million people will suffer with OA, constituting a significant societal burden (2). In Egypt, more than five million people have OA, percentage of years of healthy life lost due to OA disability per 100.000 people is 67.6 %. The rate of years of healthy life lost from osteoarthritis has changed over time and relative to the parent region of North Africa & Middle East and the world at large (3).
Self-management intervention has an important role to play in managing osteoarthritis, and suit an integrative model of shared care between conventional and complementary medicine practitioners. Self-management program help to promote health and management of diseases, increasing motivation of elderly and decreasing the negative effects on their daily function (4). It including low-impact activity, balance of rest, range-of-motion exercises, relaxation, heat or cold, taking medication, joint protection, massage, splints and nutrition (5,6).

**Aim of the Study**

The study aimed to examine the effectiveness of Self-management of elderly patients with osteoarthritic knee on recovery outcomes.

**Hypotheses:**

- Elderly patients who will receive self-management will be more likely to have a different score of self-efficacy than elderly patients who will not receive the intervention.

- Elderly patients who will receive self-management will be more likely to have change in their physical function than elderly patients who will not receive the intervention.

**Subject and method:**

**Design:** A quasi-experimental design (study and control) was utilized to conduct this study.

**Setting:** This study was conducted at outpatient clinic of Shebin-Elkom University and Educational Hospital, and then they were following up at their homes.

**Sample:** A convenience sample of 100 elderly patients with osteoarthritic knee, were recruited. They were assigned randomly by tossing a coin for two groups (study group from University Hospital and control group from Educational Hospital).

**Tool I:** A constructed interviewing questionnaire

This questionnaire was developed by the researcher for demographic data: age, gender, marital status, and level of education......etc, history of chronic diseases and medication taken.

**Tool II:** Arthritis self-efficacy scale (ASES) (7) used to assess self-efficacy of patients with osteoarthritis.

**Tool III:** Western Ontario and McMaster Universities Osteoarthritis (WOMAC) (8) used to assess physical function among OA elderly patients.

**Validity of tools:**

The validity of three tools was done by a jury of four experts (two Professors in Community health nursing and two experts in Geriatric nursing) who reviewed the questionnaire for clarity, relevance, comprehension, understanding and applicability, content accuracy and internal validity.

**Reliability of tools:** Reliability was estimated among 10 participants by using test retest method with two weeks apart between them.

Regarding tool 2 (ASES scale): It was 0.76 with the following Cronbach alpha reliability values for its subscales: Pain SE: 0.70, other symptoms SE: 0.78.

Concerning tool 3 (WOMAC): It was 0.74 with the following Cronbach alpha reliability values for its subscales: Stiffness: 0.70, Physical function: 0.78. The Cronbach alpha reliability for the three tools and their subscales indicate that the three tools are reliable to detect the objectives of the study.

**Pilot study:** conducted on 10 of elderly patients to evaluate the effectiveness of the study tools, clarity, techniques and the availability of the study sample.

**Ethical consideration:**

- Approval of the ethical research Committee was obtained at the Faculty of Nursing, Menoufia University.

- The agreements for participation of the elderly patients were taken after the aim of the study was explained to them.
The elderly patients had the full rights to refuse to participate in the study or to withdrawal at any time. Also they were assured that, the information would remain confidential and used for the research purpose only.

The researcher gave copies of general information package (booklet) about self-management to elderly with knee OA for achieving the ethical principles of research as the principle of beneficence that all subjects should benefit from the research’s knowledge.

Data collection procedure

Data collection started on February 2019 and lasted until January 2020.

Filling in the questionnaire for elderly patients (case and control) was conducted at the waiting area of orthopedic Out-patient Clinic affiliated to Shebin-Elkom University and Educational Hospital, after the elderly patients receiving medical examination and treatment.

Each subject was personally interviewed for about 45 minutes-1 hour(according to the level of patient understanding) to fill the questionnaire for case and control group and give simple intervention about knee osteoarthritis only for case group.

The researcher took address and telephone number from each patient to facilitate communication and follow up at their homes. The implementation of the home visit for self-management intervention sessions was achieved within 6 months one session per week for every individual (total duration 3weeks for every individual).

The intervention included telephone follow up to elderly patients for completing intervention sessions for reassurance, support and keep contact.

The final session (fourth): was done after 3 month (post intervention) After the implementation of the self-management intervention each elderly patients was assessed using the study tool to determine the effect of the sessions on their pain, other symptoms and physical function of knee. Time required for the session was 20 minutes.

Statistical Analysis:

- Quantitative data were presented by mean (X) and standard deviation (SD). It was analyzed using student t-test for comparison between two means; Correlation coefficient (r) was used to test the correlation between two quantitative variables. Qualitative data were presented in the form of frequency distribution tables. It was analyzed by chi-square ($\chi^2$) test.

Results

![Fig.1: Percent distribution of age of studied groups](image-url)
Fig. 1. Seventy percent of control group, and 74% of cases of the studied elderly patients with osteoarthritic knee aged between 60 to 70 years with mean of 65 ± 5.1 years and 66.2±6.4 respectively.

Table 1: Effect of self-management, on the total self-efficacy subscales scores and total Self efficacy scale

<table>
<thead>
<tr>
<th>Total self-efficacy subscales scores and total Self efficacy scale</th>
<th>Pre intervention program</th>
<th>Post intervention program</th>
<th>P pre</th>
<th>P post</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control X± SD</td>
<td>Case X± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain SE score</td>
<td>9.3±21.8</td>
<td>7.8±17.6</td>
<td>t=2.4</td>
<td>t=3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.8±21.7</td>
<td>P&lt;0.02</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Other symptoms SE score</td>
<td>11.4±27.6</td>
<td>22.6±9.6</td>
<td>t=2.3</td>
<td>t=6.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.7±27.1</td>
<td>P&lt;0.02</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Total SE (pain +symptoms) scale</td>
<td>49.4 ±20.1</td>
<td>40.2±16.7</td>
<td>t=2.4</td>
<td>t=5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48.4 ±21.1</td>
<td>P&lt;0.02</td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

Table (1) Highlights that, there was a highly significant improvement in the mean total pain SE score, among case group than control group Post-intervention (28.7 ± 9.4 vr 21.7 ± 9.8, P<0.0001). Similar pattern was observed concerning other symptoms SE subscale. In addition, total SE scale (pain +symptoms) showed similar pattern where mean total SE score, among case group was higher than control group and the difference was highly statistically significant (P<0.0001).

Fig. 2: Post intervention Mean pain SE score in mild, moderate and sever osteoarthritis among case and control groups
Fig 2. Reveals that post-intervention mean total score in pain SE in both case and control groups were decreased when degree of osteoarthritis increased.

Table 2: Relation between post intervention mean total score of pain and other symptoms SE, with post intervention degree of osteoarthritis and pain intensity.

<table>
<thead>
<tr>
<th>Total self-efficacy subscales scores</th>
<th>Degree of osteoarthritis</th>
<th>Levels of pain intensity</th>
<th>P Osteo</th>
<th>P Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild X± SD</td>
<td>Moderate X± SD</td>
<td>Sever X± SD</td>
<td>Mild X± SD</td>
</tr>
<tr>
<td>Cases group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain SE score</td>
<td>(N=17) 36.3±7.3</td>
<td>(N=18) 28.1±5.4</td>
<td>(N=15) 20.8±8.7</td>
<td>(N=20) 32.5±10.8</td>
</tr>
<tr>
<td>Other symptoms SE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45.7±9.5</td>
<td>43.6±7.5</td>
<td>32.9±11.0</td>
<td>43.6±7.7</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain SE score</td>
<td>(N=19) 30.4±7.6</td>
<td>(N=19) 28.1±5.3</td>
<td>(N=12) 18.5±4.8</td>
<td>(N=9) 34.0±6.7</td>
</tr>
<tr>
<td>Other symptoms SE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36.6±7.1</td>
<td>25.7±7.1</td>
<td>14.2±7.1</td>
<td>41.7±5.1</td>
</tr>
</tbody>
</table>

Table 2 reveals that post-intervention mean total score in pain SE and other symptoms SE in both case and control groups were decreased when degree of osteoarthritis and the pain intensity increased.

Fig.3: Mean total WOMAC score pre and post intervention
among control and case elderly groups

Fig 3: shows post-intervention decreases in total WOMAC score in the two groups; however, the case group had more pronounced significant pre–post differences (43.8 and 33.4) compared to the control group (40.2 and 41.4) respectively. This difference was statistically high significant (p <0.001).

Table (3): Relation between post intervention mean total score of stiffness, and physical function with post intervention degree of Osteoarthritis, as well as Pain intensity levels among cases and control groups (N=100).

<table>
<thead>
<tr>
<th>Total stiffness and physical function scores</th>
<th>Osteoarthritis Degree</th>
<th>Pain intensity</th>
<th>P Osteo</th>
<th>P Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild X± SD</td>
<td>Moderate X± SD</td>
<td>Sever X± SD</td>
<td>Mild X± SD</td>
</tr>
<tr>
<td>Case group Stiffness</td>
<td>0.51±1.0</td>
<td>3.2±1.3</td>
<td>5.4±1.3</td>
<td>1.7±1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical function</td>
<td>22.5±9.1</td>
<td>32.6±8.6</td>
<td>46.2±5.8</td>
<td>28.9±15.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group Stiffness</td>
<td>2.2±1.6</td>
<td>4.0±1.3</td>
<td>6.3±1.6</td>
<td>2±1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical function</td>
<td>36.3±10.3</td>
<td>40.5±7.6</td>
<td>48.8±5.8</td>
<td>31.3±10.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3) post-intervention mean total scores in stiffness and physical function were increased in both case and control groups when degree of osteoarthritis and the pain intensity categories increased.

Discussion

Knee osteoarthritis causes significant pain and function loss among elderly people. As the disease progresses, it can cause chronic joint pain, muscle weakness, deformity and functional deficiency and leads to dependency for daily life activities and decreased quality of life, thus increasing the need of the elderly for healthcare services (9). The present study aimed to examine the effectiveness of Self-management of elderly patients with osteoarthritic knee on recovery outcomes.

Regarding the effect self-management on pain self-efficacy the current study revealed that there was a highly significant improvement in the mean total pain self-efficacy, among case group than control group post intervention. This result is supported by study conducted by (10) who studied “The Effect of Self-management Training on Self-efficacy of Elderly Patients with Knee Osteoarthritis” in Iran. They reported that implementation of self-management training is effective in improving the self-efficacy of the elderly with knee osteoarthritis.

Also, this finding is consistent with the results of a study by (11) who studied “Feasibility of Imported Self-Management Program for Elderly People with Chronic Pain” in Japan. They concluded that pain self-efficacy was significantly improved immediately after the program compared with baseline and these effects
were maintained at 3-month follow-up, this finding has important implications for the development of pain services in community-dwelling elderly Japanese.

Concerning the effect self-management on other symptoms self-efficacy the current study revealed that there was a highly significant improvement in the mean total other symptoms self-efficacy, among case group than control group post intervention. This result is congruent with study by (12) who studied “Effect of self-management program on outcome of adult knee osteoarthritis” in Iran. They reported that the self-management program, which included pain relief methods, proper diet and exercise, aimed to improve pain, symptoms, function and quality of life. They have demonstrated that participants in the self-management program experienced improvements in the outcome of knee osteoarthritis.

This finding is consistent with the results of study by (13) who studied “The better management of patients with osteoarthritis program; outcomes after education and exercise delivered nationwide” in Sweden. They indicated that patients with knee osteoarthritis showed significant improvements in scores for arthritis self-efficacy other symptoms subscale and arthritis self-efficacy painsubscale.

In addition, this finding is agreement with the results of study by (14) They showed that knee symptoms scores significantly improved and quality-of-life scores significantly increased. Moreover, is consistent with the results of study by (15) They indicated regarding self-efficacy, there was improvement in the confidence that studied elderly abilities to perform a specific task with a significant difference was observed at 8th week post intervention and 6th month follow-up among study group compared to control group. As the exercises, proper diet and pain relief measures included as part of the self-management program (SMP), that can reduce stiffness and help knee pain relief.

In contrast, (16) who studied “Challenges in evaluating an Arthritis Self-Management Program for people with hip and knee osteoarthritis in real-world clinical settings” in Australia. They showed that there wasn’t any difference in improving the quality of life, pain, stiffness of the joints, physical function and psychosomatic symptoms at 3 and 12 months after the intervention. This may be attributed to patients with hip and knee osteoarthritis may have more complication that affect quality of life of elderly patients.

Regarding the effect of self-management on physical function results of the current study revealed a highly significant improvement in function WOMAC score after intervention than control group. This finding is similar to what was reported by (17) They showed that their study supports the importance of a combined self-management and exercise intervention to improve functional lower limb strength and aerobic capacity in a Portuguese sample. Additionally, pain and other symptoms have improved. Also, result is supported by (18) They showed that a self-management program improved the health status of patients with knee osteoarthritis.

In addition, this result came on the same line with study conducted by (15) They suggested that concerning physical function subscales for study group, a statistically significant difference was found between pretest and posttest after implementation of interventions regarding to descending stairs, rising from sitting, standing walking on flat surface, lying in bed, getting in/out of bath and sitting.

Moreover, study by (19) They found that self-efficacy at baseline was associated with change over time in pain intensity and physical activity at follow-up after the intervention. High self-efficacy had a positive effect on pain intensity and physical activity, that increasing the need for exploring and strengthening patients’ self-efficacy. This result may be related to implementation of pain relieve measures and physical exercise that can improve physical function for elderly patients with knee osteoarthritis.

Regarding the relation between elderly knee osteoarthritis, socio demographic characteristic and their mean of post intervention self-efficacy and physical function scores, the current study revealed that, young
age of studied elderly patients from 60-70 years had high self-efficacy and improved physical function compared with other ages from 71-80 years. Also, male, university education, had high self-efficacy and improved physical function compared with others. This results supported by a study by (20) They reported that female gender are significantly related to less self-efficacy and higher disability, and low educational level negatively influences the amount of perceived pain and disability, and older age can affect degree of disability and pain intensity.

Also, study by (21) who reported that indicators of quality of life are equal or even higher in older compared to younger patients. Moreover, Study by (22) they stated that subjective disability was affected by education level. In contrast, study by (20) who studied “Is there a relationship between self-efficacy, disability, pain and sociodemographic characteristics in chronic low back pain?” in Italy. They reported that they did not find any correlation between educational level and pain self-efficacy. This may be related to difference in community cultures.

**Conclusion**

Implementation of self-management for elderly patients was effective in management symptoms of knee osteoarthritis among study group compared to control group which was resulting in increasing their self-efficacy regarding pain and other symptoms. Moreover, it was effective in increasing physical function in study group than control group.

**Recommendations:** Awareness programs, targeted for all elderly patients, their family caregivers and general public about self-management and its importance for increasing self-efficacy and improving physical function.

**Ethical Clearance:** Institutional review committee was informed and study cleared

**Source of Funding:** Self

**Conflict of Interest - Nil**

**References**


The Impact of Exclusive Breast Feeding on Infant Morbidity in The First Six Months of Infants Life

Maha A. Muhsen¹, Jabbar T. Ahmed²

¹Researcher, ²Prof., Southern Technical University – College of Medical and Health Technology/Basra - Community Health Department

Abstract

The breast feeding was basic and ideal type of feed for infants especially during first six months of their life, breast feeding has a main role in infant’s life as maintaining on mental and physical health, protecting from many disease and play important role in health of mothers and national and international strategies. The goals of this study is to estimate the prevalence exclusive breast feeding (EBF) in Basra (Southern of Iraq) during period of study, to determine the impact of exclusive breast feeding on morbidity of infant during first six months of life and to assess relationship between some character’s for mothers and babies (Age of mothers at marriage, Age of mothers at baby birth, educational level, occupation, birth interval, methods of delivery, parity, gestational age, age, gender and birth weight of infant). The study found prevalence of exclusive breast feeding in infants for first six months of life is 54.75%, while (23.75%) and (21.5%) for partial and exclusive formula feeding respectively and showed significant relationship between diarrhea disease, respiratory tract infection and gastroenteritis and type of feeding in first six months of life at level p<=0.05. also showed EBF associated with age of mothers at baby birth, education level, occupation of mothers, and mode of delivery, gestational age and parity and found no relation between age of mothers at marriage, birth interval and sex of infant with EBF.

Keyword: type of feeding, exclusive breast feeding, morbidity, infants.

Introduction

The breast feeding was basic and ideal type of feed for infants especially during first six months of their life to achieve good health and growth this was recommended by world health organization ( WHO )¹(1). Breastfeeding had a religious basis in Islam where The Holy Quran it was recommended to the mothers for breastfeed her infant for a period of full two years if possible (2).

Breast feeding has a main role in infant’s life as maintaining on mental and physical health, protecting from many disease and play important role in health of mothers and national and international strategies (3). Breastfeeding contributed to a reduction in incidence of preventable infant diseases, such as respiratory, otitis media, necrotizing entercolitis, gastroenteritis and atopic disease irrespective of family history (4).

The breastfeeding can prevent the development overweight and obesity which reduce risk of several chronic disease as diabetes, cancer and cardiovascular disease, this impact for long term not only in early life⁵. Infants who depend on breastfeeding have a high intelligence rate compared to babies who do not drink breast milk, in addition to this, good development in vision and less retinal disease in premature infants (6).

Breastfeeding also help mothers to return their health after delivery, in terms of help to construction of uterus after birth and reduce bleeding at the time of birth, less cancers of breast and ovarian and reduce chance to fractures in later ages (7). Experts have proven that breastfeeding had a role in preventing conception (lactation amenorrhea method), it have a role in return ovulation, the breast feeding was effective way to prevent pregnancy by 98-99% if follow guides as nursing any six
times a day and not giving any pacifiers or formula, and breastfeeding no more than six months after childbirth (8).

Breastfeeding lengthens post-partum amenorrhea and post-partum weight loss and this amenorrhea also helps to replenish maternal iron stores that were lost during delivery, and thus reducing the risk of anemia. (9).

Also there were ecological benefits of breastfeeding to society include decreased energy demands for the production of infant formula and less solid waste such as formula cans and bottles (10).

Human milk (HM) help to facilitate the infant’s transition from enter to extra uterine life by providing immunomodulatory component provides good nutrition. The HM nutrient affected by the maternal genetics and varying degrees by maternal diet, fat composition and fat-soluble and water-soluble vitamins content were influenced by maternal diet but lactose, minerals, protein and trace elements were not affected by maternal nutrition, also oligosaccharide composition affected by maternal genetics, HM provides all nutrient for infants except vitamins D, K and iron so should be give supplements of vitamins to infants (11).

Patients and Methods:

A cross-sectional study conducted on 400 infants that less than six months of life and their mothers when coming to health care centers for the purpose of vaccination or treatment the infants. The types of variables were included in this study. The first (dependent) variable was the morbidity affecting the infant in the period of the study. The second (independent) variable was the type of feeding from birth to the time of the study. Also we studied other variables as such factors associated with infant as age, weight and gender. Together with the mother’s factors as age, age at marriage, profession, parity, level of education, type of delivery and birth space to explain their affected on selected of mothers to type of feeding. The data was obtained from mothers of infants by interviewing the breast fed currently or discontinued mothers face to face and answering the questions in the questionnaire, and participation rate high in this study.

Statistical Analysis

After collected data entered statistic package social science program (SPSS)22 version for analyzed by descriptive statistic frequencies and percentages to demographic characters for mothers and infants to determine data prevalence, inferential statistic crosstab table, Pearson chi square used to analyzed correlations among variables, statistical significant for (P value ≤ 0.05).

Results

The finding of the 400 mothers according to the feeding type in the first six months of life reported: 219 had EBF at percentage (54.75%) while 95 (23.75%) and 86 (21.5%) had partial and exclusive formula feeding respectively as shown in table (1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>No</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Exclusive breastfeeding</td>
<td>219</td>
</tr>
<tr>
<td>2</td>
<td>Partial breastfeeding</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>Exclusive formula feeding</td>
<td>86</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>400</td>
</tr>
</tbody>
</table>

The result in table(2) shows that there was statistically significant relationship between (mother’s age at baby’s birth, mother’s level of education, occupation of mother’s parity, mode of delivery ,gestational age) and type of feeding in the first six months of life in Basra city at P-Value(0.000, 0.000, 0.020, 0.001, 0.027, 0.050) respectively.
### Table (2) The Relation between socio-demographic for mothers and type of feeding:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Type of Feeding in the first six months of life</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EBF</td>
<td>PBF</td>
</tr>
<tr>
<td>Mother’s age at marriage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;=15 years</td>
<td>59</td>
<td>62.8</td>
</tr>
<tr>
<td>16-25 years</td>
<td>140</td>
<td>52.8</td>
</tr>
<tr>
<td>26-35 years</td>
<td>17</td>
<td>50</td>
</tr>
<tr>
<td>more than 35 years</td>
<td>3</td>
<td>42.9</td>
</tr>
<tr>
<td>Mother’s age at birth of baby</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;=15 years</td>
<td>9</td>
<td>32.1</td>
</tr>
<tr>
<td>16-25 years</td>
<td>129</td>
<td>66.8</td>
</tr>
<tr>
<td>26-35 years</td>
<td>54</td>
<td>43.2</td>
</tr>
<tr>
<td>more than 35 years</td>
<td>27</td>
<td>50</td>
</tr>
<tr>
<td>level of education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not educated</td>
<td>50</td>
<td>54.9</td>
</tr>
<tr>
<td>Primary</td>
<td>57</td>
<td>49.1</td>
</tr>
<tr>
<td>Secondary</td>
<td>88</td>
<td>71.5</td>
</tr>
<tr>
<td>High education</td>
<td>24</td>
<td>34.3</td>
</tr>
<tr>
<td>occupation of mothers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>housewife</td>
<td>200</td>
<td>58</td>
</tr>
<tr>
<td>Employed</td>
<td>14</td>
<td>37.8</td>
</tr>
<tr>
<td>Student</td>
<td>5</td>
<td>27.8</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primipara</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Multipara</td>
<td>180</td>
<td>60</td>
</tr>
<tr>
<td>Birth interval</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (2) The Relation between socio-demographic for mothers and type of feeding:

<table>
<thead>
<tr>
<th>Type of baby birth</th>
<th>&lt;=1year</th>
<th>2-3 years</th>
<th>more than 3 years</th>
<th>Correlation</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (spontaneously)</td>
<td>159</td>
<td>59.3</td>
<td>59</td>
<td>22</td>
<td>50</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>60</td>
<td>45.5</td>
<td>36</td>
<td>27.3</td>
<td>36</td>
</tr>
</tbody>
</table>

Gestational age

| Gestational age | Preterm | Full term | | Correlation | P-Value |
|-----------------|---------|-----------| | 6.006 | 0.050 |

The result showed significant relationship between diarrhea disease, respiratory tract infection and gastroenteritis and type of feeding in first six months of life at P-Value (0.036, 0.049, and 0.026) respectively as show in table (3).

Table (3) Relationship between morbidity of infant in the first six months of life and type of feeding:

<table>
<thead>
<tr>
<th>Type of disease</th>
<th>Type of Feeding in the first six months of life</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EBF No.</td>
<td>EBF %</td>
</tr>
<tr>
<td>Diarrhea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>22.5</td>
</tr>
<tr>
<td>No</td>
<td>40</td>
<td>40.8</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>48</td>
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</table>

**Discussion**

The finding of the 400 mothers according to the feeding type in the first six months of life reported: 219 (54.75%) had EBF while 95 (23.75%) and 86 (21.5%) had partial and exclusive formula feeding respectively as shown in table (1).

This result nearly matches another’s cross sectional study was conducted between October 2006 and November 2007 in four primary health care centers in Thi – Qar, showed EBF (55.1%) (12). This increasing in EBF beyond to that mothers were educated on the advantages, practices, length, and exclusivity of BF, also societal beliefs and traditional attitudes play a significant role in society; this may justify the second and third most prevalent reasons (13).

Poverty is a significant factor in rural women’s decision to breastfeed their children (14). Furthermore, maternal education, social status, cultural background, and religion are associated with the decision to begin and maintain BF (15).

The result of study showed that mother’s age at baby’s birth was for the age interval (16-25) years (66.8%), with secondary education level (71.5%), (58%) house wives mothers, (60%) was for multipara mothers, (59.3%) delivered vaginally mothers, (56%) was for mothers of full term infant all these socio-demographic characters for mothers had statistically significant and highest percentage in using EBF to breastfeed their infant.

This study agreement with study showed the risk of cessation of breastfeeding increased with increasing maternal education (16). Also this result agree with study done by Al-Kohji et al. showed that exclusive breastfeeding is more common among housewives than among employed mothers, owing to the fact that unemployed mothers have more time to practice on-demand breastfeeding, as the WHO recommends (17).

The study agree with several study like this done by Al-Nuaimee et al. showed multipara women more than primipara using EBF may be due to psychological pressure of the pregnancy on these women who didn’t have experience and visit the health center more frequently (18), corresponding with the study by Al-Kohji.
et al showed many factors explains a higher incidence of breastfeeding initiation in mothers who delivered vaginally than those who delivered via cesarean section are: discomfort of surgery, the baby isolation from the mother, and the mothers’ failure to sit in the appropriate position for breastfeeding were all factors in mothers who delivered cesarean section (17).

The result showed significant relationship between diarrhea disease, RTI and gastroenteritis and type of feeding in first six months of life at level p<=0.05. Also another’s study showed same effect 14 (22%) of breast-fed infants developed diarrhea, compared to 29 (42%) of bottle-fed infants and 25 (36%) of combined breast-and-bottle-feeding infants (19). this due to human milk have antiviral, antibacterial, and antiprotozoal properties have been found in fatty acids and monoglycerides, this operation could strengthen the stomach’s ability to serve as a protector against ingested pathogens (20).

This result same result of another’s study showed infant of 6 months of exclusive breastfeeding is often associated with lower rates of gastro-intestinal tract infections (21). Antibodies pass on maternal immunity to the fetus. Both transplacentally transferred maternal IgG and breast milk IgA can protect the infant from infections by neutralizing microbe infectivity, Breast milk also contains other antimicrobials. Substances that could also play a role Breast milk reduces the morbidity of gastroenteritis (22).

Our result agree with study conducted in Erbil city showed that 69% of bottle-fed infants developed lower respiratory tract infections compared with 31% infants of breast-fed (23). Breast milk appears to influence the infant’s systemic immune response through a variety of mechanisms, including maturational, antiinflammatory, immunomodulatory, and antimicrobial, many changes in immune phenotype of newborns following exposure to maternal milk, such as rises in post vaccination interferon-Y levels and natural killer cell numbers, which are associated with age-related changes and indicate increased immune maturation, these immune effects that may result in extended defenses against respiratory infections. (24).

Conclusion

Prevalence of EBF among mothers who ever breastfed was 54.75%. Most mothers in Basra exclusively breastfeed their babies in the first 6 months means positive trend for the rate of EBF in Basra / Iraq. Breastfeeding exclusively until the age of six months, was associated with a significantly decrease in respiratory and diarrhea and gastrointestinal infectious diseases.

Ethical issue: Gaining formal approval from ministry of higher education then public health department in Basra province to conduct this study, also permission was obtained from each Primary health care centers. All data was obtained from mothers who agreed to take part in this study .this participation was voluntary and anonymous.

Conflict of Interest: Nil

Source of Funding: Self-financing

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Food Hygiene Knowledge and Practice among South Indian Women

Mahadeva1, Manasa P1, Melby Elizabeth1, Nancy Ruby1, Priya Joy1, Hezil Reema Barboza2

1Student, 2Lecturer, Department of Medical Surgical Nursing, Yenepoya Nursing College, Yenepoya Deemed to be University, Mangalure

Abstract

Background: Food is essential for the existence of all living organisms. The main purpose of food hygiene is to prepare and provide safe hygienic food and consequently contribute to a healthy family. Lack of adequate food hygiene can lead to a number of food-borne diseases and death.

Methods: A descriptive study design was adopted in order to assess the level of knowledge and practice among women regarding food hygiene. The tool used for this study was structured knowledge questionnaire and self-reported practice checklist. The content validity of the tool was established in consultation with 7 experts. The reliability of the knowledge questionnaire was tested by split-half method and self-reported practice checklist by Cronbach’s Alpha. The reliability of the knowledge questionnaire was 0.8 and the practice checklist was 0.9. Both the tool was found statistically significant. Data collected from the 132 women were analyzed by descriptive and inferential statistics using SPSS.

Conclusion: The results revealed that majority of the women (80.4%) belong to age group of 20-30 years. There was a positive correlation found between food hygiene knowledge and practice. Majority of the women had good knowledge (77.3%) and had good practice (70.3%) towards food hygiene.

Key words: Foodborne diseases, Food hygiene, Knowledge, Practice.

Introduction

Food poisoning is a common health problem worldwide. All over the world people are seriously affected every day by diseases that are caused by consuming unhygienic and unsafe food.1 Initially food-borne diseases were estimated to be responsible for 6-8 million illnesses and 9000 deaths each year. The US Centers for Disease Control and Prevention (CDC) estimates 1 in 6 Americans (48 million people) are affected by food-borne illness annually. The 31 known pathogens account for an estimated 9.4 million annual cases, 55,961 hospitalizations, and 1,351 deaths.2-4 In 2011 about 100 million cases of food borne diseases reported in India. Researchers found that number of cases expected to rise 150-177 million in 2030.5,6 The data from the World Health Organization food borne disease epidemiology reference group in 2015 reported urban and rural population will be affected disproportionally, where every third person fall sick from food borne diseases.7 Ensuring food safety at the household level is important and an understanding of the status of the food handling knowledge and practices is very much needed to prevent the food borne diseases.8-10

Objectives

The objectives of the study were to:-
to assess the knowledge on food hygiene among women

• to determine the food hygiene practice among women

• to find correlation between knowledge and practice score on food hygiene among women

• to find association between knowledge score with the selected demographic variables

• to find association between practice score with the selected demographic variables

Materials and Methods

The aim of this study is to understand the status of knowledge and practice among South Indian women regarding food hygiene. In this study food hygiene is referred to as all the conditions and measures that are necessary during the purchase, storage, preparation and serving of food to ensure that it is safe, wholesome and fit for human consumption.

This study assumes that safe food hygienic practices can reduce the incidence of food borne diseases. When individuals adopt better practices that promote health, food contamination can be minimized.

The descriptive design was selected for the study. The data was collected from women (n=132) who visited the tertiary care hospital OPD at Mangalure. Subjects were selected by non-probability convenient sampling technique. Women between 20-50 years of age and who were involved in the food preparation, preservation, storage and serving were selected as study participants.

Description of the tool:

As per the review of literature the following tools were constructed by the researchers.

(a) Demographic Proforma

(b) Structured Knowledge Questionnaire

(C) Self-Reported Practice Checklist

Demographic Proforma: It consisted of items for obtaining information regarding age, marital status, occupation, educational status, type of family and religion.

The Structured Knowledge Questionnaire on Food Hygiene: It consisted of 20 Multiple Choice Questions (MCQ’s) with one correct answer and four distracters. Each right answer carries one score and the total score of the questionnaire was 20.

Self Reported Practice Checklist: It was prepared to assess the practice of food hygiene with 15 questions. The total score of the Self reported practice checklist was 15.

To determine the content validity, the prepared tools were given to 7 experts. For establishing the reliability the tools were administered to 6 subjects who met the inclusion criteria. The reliability was calculated by split-half method for structured questionnaire and test-re-test method for self reported practice checklist. The reliability of the Structured Knowledge Questionnaire on Food Hygiene was 0.8 and Self-Reported Practice Checklist was 0.9. Both the tools were found to be reliable.

Data collection method:

A formal permission was obtained from Scientific Review Board and Institutional Ethics Committee before the data collection process. A pilot study was conducted for a smaller sample size to assess the feasibility of the study.

The Structured Knowledge Questionnaire and Self-Reported Practice checklist was administered to women (n=132) through Google Forms. The data obtained was analyzed in terms of objectives of the study using descriptive and inferential statistics. The collected data was coded and transformed to master sheet. Demographic data was computed using frequency and percentage. Mean, median, mean percentage, and standard deviation for knowledge and practice score was computed. Karl Pearson’s Correlation Co-efficient
was used to find the correlation between knowledge and practice score. Chi-square test was used for finding association between knowledge and practice score with selected demographic variables.

**Results and Discussion**

Statistical Package for the Social Sciences (SPSS) version 24 was used to analyze the data. Majority of study participants were between 20-30 years of age and also many of them were graduates. The results of other studies highlight the importance of education and educational programs to improve food safety knowledge and practices among mothers.\textsuperscript{11,12} World Health Organization (WHO) also suggests that advance education is comprehensively required to reduce the food-borne illnesses globally.\textsuperscript{13}

| Table 1: Level of knowledge and practice regarding food hygiene among women |
|---|---|---|---|---|
| Variable | Grade | Score | Frequency (f) | Percentage (%) |
| Knowledge | Poor | < 6 | 18 | 13.6 |
| | Good | 7-13 | 102 | 77.3 |
| | Very Good | 14-20 | 12 | 9.1 |
| Practice | Poor | 1-6 | 10 | 7.0 |
| | Average | 7-10 | 29 | 21.5 |
| | Good | 11-15 | 93 | 71.5 |

Table 1 depicts 77.3\% were had good knowledge on food hygiene. The mean knowledge score of women was 9.74±2.81. In other studies conducted in Nigeria and Sri Lanka showed good level of knowledge among study participants.\textsuperscript{14,15} In a community based study conducted in Ethiopia, almost 72\% of food handlers in food establishments had a good level of food hygiene knowledge.\textsuperscript{16}

In the present study practice of food hygiene among women was good which was assessed by self reported practice scale as depicted in the table 1. The mean practice score of women regarding food hygiene was 2.70±.475. In another study conducted in Saudi Arabia showed that food handlers had excellent practices towards food and personal hygiene. In a cross sectional study conducted among the street vendors in Kuching City revealed food safety knowledge, attitude, training and age of the food vendors influence the food safety practice.\textsuperscript{17,18}

**Correlation between food hygiene knowledge and practice score**

The hypothesis will be tested at 0.05 level of significance

\[ H_1: \text{There will be a significant correlation between knowledge and practice score on food hygiene} \]
Table 2: Correlation between food hygiene knowledge and practice score

<table>
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<th>Standard deviation</th>
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<th>Remarks</th>
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<td>Knowledge</td>
<td>9.74</td>
<td>2.82</td>
<td>0.043</td>
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<tr>
<td>Practice</td>
<td>2.70</td>
<td>.475</td>
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<td></td>
</tr>
</tbody>
</table>

Table 2 shows that there was a positive correlation between food hygiene knowledge and practice among women. Hence H₁ is accepted. In another study conducted on food safety among women of Khaza bazar, Karnataka depicted a positive correlation between knowledge and practice. In a survey conducted in Poland and Thailand also showed correlation between food hygiene knowledge and practice.

Association between the knowledge score and selected socio demographic variables:

H₂: There will be a significant association between knowledge score with selected demographic variables

Table 3: Association between the knowledge score and selected socio demographic variables

<table>
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<th>Sl No</th>
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<th>median &gt;10</th>
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<td>Post graduation</td>
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<td>Widower</td>
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</table>
There was a significant association between knowledge score and selected demographic variables such as marital status ($x^2 = .002$) and religion ($x^2 = .002$). Similar result was found in another study conducted in Ghana showed the knowledge on food safety was associated with marital status ($p$ value $<0.001$), and religion ($p$ value $\leq 0.038$).

**Association between the practice score and selected socio demographic variables:**

H$_3$: There will be a significant association between practice score with selected demographic variables

**Table No 4: Association between the practice score and selected socio demographic variables**

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<td>Post graduation</td>
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</table>
The findings of the study revealed that there was no significant association between practice score and demographic variables. Hence $H_3$ is rejected.

**Limitations of the study:**

The study participants were finding it difficult to recall the information regarding the practice of food hygiene. Direct observation of food hygiene practice by the researcher is one of the limitations which influenced the study results. No other record was available to verify the practice responses given by the participants. It was a cross-sectional study, limited to smaller a small sample size; hence results cannot be generalized to the entire population.

**Conclusion**

The practice of food hygiene needs to be based on scientific knowledge. Every woman involved in the preparation, storage and serving of food to the family has to gather information regarding new trends, changes and myths about food hygiene. Research helps in carrying out this in a better way. This can possible through mass media. Communication through media can play a very important role in giving information to the women with simple messages in newspapers, pamphlets, magazines and television in local language which help in dispelling the myths and propagating the concepts and importance of food hygiene.

**Ethical Clearance:** Ethical clearance was obtained from Institutional Ethics Committee (Protocol No:
Source of Funding: Self funding

Conflict of Interest: Nil

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How Can the Maternal Age and the Basal Level of Serum FSH Predict the ICSI Outcome?

Maher Abbood Mukheef1, Rihab Abbas Ali2, Hind Hadi Majeed3

1Lecturer at University of Karbala/ College of Medicine/ Department of Biochemistry, Iraq, 2Assistant Lecturer at University of Karbala/ College of Medicine/ Department of Anatomy, Histology And Embryology, Iraq, 3Assistant Lecturer at Jabir IBN Hayyan Medical University/College Of Medicine/ Department of Human Anatomy

Abstract

Background: Age is an important factor affect female fertility, it is important to consider it while planning for intracytoplasmic sperm injection (ICSI). Basal follicular stimulating hormone(FSH) level can be an indicator of the ovarian reserve as the high level is associate with poor ovarian reserve and subsequently poor intracytoplasmic sperm injection outcome.

Objective: we aimed in this study to emphasize the role of age and basal serum follicular stimulating hormone level in predicting the intracytoplasmic sperm injection outcome.

Patients and Method: a sixty sub-fertile couple were involved in this study. They were divided into two groups according to the age of female partner. Group 1 ≤35 years old and group 2 >35 years old. We compared the difference in the basal follicular stimulating hormone level and the intracytoplasmic sperm injection outcome. Then we studied the association between the basal follicular stimulating hormone level and the intracytoplasmic sperm injection outcome.

Results: the study revealed that female ≤35 resulted in significantly better the intracytoplasmic sperm injection outcome. The no. of follicles, the no. of collected oocytes, no. of injected metaphase II oocytes (MII), no. of two pronuclei (2PN), total no. of embryos and no. of transferred embryos all were significantly higher in younger patients (P-value <0.05. The pregnancy rate was significantly higher in younger age group (47.5 % vs 25%, P-value = 0.04). There was a negative association between the basal follicular stimulating hormone level and the no. of follicles and no. of oocytes.

Conclusion: Maternal age is a good indicator for the success of the intracytoplasmic sperm injection procedure. The younger the female partner the better the intracytoplasmic sperm injection outcome. Basal level of follicular stimulating hormone is closely related to the ovarian reserve and subsequently the intracytoplasmic sperm injection outcome.

Key words: maternal age, basal FSH, ICSI.

Introduction

The assisted reproductive techniques involve both intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF). In 1992 the Intracytoplasmic sperm injection (ICSI) was introduced to treat subfertility in couples due to severe male factor to allow fertilization and subsequent pregnancy whatever the semen characters were (1,2). However, (IVF) or (ICSI) was also helpful to patients with tubal factor subfertility, unexplained subfertility as well as some cases of polycystic ovarian syndrome (3).
The age of female partner in assisted reproductive techniques (ART) cycles is an important influencing factor, the association between the advanced maternal age and the poor ART result is well known (4). The diminishing ovarian reserve together with the decreased endometrial receptivity caused by aging are the possible explanation for this fertility reduction (5), this is beside increasing the follicular disappearance starting after the 37 years old (6). It is important to mention that advanced age is significant cause of increasing aneuploidy and spontaneous abortion rates (1). Different responses to the controlled ovarian stimulation were obtained from women in the same age. Moreover, diminished ovarian reserve and poor IVF outcome have been noticed in women under the age of 35 years old (7).

Basal level (cycle day 2 or 3) of serum follicular stimulating hormone (FSH) has been studied as additional factor predicting the ICSI outcome (8). Elevated basal level of serum FSH in sub-fertile women was associated with poor responses to the ovarian stimulation and subsequently lowering the pregnancy rate regardless the age (9). Conversely other authors, mentioned that basal serum FSH level could be unreliable predictor for reproductive potential in older group of women as measuring FSH concentration represent an indirect ovarian reserve assessment (10, 11).

Basal FSH level and maternal age are associated with the ART outcome independently. They are related to the ovarian reserve phenomenon, which reflect the quality and quantity of remaining follicles in the ovary (12).

In this study we aimed to estimate the role of maternal age and basal FSH level in predicting the ICSI outcome.

Patients and Method

This is a retrospective cohort study was done in the sub-fertility center of AL-Sader Medical city in Iraq- Al Najaf governorate. It involved retrieving data of sub-fertile couples who were referred to the center by their gynecologist for ICSI procedure during the period from October 2017 to May 2019. The study involved randomly collected 60 couples with different causes of sub-fertility (male factor n= 36, tubal factor n= 4, unexplained factor n=10 and anovulatory cause n= 10). For the female patients age, type of infertility (primary infertility: the patient had never get pregnant previously or secondary infertility: the patient had get pregnant at least once regardless the fate of the pregnancy) and duration of infertility were recorded. The female age ranged from 21 years old till 44 years. The female gynecological, medical and surgical history were taken into account. On the menstrual cycle day 2 (CD2) baseline transvaginal ultrasound (TVUS) was done to assess uterus, endometrium and ovarian condition. Blood sample was collected in the second day of the menstrual cycle for the measurement of basal level of serum FSH, serum estradiol (E2) and serum leulnizing hormone (LH) level.

Male partners were evaluated by seminal fluid analysis; biopsy was done for azoospermic patients. Sever endometriosis, anatomical uterine abnormalities, female age > 44, cancelled cycles and male partner with testicular atrophy were excluded. The whole study was clarified to the patients and an informed verbal consents were taken. The patients were grouped according to the maternal age into two groups: Group1≤35 and Group2 > 35. The ICSI outcome was compared between the two groups. CD2 serum FSH level was measured and the correlation between it and the ICSI outcome and patients age were analyzed.

Ovarian Stimulation, Oocytes Pick up and ICSI: The patients were involved in controlled ovarian hyperstimulation (COH) programs using recombinant follicular stimulating hormone +/- human menopausal gonadotropin (HMG) with pituitary desensitization by agonist /antagonist protocols which were chosen according to the patient’s age, body mass index (BMI) and cause of subfertility. Follicular maturation was followed by U/S and serial measurement of serum E2. Ova pick up was performed under general anesthesia guided by transvaginal ultrasound 36 hour after ovulation trigger with 0.1 mg decapetyl (gonadotropin releasing hormone
GnRH agonist) or 10000 IU of pregnyl (the human chorionic gonadotropin Hcg). In the ICSI procedure the good quality metaphase II (MII) oocytes were injected and incubated in special media. The fertilization was assessed 16_18 hr. after injection by identifying the two pronuclei(2PN). On the day 2 or 3 post injection the top quality embryos were transferred, not more than 3 embryos. The number and quality of picked up oocytes and the ICSI outcome in form of number of 2PN, number and quality of embryos, number of transferred embryos, fertilization rate, cleavage rate and pregnancy rate all recorded and statistically explained.

Statistical Analysis
The data analysis was done using Microsoft Office Excel 2016 and the SPSS 21 program (Statistical Package for social sciences). The categorical data were represented as number and percentage while numeric data were represented as mean ±SE. The difference between two numeric variables was studied by independent sample t-test and the association of categorical data analyzed by Chi-Square test. Pearson’s correlation test shows the correlation between variables. The significant P value is < 0.05.

Results

Table 1: Demographic data of the patients.

<table>
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<th>Variables</th>
<th>Value</th>
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</tr>
<tr>
<td>Age in years</td>
<td>31.3 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>6.1042</td>
</tr>
<tr>
<td>Type of sub-fertility</td>
<td></td>
</tr>
<tr>
<td>Primary n (%)</td>
<td>45 (75%)</td>
</tr>
<tr>
<td>Secondary n (%)</td>
<td>15 (25%)</td>
</tr>
<tr>
<td>Duration of sub-fertility in years</td>
<td>8.26± 0.26</td>
</tr>
<tr>
<td>Cause of subfertility</td>
<td></td>
</tr>
<tr>
<td>Male factor (%)</td>
<td>36 (60%)</td>
</tr>
<tr>
<td>Unexplained (%)</td>
<td>10 (16.7%)</td>
</tr>
<tr>
<td>Tubal (%)</td>
<td>4 (6.7%)</td>
</tr>
<tr>
<td>Anovulatary (%)</td>
<td>10 (16.7%)</td>
</tr>
<tr>
<td>Type of protocol</td>
<td></td>
</tr>
<tr>
<td>Agonist (%)</td>
<td>28 (46.7%)</td>
</tr>
<tr>
<td>Antagonist (%)</td>
<td>32 (53.3%)</td>
</tr>
<tr>
<td>Basal FSH (mlu/ml)</td>
<td>6.22± 0.36</td>
</tr>
<tr>
<td>Basal LH (mlu/ml)</td>
<td>4.2± 0.31</td>
</tr>
<tr>
<td>Basal E2 (pg/ml)</td>
<td>38.89± 2.3</td>
</tr>
</tbody>
</table>
Table 2: Comparing the day 2 serum FSH and ICSI outcome between the patients according to the age.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age ≤ 35 (N=40)</th>
<th>Age &gt; 35 (N=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mlu/ml)</td>
<td>5.79 ± 0.37</td>
<td>7.07 ± 0.37</td>
<td>0.09</td>
</tr>
<tr>
<td>No. of preovulatory follicles</td>
<td>12.97 ± 0.91</td>
<td>7.9 ± 1.26</td>
<td>0.002</td>
</tr>
<tr>
<td>No. of collected oocytes</td>
<td>10.9 ± 1.26</td>
<td>7.500 ± 0.90</td>
<td>0.033</td>
</tr>
<tr>
<td>No. of injected MII</td>
<td>9.27 ± 0.88</td>
<td>6.200 ± 1.21</td>
<td>0.048</td>
</tr>
<tr>
<td>No. of 2PN</td>
<td>7.02 ± 0.58</td>
<td>3.800 ± 0.80</td>
<td>0.002</td>
</tr>
<tr>
<td>Total no. of embryos</td>
<td>6.55 ± 0.5546</td>
<td>3.7 ± 0.78</td>
<td>0.04</td>
</tr>
<tr>
<td>Grade I embryos</td>
<td>1.8 ± 0.33</td>
<td>1.1 ± 0.31</td>
<td>0.12</td>
</tr>
<tr>
<td>Grade II embryos</td>
<td>3.61 ± 0.46</td>
<td>1.7 ± 0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Low quality embryos</td>
<td>0.74 ± 0.18</td>
<td>0.9 ± 0.49</td>
<td>0.7</td>
</tr>
<tr>
<td>Fertilization rate %</td>
<td>75.6 ± 3.99</td>
<td>65.30 ± 7.64</td>
<td>0.5</td>
</tr>
<tr>
<td>cleavage rate %</td>
<td>92 ± 2.88</td>
<td>88.12 ± 6.87</td>
<td>0.1</td>
</tr>
<tr>
<td>No. of transferred embryos</td>
<td>2.71 ± 0.1</td>
<td>1.9 ± 0.25</td>
<td>0.02</td>
</tr>
<tr>
<td>Pregnancy rate %</td>
<td>47.5 % (19/40)</td>
<td>25% (5/20)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table 2 shows that when we divided the patients according to the age, the group whom age was younger than 35 years old had lower CD2 serum FSH and better ICSI outcome. The no. of follicles, the no. of collected oocytes, no. of injected MII, no. of 2PN, total no. of embryos, no. of transferred embryos and pregnancy rate all were significantly higher in patients group younger than 35 years old.

Table 3: Correlation between basal FSH with patients age and ICSI outcome.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation coefficient</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.18</td>
<td>0.14</td>
</tr>
<tr>
<td>No. of preovulatory follicles</td>
<td>-0.33*</td>
<td>0.01</td>
</tr>
<tr>
<td>No. of collected oocytes</td>
<td>-0.26*</td>
<td>0.04</td>
</tr>
<tr>
<td>No. of injected MII</td>
<td>-0.21</td>
<td>0.1</td>
</tr>
<tr>
<td>No. of 2PN</td>
<td>-0.21</td>
<td>0.1</td>
</tr>
<tr>
<td>Total no. of embryos</td>
<td>-0.19</td>
<td>0.1</td>
</tr>
<tr>
<td>Grade I embryos</td>
<td>0.02</td>
<td>0.8</td>
</tr>
<tr>
<td>Grade II embryos</td>
<td>-0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Table 3 show the correlation between the CD2 serum FSH and the ICSI outcome. The CD2 serum FSH correlate negatively with no. of follicle and no. of collected oocytes.

**Discussion**

Female age and basal FSH level are independently associated with IVF outcome (7). The increased maternal age and its effect on the reproduction is a subject of interest because it affects negatively oocytes and embryos quality and pregnancy rate (13,14). In addition to that the rates of obstetrical and maternal complication such as maternal death, prematurity, fetal and early neonatal death, and caesarian section deliveries (15).

The elevated basal FSH in many women discourage the option of ICSI/IVF as treatment option for them. This is due to that the high basal FSH is associated with lower assisted reproduction treatment results (16). The age and elevated serum level of basal FSH in women are used as predictive criteria associated with diminished fertility (17). In this study we re-evaluated the association between the ICSI outcome and the maternal age and basal FSH level.

The study showed significantly better ICSI outcome in women younger than 35 years old. This is probably due to the better oocytes quality and better ovarian reserve in young women. The no. of follicles, no. of oocytes, the no. of injected oocytes, no. of 2PN (fertilized oocytes), no. of embryos and pregnancy rates were all significantly higher in younger group of patients. Many studies were in agree with this results (12,15, 18, 19,20, 21,22). On the other hand, the level of basal serum FSH was higher in older women group but still the difference was not significant may be due to the minimum differences in the age of the individuals between both groups. The higher level of FSH in older women is indicative of decreased ovarian reserve. A study reported that there were no significant differences in level of FSH with age (23). When we studied the association of the basal serum FSH level and the ICSI outcome, there was significant negative correlation between the FSH level and the no. of follicles and the no. of collected oocytes. This is because the FSH secretion from the pituitary gland increases with diminished ovarian reserve and women with high basal FSH frequently have lower no. of collected oocytes. There were studies which were in agreement with our finding in that the elevated FSH level is associated with poor ICSI outcome (7,12,24).

The limitation for this study

Conclusion: the maternal age is a cornerstone factor affecting the outcome of ICSI. The basal FSH is a good indicator of the ovarian reserve. Both of age and basal FSH are a good predictor for the success of the ICSI procedure.

Conflict of Interest: Non.

Source of Funding: Self-Funding.

Ethical Clearance: We obtained the ethical approval for our study from the institutional ethical committee. The patients were voluntarily participated in the study after they understood the aim of the research and verbal consents were taken from them. The participants were awarded that their information would remain strictly
confidential and would be used for research purpose only.

References


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A Cross Sectional Study of Deaths Due to Snakebite: Autopsied at a Tertiary Care Centre, Hubballi

Mahesh M Devadas¹, Vijay N Tasgaonkar², Suresh Kumar Karthikean³, Anand Patil⁴

¹Assistant Professor, Department of Forensic Medicine & Toxicology, KJSMC, SION, Mumbai, ²Associate Professor, Department of Forensic Medicine & Toxicology, BKLWMC&H, Sawarda, ³Assistant Professor, Department of Forensic Medicine & Toxicology, ACS Medical College and Hospital, Dr MGR Educational and Research Institute, Chennai, ⁴Assistant Professor, Department of Forensic Medicine & Toxicology, GIMS, Gadag

Abstract

Worldwide, Snakebite is an important and preventable health hazard in many of the tropical and subtropical countries. Death following snake bite is a cumulative effect of all the toxic reactions that are produced by the snake venom in the body of the victim. Globally it is estimated that the true incidence of snake envenomation could exceed 3 million per year and about 1,00,000 of these develop severe sequelae. With this background, a study has been conducted to determine the pattern of snakebite death cases, autopsied at KIMS, Hubballi, Karnataka. The study revealed that maximum number of snakebite cases were recorded in the rainy season (34.9%) followed by the winter season (31.7%). In a greater number of cases the incidence of snake bite occurred predominantly in lower limb (54%) as compared to upper limb (46%). Peak incidence of snake bite was recorded in the time between 08:00 am to 04:00 pm (41.3%) followed by 04:00 pm to midnight (30.2%). Majority of the cases were declared dead (47.61%) within 24 hours of initiation of anti-snake venom. The cause of death in majority of the study population was respiratory failure (92.06%) followed by shock (7.93%).

Key words: Snakebite, Death, Autopsy, Survival period and Cause of death.

Introduction

The word ‘Snake’ is derived from the Anglo-Saxon word ‘Snaca’ meaning the creeping. Nearly 240 million years ago the creeping animals were seen and were called as ‘reptiles’. They became enormous in size and predominated till the time mammals were about to be born. Evolution of reptile’s dates back to 240 million years during the cretaceous period, crocodiles, turtles, lizards and snakes all of them belong to reptiles, once inhabited the earth surface in enormous numbers. Origin and evolution of peculiar structure of snakes is not clearly known, so many hypotheses have been formed to explain it. There are more than 3500 species of snakes in the world and about 216 species are found in India, out of which only about 52 are poisonous. Snake bite remains a major public health problem in most of the countries even though it is difficult to be precise about the actual numbers involved. Various estimates have shown almost the same statistics that 15,000 to 25,000 people die annually in India due to snake bite envenomation. Maharashtra state with highest incidence of snakebite reported, 70 bites per 1,00,000 population and mortality of 2.4 per 1,00,000 per year. With this background present study has been carried out at KIMS, Hubballi, Karnataka to find out the most common site of snakebite in humans, most common season of the year, where maximum number of snakebites are occurred, most common time of snakebite in a day, most common cause of death and survival period of a snakebite victim after initiation of anti-snake venom.

Materials and Methods

This study has been carried out at the department of Forensic Medicine and Toxicology, KIMS, Hubballi, Karnataka, by taking all the cases brought for medico
legal autopsy with the history of snakebite and cases that were diagnosed as snakebite injury case after post mortem examination during the period of two and half years, from July 2016 to December 2018. Total 63 cases were selected for this prospective study. In all the cases of snakebite, the detailed history and information were collected from the police and the relatives of the deceased with the help of questionnaire, and post mortem findings were analyzed. In case of hospital admitted and treated cases the information’s were collected by the perusal of hospital records. The cases with the history of scorpion bites, bee sting and any other insect bite envenomation were excluded from the study group. Meticulous autopsy was done in all cases.

The present study has been carried out after obtaining the ethical clearance and consent from the relatives to take the relevant information.

## Results

Total 63 cases were selected for the present study and the following observations were made. The study revealed that Maximum incidence of snakebite i.e. 22(34.9%) were in third quarter (rainy season) July to September month, followed by in fourth quarter (winter season) October to December 20(31.7%) and 13(20.6%) in second quarter. Least incidence was seen in between January and March.

### Table No 1: Distribution of Seasonal Variation in snake bite.

<table>
<thead>
<tr>
<th>Season</th>
<th>Number of cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan – Mar</td>
<td>8</td>
<td>12.7</td>
</tr>
<tr>
<td>Apr – June</td>
<td>13</td>
<td>20.6</td>
</tr>
<tr>
<td>July – Sept</td>
<td>22</td>
<td>34.9</td>
</tr>
<tr>
<td>Oct – Dec</td>
<td>20</td>
<td>31.7</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>100.0</td>
</tr>
</tbody>
</table>

In this present study population, it also observed that incidence of snake bite occurred predominantly in lower limb i.e. 34(54%) as compared to upper limb i.e. 29 (46%).

### Table No 2: Site of bite mark distribution of snake bite.

<table>
<thead>
<tr>
<th>Site</th>
<th>Number of cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper Limb</td>
<td>29</td>
<td>46.0</td>
</tr>
<tr>
<td>Lower limb</td>
<td>34</td>
<td>54.0</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>100.0</td>
</tr>
</tbody>
</table>

From the above study it is observed that maximum incidents of snakebite poisoning occurred between 8:00am to 4:00pm i.e. 26 cases (41.3%) as in this period, people commonly will be working in outdoor field, followed by evening time 4:00pm to midnight i.e. 19 cases (30.2%) and least incidence was seen from midnight to morning 8:00am.
Table No 3: Time of snake bite wise distribution of snake bite.

<table>
<thead>
<tr>
<th>Time in 24 Hrs</th>
<th>Number of cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00:00 to 07:59</td>
<td>18</td>
<td>28.6</td>
</tr>
<tr>
<td>08:00 to 15:59</td>
<td>26</td>
<td>41.3</td>
</tr>
<tr>
<td>16:00 to 24:00</td>
<td>19</td>
<td>30.2</td>
</tr>
<tr>
<td>24 Hours</td>
<td>63</td>
<td>100.0</td>
</tr>
</tbody>
</table>

From the above study it is observed that among total 63 cases of study population, 42 cases were admitted in hospital after a snakebite, and were managed with anti-snake venom initiative and 21 cases were brought dead. Among those snake bite admitted cases maximum number i.e. 20(47.61%) were declared dead within 24 hours of initiation of anti-snake venom.

Table No 4: Distribution of survival period after initiation of anti-snake venom.

<table>
<thead>
<tr>
<th>Duration of survival after initiation of ASV</th>
<th>Number of cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 24 Hrs</td>
<td>20</td>
<td>47.61</td>
</tr>
<tr>
<td>1 – 3 days</td>
<td>10</td>
<td>23.81</td>
</tr>
<tr>
<td>More than 4 days</td>
<td>12</td>
<td>28.57</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>100</td>
</tr>
</tbody>
</table>

In this present study population, it also observed that in maximum number of snakebite poisoning cases the cause of death is respiratory failure i.e. 58 cases (92.06%) followed by shock i.e. 5 cases (7.93%).

Table No 5: Cause of Death wise distribution of Cases.

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Number of Cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shock</td>
<td>5</td>
<td>7.93</td>
</tr>
<tr>
<td>Respiratory failure</td>
<td>58</td>
<td>92.06</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>100</td>
</tr>
</tbody>
</table>

Discussion

The objectives of the present study are to find out the common site of snakebite in humans, most common season of the year; where maximum number of snakebites are occurred, most common time of snakebite in a day, most common cause of death in snakebite poisoning and survival period of a snakebite victim after initiation of
anti-snake venom.

Our present study has showed the highest similarities with all the parameters mentioned above, when compared with other similar studies done in the past.

In our present study majority of snakebite cases 22(34.9%) occurred during month of July to September. Similar findings were observed with the study conducted by Sharma et al7, where in maximum cases of snake bite were seen in July to October i.e. 54%. This study proves the fact that there is a definite seasonal pattern in cases of snakebites. This is due to the fact that this is the monsoon season and rainfall forces snakes to venture out of their water-filled pits and also there will be increased human activity in fields at this period as it is the sowing season. A similar trend was observed in studies conducted by Viramani SK et al8 and Sani UM et al9.

In our present study many of study population was got bitten by snake on the lower limbs i.e. 34(54%). Similar findings were observed with the study conducted by Rao et al10, where in out of 60 cases of snake bite poisoning, 27(45%) of bite sustained in lower limbs. Another study done by Ashok Kumar Shetty et al11, observed maximum number of snake bite was found in lower limb (75%).

In the present study, the maximum incidence of snakebite occurred between 08:00 to 15:59 hours i.e. 26(41.3%), followed by 16:00 to 24:00 hours i.e. 19(30.2%). Similar study done by Rao et al10 found that maximum bites were seen in between 12:00 PM to 04:00 PM. Another study conducted by Halesha BR et al12, it is found that maximum incidence of snake bite occurred during the day time (70.5%). These findings are in agreement with the present study.

In our present study 42 patients received anti-snake venom treatment. Out of it 20(47.61%) victims died within 24 hours after giving treatment with anti-snake venom. Similar findings were observed with study conducted by Ramakrishna Set al13 and UM Natarajan et al14. When treatment was started before 12 hours of bite, the mortality rate was 2.6% and if treatment was delayed for more than 12 hours of bite, the mortality rate was 13.5%.

In this present study, it also observed that in maximum number of snakebite poisoning cases the cause of death is respiratory failure 58(92.06%) followed by shock 5(7.93%).

Conclusion

In the present study it is concluded that, maximum number of snakebite cases were recorded in the rainy season (34.9%) followed by winter season (31.7%). In a greater number of cases the incidence of snake bite occurred predominantly in lower limb (54%) as compared to upper limb (46%). Peak incidence of snake bite was recorded in the time between 08:00 am to 04:00 pm (41.3%). Majority of the cases were declared dead (47.61%) within 24 hours of initiation of anti-snake venom. The cause of death in majority of the study population was respiratory failure (92.06%).

To reduce the incidence of snakebite poisoning, we advise people not to work in an endemic area without footwear, especially at night. People working in the field are motivated to use low cost footwear’s. Especially in rainy season, people should be very cautious while working to avoid stepping on the snake. Area surroundings of any human habitation or working fields should be kept clean from all kind of debris, garbage and rubbish.

Mortality and morbidity due to snakebite poisoning can be reduced by providing first aid as early as possible. All primary health centres should be fully equipped with facilities to give first aid measures and administer specific anti-snake venom if required. Admit the patient to the nearest hospital for further management. Since snake bite is primarily a rural occupational hazard, dissemination of information regarding readily available effective treatment may drastically bring down the morbidity and mortality associated with treatment by quacks.

Conflict of Interest: Nil
Source of Funding: Self

Ethical Clearance: Taken from IEC, KIMS, Hubballi.

References
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Comparative Study Thymol Oil and Some Gel Face Washing on Resistance Propionibacterium Acnes

Mais E. Ahmed\(^1\), Jenan A. Ghafil\(^1\), Walaa Najim\(^2\)

\(^1\)Assistant Professor, Lecturer, College of Science, Department of Biology, University of Baghdad, Baghdad, Iraq, \(^2\)Instructor, Institute of Laser Graduate Studies, Baghdad, Iraq, Department of Biology /College of Science /University of Baghdad

Abstract

Acne is elevated of skin secretion with hyperkeratosis causing Propionibacterium acnes. The goal of study was to assess the anti-inflammatory, and antibacterial potential of Thymol oil compare Gel face, and is the main bacterium involved in the acne. The unpredictable use of antibiotics for the treatment of acne may end up within the advancement of bacterial resistance.

Key words: Thymol oil, P. acnes, oil, Gel face, Hyperkeratosis

Introduction

A Gram-positive P. acnes is, anaerobic bacteria which colonizing the lipid-rich sebaceous surface of the human skin\(^1\). It has a place to the clinical gather of skin commensals, but can very soof ten cause genuine contaminations deciding the nearness of P. acnes contamination can be troublesome. Due to its less harmfulness, contaminations are less dolente \(^2\).

Skin break out could be an incessant incendiary illness of the pilosebaceous joins together, fundamentally of the confront, nose, shoulders and upper arms. It is one of the most common dermatoses affecting high school populace \(^3\). Acne vulgaris one of the most common skin disarranges, and dermatologists are still have long struggled to treat acne completely. It primarily influences young people, in spite of the fact that it may display at any age. It is nearly all inclusive malady happening in all races. The rate of seriousness of skin break out, top at (40%) in 14-17 year ancient young ladies and (35%) in boys matured 16-19 year \(^4\). It influences the skin of the confront, neck and upper trunk. These specific sebaceous follicles have capacious follicular channels and voluminous, multiacininar sebaceous organs. Skin break out creates when these specialized follicles experience pathologic change that comes about within the arrangement of non-inflammatory injuries (comedons) and fiery injuries (papules, pustules and knobs \(^5\). The intemperate utilize of anti-microbial for long periods has led to expanded resistance in skin break out causing microbes i.e. P. acnes and S. epidermidis against a number of anti-microbial utilized to treat skin break out \(^6\).

The antibacterial properties of EO has long been recognized and broadly tried in vitro against a broad run of pathogenic microscopic organisms, counting both (G\(^+\)ve) and (G\(^-\)ve) bacteria\(^7\). One of the foremost copious bunches of normal compounds is spoken to by the fundamental oils, Fundamental oils (EOs) are fragrant sleek fluids gotten from plant fabric.

They can amplify the rack life of natural or prepared nourishments by decreasing microbial development rate or reasonability. A few of these substances are moreover known in its contributions to self-defense of plants against irresistible life forms \(^8\).

Thymol a phenolic compound show in fundamental oils, may be a common monoterpene and carvacrol isomer that extricated from thyme and the other sorts of plants. Thymol is less water dissolvable at unbiased pH,
but it is as well dissolvable in some natural solvents and liquor \[9\].

The carvacrol and thymol antioxidant impacts of have been affirmed in a few considers, recommending their organization as nutritious components within the enhancement of novel useful nourishments. Thymol defensive nature against chars the field of drugs in dental caries\[^{10,11,13}\].

**Material and Methods**

Isolates strain were identified by microscopy and microscopy tests for identification of \textit{P. acnes}\[^{16, 18}\]. Directly transport from the pus and sterile disposable cotton swabs, transport media and carry to the laboratory.

Final identification by ViteckSystem 2: All the presumptive isolates (on blood agar (BA) medium and Brain heart infusion (BHI) agar were tested by Viteck2 system (Biomerix, France). This system used for diagnosis of \textit{Propionibacterium acnes}.

### Antibiotic sensitivity test \textit{P. acnes}:

The antibiotic sensitivity test was done accordance to \[^{14}\] by disc well diffusion using 5 type antibiotics.

### DNA extraction and Electrophoresis:

According to the extraction kit the samples’ examine the accuracy of the DNA extraction, photo absorption between (260 and 280) nm using a bio photometer.

The gel was then transferred to a transilluminator and the duplicated 1202 base pairs were examined under UV light.

### Emulsion preparation:

Three samples of anti-acne O/W washing with different compositions were obtain commercial market (Table I).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount/ ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esknol</td>
<td>100(\mu)l</td>
</tr>
<tr>
<td>Pharmacies</td>
<td>100(\mu)l</td>
</tr>
<tr>
<td>Bio Balance</td>
<td>100(\mu)l</td>
</tr>
</tbody>
</table>

**Table 1- The Gel face washing**

### Formulation of Face wash Gel:

**Antibacterial test was determined using -well diffusing method according to the CLSI Clinical Laboratory Standards Institute\[^{26}\].**

**Extraction of essential oils:**

About 20\(g\) of herb soaked in distilled water. The volatile vapour that condensed at water temperature of 80\(^\circ\)C was called essential oils. The distilled oils were labeled and placed in a fridge until ready for use.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount/ ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Result**

The cases Most were obtain patients between the ages \[^{16-21}\] years old in both sexes, and the distribution of acne among female was higher than that of male (14% and 4%) respectively.
Table (1) *P. acnes* infection according to age and gender groups

<table>
<thead>
<tr>
<th>Age group (year)</th>
<th>Sample No.</th>
<th>Male Positive No. (%)</th>
<th>Female Positive No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-15</td>
<td>17</td>
<td>3 (4.8)</td>
<td>4 (6.4)</td>
</tr>
<tr>
<td>16-20</td>
<td>20</td>
<td>13 (20.9)</td>
<td>16 (25.8)</td>
</tr>
<tr>
<td>21-25</td>
<td>13</td>
<td>2 (3.2)</td>
<td>2 (3.2)</td>
</tr>
<tr>
<td>Chi-square Value</td>
<td></td>
<td>9.135 **</td>
<td>9.852 **</td>
</tr>
</tbody>
</table>

** (P≤0.01)

Macroscopic examination

Colonies appeared as circular, opaque and glistening colonies with different colors, where may be white, gray or yellow. Bacterial colonies showed weak or no hemolysis when grown on BA. Figure (1)

![Figure 1](image1.jpg)

**Figure (1): *P.acnes* A) BHIA B) Blood agar at 37°C for 48 hrs**

Microscopic examination

After stained by Gram stain, all presumptive of isolates *P. acnes* isolates were Gram positive, in different forms polymorphism cells Figures(2).

![Figure 2](image2.jpg)
Figure (2): P. acnes arrangements (1000 x).

Antibiotic sensitivity test (AST) of P. acnes:

Result of AST showed in (Figure 3) the highest sensitivity P.acnes was to Levofloxacin then Clindamycin, while appeared highly resistant to Azithromycin, erythromycin, and metronidazole.

Figure (3): Antibiotic susceptibility test of Propionibacterium acne.

Used the morphological and biochemical tests and PCR technique to identify the P. acnes in lesions. The Figure(4) presents the PCR product electrophoresis on agarose gel (1.5%).

Figure 4. Amplification of 16SrDNA(1400bp) bacterial isolates. Agarose (1.2%), 5 V/cm for 2 h, stained with Red safe and visualized under UV transilluminator. M. 100 bp DNA marker. Lane 1-10: bacterial isolates.
Table (2): comparative results used against 
*P. acnes*

<table>
<thead>
<tr>
<th>Treatment used against P. acnes</th>
<th>Inhibition zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levofloxacin</td>
<td>9± 0.04 d</td>
</tr>
<tr>
<td>Thyme oil (commercial)</td>
<td>20 ± 1.26 ab</td>
</tr>
<tr>
<td>Eskolin</td>
<td>10.4 ± 0.37 d</td>
</tr>
<tr>
<td>Pharmacies</td>
<td>10.5 ± 0.42 d</td>
</tr>
<tr>
<td>Bio Balance</td>
<td>16 ± 0.79 bc</td>
</tr>
<tr>
<td>LSD value</td>
<td>4.282 **</td>
</tr>
</tbody>
</table>

** (P<0.01).

Figure (6): Comparison of the inhibitory effects against *P. acnes* A) Thyme oil B) Bio Balance C) Pharmacies D) Eskolin E) Levofloxicin that cultured on Moller Hinton Agar at 37 °C for 48 hrs. under anaerobic condition.

**Discussion**

The current results were coincides with previous results studied in Iraq of *P. acne* in different age (15). that acne prevalence was more in female than male, agree with most cases were in ages group between (15-20). Also agree with study in Iraq isolated *P. acne*.[17]

These results for identification *P. acne* were in agreement with[18,19] Viteck 2 System was a rapid, sensitive, to distinguish clinical isolates (20). The result agree with [21] The most using treatment (erythromycin) were anti *P. acne*.[22]
The antibiotics is topical application like clindamycin, tetracycline most common treatment of acne but the major problems growing in resistance of P. acnes to antibiotics caused[23].

The acne-gel with retinol had lower effect in agar dilution test. But shown that is highly effective at high concentrations against aerobic and anaerobic bacteria including P. acnes[24] the antiacnemore acts of oregano EO that of other evaluated including commercialized overthecounter acne treatment tea tree [25].

Conclusion

The antibiotics play an important treatment in acne administers. Resistance P. acnes treatment by antibiotic compares using natural oil most affections alternative commercial washing gel in pharmacy.

Conflict of Interest: The authors declare that there is no conflict of interest regarding this study.

Fund: This project was funded by University of Baghdad, Ministry of Higher Eduction and Scientific Research.

Ethical committee approval: This work was approval by the ethical committee of Department of Biology, College of Science, University of Baghdad.

References

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Drug Abuse Prevalence and Effects in Coffee Shop Workers: A Cross Sectional Study

Makloph MG¹, Mohamed Masoud²

¹Lecturer of Forensic Medicine and Clinical Toxicology, Faculty of Medicine Fayoum University, Fayoum,  
²Assistant Prof. of Public Health, Faculty of Medicine, Fayoum University, Fayoum, Egypt

Abstract

Background: Drug abuse among population have become more common than before, coffee shops workers are of increasing chance to drug abuse, the facing of such big problem necessitates collection of information regarding the prevalence of drug abuse types and effects on health, and this will result in prevention of its hazardous on population at all.

Patients and Methods: cross-sectional study during the first day of April 2019 till the last day of September 2019, one hundred and twenty coffee shop workers were included. The workers were chosen from 12 randomly selected coffee shops located in rural and urban areas. The data were gathered through a questionnaire and urine samples from participants. The collected samples were taken to the lab and screened for drug abuse using dip stick, thin layer chromatography (TLC) and gas chromatography (GC-MS). The collected data were organized, tabulated, and statistically analyzed using statistical package for social science (SPSS Inc, version 22).

Results: the study showed that The prevalence of tramadol and cannabis and other synthetic drugs among coffee shop workers in urban and rural area nowadays more than before and their detection easily done by using GC-MS. hazardous on health among abusers more than non abusers.

Conclusion: The prevalence of drug abuse among coffee shop workers of increasing popular especially tramadol, cannabis and other synthetic drugs, between all age groups in urban and rural area, these drugs of abuse can be detected using dip stick, TLC and the most specific method of detection using GC-MS to confirm the results. Countries should plan for facing this cancer due to its bad hazardous and consequences on health, family and society.

Keywords: abuse, coffee shops, drugs, gas chromatography.

Introduction

Drug abuse is one of the important problems facing governments and people as it affects the population in their productive period of life resulting in decreased work productivity and national income of countries. Drug abuse results in destruction to patients and societies. For example, 25% of condemned prisoners have committed crimes under the influence of drugs.

Cannabis is one of the most common illicit drug in most of the world and its abuse increasing progressively. A large number of people have become users of cocaine, amphetamine and other new synthetic medications.

Tramadol has become increasingly popular among people for consumption and for sexual gratification especially in young adults, despite its prevention nowadays.
in Egypt, due to its low price and easy availability[6].

The real prevalence of drug abuse in developing countries not accurately detected due to lack in data regarding that problem[7].

In 2005, the Egyptian government has declared the report of the National Addiction Research Program which found that more than 12% of the Egyptian citizens are drug abusers. Cannabis was the most commonly abused substance (77%) followed by opiates in upper Egypt and the 3rd most common was alcohol, then pharmaceutical substances, stimulants and organic solvents, respectively[8]. Since 2008 tramadol abuse in Egypt has increased dramatically in all age groups and among a lot of people[9].

**Subjects and Methods**

**Study design**

This study was a cross-sectional study.

**Study sample**

One hundred and twenty coffee shop workers were included. The participants were chosen randomly from 12 selected coffee shop located within Fayoum governorate, 6 in rural and 6 in urban area.

The current study was conducted for 6 months from the first day of April 2019 till the last day of September 2019

Inclusion criteria includes coffee shop workers work for more than 2 year at this field, their age should be between 25 and 44 years old. We exclude those refuse to participate, those beyond the age and work limit .

**Data collection**

The following data were gathered through a questionnaire constructs of two parts:

The first part: demographic data such as Age from their birth certificate, education level, residence, marital state, social problem and smoking habit .

Second part: type of drug abuse, method of drug abuse and health state of the participant.

Pretested Pilot test of 30 coffee shop student was done to detect reliability of the questionnaire and cronbach alpha test was calculated where it was 0.877. According to its result certain questions were omitted and others were changed.

Followed by open ended questions containing the above mentioned data urine samples were collected from each individual accept to participate in the study, 50 ml of urine sample were collected in plastic container and freezed at minus 20 degree celesius at ice box. Samples were collected in front of examiner to prevent adulteration.

The collected samples were taken to the lab of forensic department Fayoum university to screen for drug abuse using dip stick (tramadol and cannabis) and thin layer chromatography(TLC).

Confirmation was done using Gas chromatography at the faculty of science Fayoum university. Chemicals for TLC had brought from Alnasr pharmaceutical co, Sleeve Hanover co Switzerland and Adwic chemicals co Egypt, while in G.C the column used was C18 and the reagent used was G.C grade using flame ionizing detector(FID).

**Statistical Analysis**

The collected data were organized, tabulated, and statistically analyzed using statistical package for social science (SPSS Inc, version 22). Data were presented as frequencies and percentages; chi-square ($\chi^2$) was used as a test of significance. For interpretation of the results of tests of significance, significance was adopted at $P \leq 0.05$ in the results. Sensitivity and specificity of the detection methods of the drug abuse in the collected samples were done using ROC curve (receiving operating characteristic).

**Results**

Descriptive statistics.
120 middle aged workers are working for 2 years or more in coffee shop were included in this study. 40 of them (1/3) were abuser, Basic characteristics were presented in table (1) with statistically significant $P \leq 0.05$ regarding smoking habit and marital status.

**Analytical Statistics:**

**Regarding** Prevalence of drug abuse according to types of drugs in descending manner

About one third of the participant 16/120 (13.1%) reported abusers of tramadol followed by cannabis (11.6%), tramadol and cannabis (8.3%), benzodiazepine (2.5%), morphine and MDMA (1.6 % for each), amphetamine and parkinol (.8% for each).

**Regarding** drug abusers (16/40) about 40% of the drug abusers are tramadol abusers followed by 35% for cannabis abusers, 25% for both drugs, 7.5% for benzodiazepine abusers, 5% for morphine and MDMA.
Regarding relation between smoking and drug abuse, the percentage of smokers among abusers were found more than 80%.

Regarding Age group and drug abuse the prevalence of abuser among age groups it was found that cannabis abuser among the first two age groups (25-29,30-34) higher than the other two age groups(35-39,40-44), while in tramadol the prevalence in the first age group less than other 3 age groups with the most higher level at the 3rd age group (cannabis abuse at younger age groups than tramadol), with non statistically significant p-value.

Regarding distribution of abuse in relation to residence, it was found that drug abusers more in urban than rural while in relation to type of drug abuse tramadol and other drug of abuse more in urban than rural and the reverse for cannabis.

<table>
<thead>
<tr>
<th>Table 2: Health status of study participant.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>nil</td>
</tr>
<tr>
<td>Chronic Hepatitis C</td>
</tr>
<tr>
<td>Respiratory problem</td>
</tr>
<tr>
<td>Varicose vein</td>
</tr>
<tr>
<td>Flat foot</td>
</tr>
<tr>
<td>Poliomyelitis</td>
</tr>
<tr>
<td>Visual trouble</td>
</tr>
<tr>
<td>Musculoskeletal</td>
</tr>
<tr>
<td>Eczema</td>
</tr>
<tr>
<td>DM</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Sexual problems:</td>
</tr>
<tr>
<td>Erection problems</td>
</tr>
<tr>
<td>Ejaculation problems</td>
</tr>
</tbody>
</table>

Regarding Health status of the study participants, 46.6% of the study population were free from disease other than sex related problem , while the remaining participants suffering chronic hepatitis C were 16/120 (13.3%), musculoskeletal disorder, varicose vein and visual troubles skin problem, diabetics and hypertension in descending order . While health status of abusers 30% of them were free with significant p-value(0.005), the remaining participant suffering musculoskeletal disorder 9/40(22.5%) mostly those with tramadol abuse with
significant p-value<0.05, chronic hepatitis 8/40(20%) mainly those with tramadol and morphine with significant p-value(0.019), respiratory problem mainly those abusers of cannabis with significant p-value(0.001), flat foot, visual trouble, varicose vein, poliomyelitis, DM and hypertension in descending manner of order with non significant p-value>0.05. About one third of workers had sexual problems 44/120 (36.7%), while drug abusers suffer more complications regarding sexology 18/40 (45%) than non abusers with significant p value regarding ejaculation mainly abuse tramadol p-value (0.05)

Regarding dip stick sensitivity in diagnosis of drug abuse versus TLC and GC.MS, As compared to GC.MS, both TLC and dip stick had a sensitivity and NPV of 100% in diagnosing Tramadol, Cannabis & combined Tramadol and Cannabis abuse. However, specificities were 98.1%, 99.1%, and 98.2% for TLC and 96.2%, 98.1%, and 95.5% for dip stick in identification of Tramadol, Cannabis and combined Tramadol and Cannabis abuse, respectively. As regards PPVs, they were 88.9%, 93.3%, and 83.3% for TLC and 80.0%, 87.5%, and 66.7% for dip stick in diagnosis of Tramadol, Cannabis and combined Tramadol and Cannabis abuse, respectively.

### Table 3: Pattern and cause of drug abuse (N=40)

<table>
<thead>
<tr>
<th>Cause of drug abuse*</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>For relief of pain</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>For sexual desire</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>For work trouble &amp; increasing effort</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>For pleasure and curiosity</td>
<td>6</td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mode of abuse*</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral tablet</td>
<td>28</td>
<td>70</td>
</tr>
<tr>
<td>Smoking</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Syrup</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Injection</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frequency of usage per</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Once or twice</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Frequent</td>
<td>30</td>
<td>75</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time of drug abuse</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>At night</td>
<td>30</td>
<td>75</td>
</tr>
<tr>
<td>At day time</td>
<td>10</td>
<td>25</td>
</tr>
</tbody>
</table>

*Not mutually exclusive
about one third of those reported drug abuse had taken drugs to relief pain 14/40 (35%) mainly tramadol, while the other use drugs for sexual desire and increasing work effort mainly tramadol, respectively. A little use drugs for fun and curiosity ,mainly cannabinoid. Majority 28/40 (70%) had taken drugs in the form of oral tablets followed by smoking , syrup , and injection . about 3/4 of the participant 30/40 (75%) reported that they take drugs frequently. Most workers 30/40 (75%) had used drugs at night, table (3).

**Regarding** Social effect Majority of workers with drug abuse reported that they had quarreled at work 28/40 (70%) while less than one quarter of abusers were imprisoned before 8/40 (20.%), also delivery and family problem is more in abusers (35%) than in non abusers.

**Discussion**

Drug abuse refers to the self-administration of any drug in a way that deviates from the approved medical or social pattern within a given culture and it may not necessarily involve any physical, psychological or physiological dependency like an occasional experimental use of LSD for fun is drug abuse but not drug dependence[10].

Regarding smoking habits the present study in agreement with those found by Degenhardt L, et al 2009[11] which stated that there is a strong relation between smoking and drug abuse so smoking is the door for abuse.

In reference to the drug of abuse , In the present study we found cannabis and tramadol were the predominant drug of abuse contrary to that was found in china by Hong et al,2014, where it was found that amphetamine like synthetic drugs is the predominant drug of abuse with reduction of opiate abusers due to strong control[12]. Also in Japan in a study done by Wada K.2010[13] and Yasukazu Ogai,2015[14] they found methamphetamine , cannabis and MDMA the predominant drug of abuse with shift to new designer drugs to avoid arrest and imprisonment.

Regarding the epidemiology of abuse , the present study show that a little bit more than one third of the study population were abusers the same was found by Maryse Lapeyre et al,2004[15]. abuse started at younger age with tendency to abuse polydrugs either naturally or synthetically, the same was found by Hong et al,2014[12]. multi drug abuse were found in the present study in accordance with that was found by Jones et al ,2012[16].

Tramadol widespread abuse found in this study in agreement with those found by Aboelmaged et al,2013[9] and Fawzi ,2011[17] in Egypt, spiller et al ,2010 in the USA[18] and Nazarzadeh et al ,2014 in Iran[19]. The widespread use of tramadol in Egypt was probably due to its easy availability, its low cost and its use in sexual desire.

Contrary to the present study results regarding prevalence of drugs of abuse, Hamdi et al,2016[8] found cannabis is the main drug of abuse , this is my be due to difference in the study population or the place or size of the study participants.

Opioid abuse in the present study found to be less than other drugs of abuse In consistency with those found in the report of united nation office (UNODC,2014) [20] which stated that the use of opioids in north Africa lesser than other abused drugs the same was found also by Senna et al ,2010[21].

In other countries benzodiazepine abuse as common as THC as that occur in Australian study done by Drummer et al,2012[22] also in Brazilian study done by De boni et al, 2014[23] this contradiction with the present study may be due to study population difference, legislation done by countries , availability of drugs or cost.

From the present study tramadol and other synthetic drugs were abused in urban more than rural areas while cannabis abuse in the village more in the town , Rigg and monnat ,2015 found that urban residents show increased prevalence of opioid in urban than rural population[24] contrary to that Domingo et al 2015 found that the prevalence of cannabis was found more in urban area.
than rural area\textsuperscript{25}. While Wang et al, 2013 found that there is no big difference in prevalence of opioids among urban and rural areas\textsuperscript{26}.

The present study show that cannabis abuse mostly occur at younger ages than tramadol, Domingo et al, 2015 found that the prevalence of cannabis was found more in younger age groups\textsuperscript{25}, contrary to that Tjaderborn et al, 2016 found increasing prevalence of tramadol among younger age groups in Sweden\textsuperscript{27}.

The present study agreed with that done by Elsawy et al, 2010 regarding the motive for drug abuse which was due to peer pressures, get pleasure and mode improvement\textsuperscript{28}. While el akabwi, 2001 found that curiosity and desire to have fun were the main motive for abuse\textsuperscript{1}. Maryse Lapyere et al, 2004 found that regarding the motive for drug abuse in work, 20% used drugs to be in good form at work, 12% used drugs at the workplace for an awkward symptom, and 18% used drugs to relax after a difficult day’s work\textsuperscript{15}.

Drug abuse definitions are used in public health, medical and criminal justice contexts. In some cases criminal or anti-social behaviour occurs when the person is under the effects of a drug, use of some drugs may also lead to criminal penalties\textsuperscript{29}, the same was found in the present study where a lot of workers with drug abuse reported that they had quarreled at work others were imprisoned before for committing a crime under the influence of drug abuse.

Regarding sensitivity for analysis of drug of abuse we found GC and TLC was better than dip stick in detection of poisoning, the same was found by kim et al, 2016\textsuperscript{30}. Where the presence of codeine may give false positive results with dip stick confounding opiates as that found by Botvin et al, 2010\textsuperscript{31}. Also ibubruphen can cause false positive results confounding for THC as that found by James, 2011\textsuperscript{32}.

**Conclusion**

The prevalence of drug abuse among coffee shop workers of increasing popular especially tramadol, cannabis and other synthetic drugs, between all age groups in urban and rural area, these drugs of abuse can be detected using dip stick, TLC and the most specific method of detection using GC-MS to confirm the results. Countries should plan for facing this cancer due to its bad hazardous and consequences on health, family and society.

**Conflict of Interest:** the author declare that there is no presence for conflict of interest.

**Source of Fund:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Ethical Consideration**

This study was approved by the Fayoum Faculty of Medicine Research Ethical Committee in regard to the declaration of Helsinki 1964. The study was conducted after explaining the study’s aims. Verbal and written consents were obtained from all participants included in the study and each person had the right to refuse to participate in the study.

**References**


[6] Salem E.A. and Wilson S.K. Tramadol HCL has promise in on demand use to treat premature


Psychosomatic Effects of Sexual Harassment and Violence on Undergraduate University Female Students; A Cross Sectional Prevalence Study

Makloph MG

Lecturer of Forensic Medicine and Clinical Toxicology, Faculty of Medicine Fayoum University, Fayoum

Abstract

Background: Sexual harassment is the plague of that century due to its tremendous prevalence in various societies, it is unwanted and unaccepted sexual conduct with a female either verbal, non-verbal or physical, and it affects the physical and psychological wellbeing of the victim.

Patients and Methods: Cross-sectional study was done during the period of October 2019 till January 2020. Five hundred and four participants aged between 19 and 25 years old randomly selected, distributed on nine faculties, a questionnaire constructed of three parts given to each participant to collect data about type of abuse and psychosomatic effect through the use of standardized questionnaire. The collected data were organized, tabulated, and statistically analyzed using statistical package for social science (SPSS Inc, version 18).

Results: The study showed significant relationship between physical harassment and psychosomatic effects also method of harassment and frequency of harassment is a risk factor for occurrence of psychosomatic effects, and family response regarding perpetrator affect profoundly psychosomatic effects. The main harasser were university students.

Conclusion: The prevalence of sexual harassment now days resemble a cancer due to its wide spread and diffusion within the societies and its profound effect on the well being of the victim causing profound psychosomatic effects. These effects are affected by the method of harassment and the frequency of exposure. Families should have strong reflex against the perpetrator as this help the victim recovery and affects its profound effects on her well being.

Keywords: harassment, students, perpetrator, psychosomatic, verbal.

Introduction

Sexual harassment is defined as verbal, non-verbal or physical contact of sexual nature with a female without her permission. It is unwanted sexual contact with a female without her consent and affects her physical and psychological wellbeing. So it is any annoying or unpleasant behavior towards someone that occurs through any sexual form either corporeal, verbal or non-verbal act.

Sexual harassment is the commonest form of abuse and violence against women world wide generally and locally and increased tremendously at the last 25 years changing physical and psychological wellbeing of the harassed victims.

Sexual harassment takes many forms namely: Verbal as sexual comments, words, jokes, threats, sounds
or whistlers. Non-verbal as pictures, photos, graphics, twinking, gestures or massages. And Physical as sexual violence, inappropriate contact, touch, fingering, attempted sexual intercourse or assault[5].

Sexual harassment has rapidly increased recently in the Middle East’s countries especially, Egyptian society. in 2006 holiday of Eid El Fetr, Egypt got up on mass sexual harassment events in Cairo governorate. Two Years after, a worse mass sexual harassment incidents occurred in Giza governorate. 62% of Egyptian men said that they had sexually harassed women; and above 80% Egyptian women reported that they were sexually harassed once before [6].

High rates of sexual harassment against female was found at the academic universities and work places and it happens to be around 50% in university[1].

Effects of harassment on individuals include

- Decline academic performance.
- Failure in exam and career loss.
- Loss of motivation, anxiety, stress.
- Loss of interest and trust in people.
- Discrimination and introversion.
- Reference loss and relocation[7].

**Subject and Methods:**

**Study design:**

A cross-sectional study was done during the period of october 2019 till January 2020.

**Study sample:**

Five hundred and four participant aged between 19 and 25 years old randomly selected, distributed on nine faculties in relation to their students percentage of the university, (social science(22%), agriculture(18%), education(15%), science(12%) rights and law(10%), nursery(8%), engineering(6%), medicine (5%), arts(4%) education they were involved in the current study after explaining the purpose of the study and taking their consent.

Inclusion criteria: undergraduate Female Egyptian population study at Fayoum university, aged more than 18 year and below 26 years old.

Exclusion criteria: those refuse to participate in the current study, the above 25 and those below 19 years old, those with chronic illness, mentally deficient or handicapped and those with bad conduct and reputation.

**Data collection**

Pretested Pilot test of 30 female student was done to detect reliability of the questionnaire and cronbach alpha test was calculated where it was 0.785, according to its results certain questions were omitted and others were changed.

**The Questionnaire constructed of three parts:**

The first part: open ended questions regarding demographic data such as Age of the victim from their identity card, faculty, residence, marital status, family response regarding harassment, whether the harassed was alone or not, who was the perpetrator, whether the harasser was a lone or in group, his smoking habit and his relative age.

Second part: We use modification of sexual experience questionnaire including 19 items resembling the three types of sexual harassment, In addition to site of harassment, time of harassment and way of dressing of the harassed[8].

The third part: about psychosomatic effects on harassment and it was taken from WHO in the form of standardized self reported questionnaire of 20 item(SRQ20) to diagnose for psychological insult if more than 6 items of the questionnaire were positive[9].

Well trained faculty employees at student affairs unit at each faculty included within the study were used to collect data after explaining the purpose of the research, information collected were ascertain privacy and only for research purpose and collected data was
under direct observation of the researcher of the study.

Statistical analysis

The collected data were organized, tabulated, and statistically analyzed using statistical package for social science (SPSS Inc, version 18). Data were presented as frequencies and percentages; chi-square ($\chi^2$) was used as a test of significance, and multinomial logistic regression analysis was used to predict for future psychosomatic effects as the dependant factor was categorical dichotomas variant. Also univariate analysis used to document relationship between certain variables. For interpretation of the results, the 2 tailed-significance was adopted as a test of significance at $P \leq 0.05$.

Results

From seven hundred and fifty interviewed female undergraduate students Only 5 hundred and four female undergraduate students accepted to participate and completed the questionnaire given, of whom only one hundred and twenty four showed exposure to harassment within a year.95% were single, sexual harassment at social science faculty and education faculty was more than other faculties (22%,18% respectively) with the least prevalence of harassment within the faculty of medicine and rights and law(6%,5% respectively), mean age (22 years+SD2.2) with non specific p-value regarding harassment.

Descriptive statistics:

Tab(1) type of harassment and perpetrators and factors affecting.

<table>
<thead>
<tr>
<th>Cross tabs</th>
<th>df</th>
<th>p-value(2-tailed)</th>
<th>Cross tabs</th>
<th>df</th>
<th>p-value(2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of harassment</td>
<td></td>
<td></td>
<td>Site of harassment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction toward harassment</td>
<td>80</td>
<td>&lt;0.001</td>
<td>perpetrator</td>
<td>64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Site of harassment</td>
<td>160</td>
<td>0.015</td>
<td>individuality</td>
<td>8</td>
<td>0.004</td>
</tr>
<tr>
<td>Family reaction</td>
<td>6</td>
<td>0.041</td>
<td>Perpetrator reaction later</td>
<td>12</td>
<td>0.001</td>
</tr>
<tr>
<td>Family reaction</td>
<td></td>
<td></td>
<td>Family reaction</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results showed that statistically significant p-value($<0.05$) regarding phi and chi ($\chi^2$) square between Reaction of harassed toward harassment and Type of harassment and Site of harassment. family reaction toward harasser strongly and significantly dependant on the type and method of harassment as pulling harassed clothes and rape attempt was faced by strong family response against harasser but verbal or non verbal harassment not faced as such by the family of the harassed victim.

The results showed also that statistically significant p-value( < 0.05) regarding phi and chi($\chi^2$) square between Perpetrator reaction later and Family reaction where the family response toward the harasser affect later recurrence of the effect. and between perpetrator and being alone or in group as harassment occurs once the perpetrators are in groups than being a lone and also with the Site of harassment.
Tab(2): Psychosomatic effects and factors affecting.

<table>
<thead>
<tr>
<th>Cross tabs</th>
<th>df</th>
<th>p-value(2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychosomatic effect</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of harassment</td>
<td>60</td>
<td>0.06</td>
</tr>
<tr>
<td>Psychosomatic effect</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of harassment with a relative</td>
<td>60</td>
<td>0.04</td>
</tr>
<tr>
<td>Psychosomatic effect</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family reaction against harasser</td>
<td>40</td>
<td>0.001</td>
</tr>
<tr>
<td>Psychosomatic effect</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of harassment</td>
<td>40</td>
<td>0.045</td>
</tr>
<tr>
<td>Psychosomatic effect</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Method of physical harassment</td>
<td>40</td>
<td>0.013</td>
</tr>
</tbody>
</table>

The results showed that statistically significant –p-value(<0.05) regarding phi and chi(χ²) square between occurrence of Psychosomatic effect to the harassed and (Type of harassment in case the relative was the harasser, Family reaction, Frequency of harassment also Method of physical harassment that is mean that the psychosomatic effects affected by the frequency of harassment, method of harassment and the response of the family toward the effect and the harasser.

Fig(1): types of harassment

Fig 1 show various types of harassment with its percentage.
The results also showed that statistically significant p-value(<0.001) regarding phi and chi(χ2) square between harassment occurrence and (the way of dressing of the victim, female being alone or in group as being alone is more risky for harassment than if in groups also most of the harassers were smokers with statistically significant p-value. so we declared from the results of the present study that the following are risk factors for harassment namely way of dressing of students and being a lone, also grouping of the harasser and their smoking habit are all risk factors for sexually abusing students.

![Fig(2) the actual perpetrator](image)

**Table 3:** The Results showed that the harassers are either in campus (students and staff) or out campus (drivers, street person or students, relatives and others) tab(3) method of harassment and psychosomatic effects.

<table>
<thead>
<tr>
<th>Method of harassment</th>
<th>%</th>
<th>Psychosomatic effect</th>
<th>%</th>
<th>Psychosomatic effect</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>verbal</td>
<td>38.7</td>
<td>headache</td>
<td>10</td>
<td>Body weakness and tingling</td>
<td>1</td>
</tr>
<tr>
<td>Non verbal</td>
<td>36.3</td>
<td>phobia</td>
<td>6</td>
<td>Acne and impetigo</td>
<td>1</td>
</tr>
<tr>
<td>Touch skin and sensitive area</td>
<td>14</td>
<td>Loss of appetite</td>
<td>5</td>
<td>Bed wetting</td>
<td>.5</td>
</tr>
</tbody>
</table>
Cont... Table 3: The Results showed that the harassers are either in campus (students and staff) or out campus (drivers, street person or students, relatives and others) tab(3) method of harassment and psychosomatic effects.

<table>
<thead>
<tr>
<th>Method of Harassment</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>fingering</td>
<td>5</td>
</tr>
<tr>
<td>Menstruation problem</td>
<td>4</td>
</tr>
<tr>
<td>others</td>
<td>4</td>
</tr>
<tr>
<td>Kissing and attempted hug</td>
<td>4</td>
</tr>
<tr>
<td>Loss of interest in people</td>
<td>3</td>
</tr>
<tr>
<td>nil</td>
<td>60</td>
</tr>
<tr>
<td>Pulling clothes and attempted rape</td>
<td>2</td>
</tr>
<tr>
<td>Skin patch</td>
<td>2</td>
</tr>
<tr>
<td>Hair fallen</td>
<td>2</td>
</tr>
<tr>
<td>Blurring of vision</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Analysis of the method of harassment showed prevalence of verbal followed by non-verbal and physical forms mainly through touching skin and sensitive area. While analysis of the psychosomatic effects showed prevalence of headache and phobia and appetite loss.

**Analytical Statistics**

Using univariate regression analysis, psychosomatic effects were statistically significant regarding physical type of harassment (p-value 0.003), but not statistically significant regarding other types also psychosomatic effect were statistically significant regarding all harassment types in case the harasser was one of the relatives of the harassed female victim p-value(<0.001).

<table>
<thead>
<tr>
<th>Tab (4) Multinomial logistic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chi -square</td>
</tr>
<tr>
<td>Frequency of exposure</td>
</tr>
<tr>
<td>Exposure to harassment by relative</td>
</tr>
<tr>
<td>Family reaction</td>
</tr>
<tr>
<td>Individuality of female</td>
</tr>
<tr>
<td>Method of physical harrassment</td>
</tr>
<tr>
<td>df</td>
</tr>
<tr>
<td>Frequency of exposure</td>
</tr>
<tr>
<td>Exposure to harassment by relative</td>
</tr>
<tr>
<td>Family reaction</td>
</tr>
<tr>
<td>Individuality of female</td>
</tr>
<tr>
<td>Method of physical harrassment</td>
</tr>
<tr>
<td>Pseudo R-square</td>
</tr>
<tr>
<td>Frequency of exposure</td>
</tr>
<tr>
<td>Exposure to harassment by relative</td>
</tr>
<tr>
<td>Family reaction</td>
</tr>
<tr>
<td>Individuality of female</td>
</tr>
<tr>
<td>Method of physical harrassment</td>
</tr>
<tr>
<td>sig</td>
</tr>
<tr>
<td>Frequency of exposure</td>
</tr>
<tr>
<td>Exposure to harassment by relative</td>
</tr>
<tr>
<td>Family reaction</td>
</tr>
<tr>
<td>Individuality of female</td>
</tr>
<tr>
<td>Method of physical harrassment</td>
</tr>
</tbody>
</table>

Using multinomial logistic regression several factors can predict psychosomatic effect of harassment on the harassed in the future with statistically significant p-value (<0.05) such as frequency of exposure, family reaction toward the perpetrator, individuality of the harassed victim and method of physical harassment.
Discussion

Sexual harassment has been known as the most frequent form of sexual abuse and violence and against women. It is a form of social control done by male to keep female in place under control\[^{[1]}\].

Regarding the prevalence of sexual harassment, the present study should that 24.6% of females within the Fayoum university were sexually harassed, contrary to that Sang et al, 2016 in their study at Eldoret found that about 50% of the interviewed students have experienced various forms of sexual harassment against their well\[^{[10]}\]. Fitzgerald et al reported prevalences of more than 70% for women from a US military sample in 1999\[^{[10]}\].

In Europians the percent of sexual harassment within the universities were 30-50%\[^{[12]}\]. One third of college victims in the USA were a victims of harassment\[^{[13]}\]. More than two third of college victims in Ethiopia were victims of harassment\[^{[14]}\]. Also Hussain JH, 2015 found that 28% of Lebanon were sexually harassed\[^{[15]}\]. Danish union survey found that, 26.5% of women had experienced some form of unwanted sexual harassment in the workplace during the last year\[^{[16]}\].

The Present study found statistically significant p-value between only physical type of harassment and psychosomatic effect except if the harasser was one of the relatives of the harassed victim, this is because some form of harassment doesn’t consider as a form of harassment in some societies and once the relative is the harasser it is shameful and destructive to the wellbeing of the harassed as the relatives are supposed to defend the victim not to harassed him. This was in contrast with that found by Almaz et al, 2015 where he found statistically significant p-value regarding all types of harassment\[^{[17]}\].

Borge et al, 2019 found that 17.1% of women, had been verbally harassed since. At a similar level, 15.2% of women reported unwanted touching, hugging or kissing since they started on the college/university studies\[^{[18]}\].

Victims of sexual harassment are more likely to suffer from short-term and long-term health problems\[^{[21]}\], those exposed to sexual harassment have more mental and psychological\[^{[22]}\] and somatic health problems\[^{[20]}\]. Harassment is related to mentality disorder such as anger and depression also related to abuse\[^{[23]}\].

The present study showed that the harassed female students were affected by psychosomatic disorder in 40% of cases contrary to those found by Ogbonya 2011 where he found More than 85% of Nigerian female students at Ebony State university suffer from psychological distress after harassment\[^{[24]}\], also 63% of Ethiopian female students at Jimma university suffer from psychological distress after harassment\[^{[17]}\].

Regarding the psychosomatic effects of harassment on the harassed victim the present study showed that the victims suffering headache (10%), phobia (6%), loss of appetite (5%) and menstruation problem (4%) contrary to that Sang et al, 2016 found that Effects of Sexual Harassment on students, most of them indicate Feeling angry" (40%), phobia (38%) and lost contact with people (36%). However, fewer students express that their daily life will be affected, such as Sleep disturbances (7%) and Eating disorders (10%)\[^{[10]}\].

The present study showed that the in campus harassers were mainly male students and to a less degree
staff persons the same was found by sang et al ,2016 they found that Most Sexual Harassment incidents is perpetrated by male students. Also teachers and teaching staff to a minor degree[10],Timmerman ,2005 in his study in Netherlands and found that most harassers were male students rather than teaching staff[25], also male Students were the most harassers in US study[26], the present study also showed drivers are the most harassers out campus, The majority of recent harassment acts were committed by someone outside of the university setting, while a student and university staff member committed the harassment in 25% and 3%, respectively, of the instances as found by Borge,2019[18].

The present study showed significant p-value regarding the way of dressing of the victim and occurrence of harassment the same was found by Hussain JH 2015 where he found that 63% of Lebanon think that their way of dressing is the cause[15].

**Conclusion**

The prevalence of sexual harassment now days resemble a cancer due to its wide spread and diffusion within the societies and its profound effect on the well being of the victim causing profound psychosomatic effects , these effects are affected by the method of harassment and the frequency of exposure. Families should have strong reflex against the perpetrator as this help the victim recovery and affects its profound effects on her well being.

**Ethical Consideration**

This study was approved by the Fayoum Faculty of Medicine Research Ethical Committee . The study was conducted after explaining the study’s aims. Verbal and written consents were obtained from all participants included in the study and each person had the right to refuse to participate in the study.

**Conflict of Interest:** The author declare that there is no presence for conflict of interest.

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**References**


Identification of Single Nucleotide Polymorphism on Bone Morphogenetic Protein 2 Gene in Non-Syndromic Cleft Lip/ Palate Patient

Mala Kurniati 1,2, RM Coen Pramono D3, Agung Sosiawan 4, David Sontani Perdanakusuma 5, Hari Basuki Notobroto 6, Andra Rizqia 3

1 Post Graduate Doctoral Program, Faculty of Medicine, Universitas Airlangga, Surabaya-Indonesia, 2 Lecturer, Department of Biology, Faculty of Medicine, Universitas Malahayati, Lampung, Indonesia, 3 Professor, Department of Oral & Maxillofacial Surgery, 4 Senior Lecturer, Department of Dental Public Health, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, 5 Professor, Department of Plastic Reconstructive and Aesthetic Surgery, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, 6 Senior Lecturer, Department of Biostatistics and Population Studies, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia

Abstract

Cleft lip/palate (CL/P) is one of the most common birth defects in humans. Haploinsufficiency in genes Bone Morphogenetic Protein (BMP) 2 is thought to play an important role in the incidence of CL/P. This study aimed to identify changes in the nucleotide (Single Nucleotide Polymorphism/SNP) BMP 2 rs235768 A>T gene in CL/P patient in Indonesia. Seventy samples of DNA that were successfully amplified and restricted consisted of patient and control samples with the three of which were used for sequencing. Based on the analysis using restriction enzymes and Finch TV and Bioedit software programs, this study identified a change from nucleotide A to nucleotide T which is a mutation missense (Serine-Arginine/TCA-TCT). Based on the results of the Fisher’s exact test, there was difference in genotype frequency between the CL/P group and the control. Meanwhile, there was no difference in allele frequencies between the two groups. The allele frequency T has a higher value than the frequency of the allele A.

Keywords: Gene BMP2, SNP, Cleft lip/palate, Indonesia.

Introduction

Cleft lip and/or palate (CL/P) is the most common congenital abnormality caused by a complex multifactorial interaction between gene variation and environmental exposure. Individuals with CL/P face many challenges, such as anatomical deformities, dental problems, eating and speaking difficulty, hearing loss, and impaired maxillofacial growth. Psychologically, individuals with CL/P also experience a low quality of life index. The incidence of CL/P varies between countries, socioeconomic status, race, or ethnic group. This variation causes the Asian population to have the highest prevalence (1/500 births) and the African have the lowest (1/2500 births) while Caucasians have the prevalence 1/1000 births.

In recent years, advances in genetics and molecular biology have begun to reveal the basis for craniofacial development. A number of genes associated with the incidence of CL/P have been identified and developed...
to study the etiology of CL/P, namely genetic and environmental. Bone Morphogenetic (BMP) is an important gene candidate in the craniofacial pattern located on chromosome 14q22-23 in humans. More than 20 BMP genes have been identified. Currently, BMP 2 and 4 are classified as subfamily of dpp (decapentaplegic) due to similarities with the dpp gene in Drosophila. BMPs 5, 6, 7, and 8 are classified as the 60A subfamily, BMP3, and 3b (GDF10) in which are classified as separate subfamilies. BMP has receptors, namely BMP (BMPR) type I and type II (BMPRI and BMPRII).

A small proportion of SNPs become biological markers for the determination of a disease on the human genome map because these SNPs are located on genes that are found to be associated with the disease. The SNPs associated with a disease can be used to find and isolate the gene that causes the disease. The SNP pattern in target genes from the results of comparative studies between case and control groups in association studies can be used to design therapeutic targets and response of drugs in a population.

In the study of Sahoo et al., haploinsufficiency in the BMP 2 gene plays an important role in cleft palate formation. Two individuals with similar cleft defects are reported to have microdeletions that only included BMP2. Microdeletions associated with CL/P have also been reported in several studies including 20p12.3 in which BMP2 was deleted. In 2018, Saket et al. published the results of a study on the variation of the BMP2 gene sequence at risk for the incidence of NSCL/P in the Iranian population. It is noted that there is a significant association between the polymorphism of BMP2 rs235768 A>T and the incidence of CB/L. This study aimed to identify changes in nucleotide bases (Single Nucleotide Polymorphism/SNP) of the BMP 2 rs235768 A>T gene in CL/P patients in Indonesia.

**Materials and Methods**

This study examined patient (CL/P) and control groups. The CL/P patient group consisted of 34 samples and 36 samples from the control group. DNA was extracted from peripheral blood using Promega A1120Wizard® Genomic DNA Purification Kit DNA at the Human Genetic Laboratory, Institute of Tropical Diseases, Universitas Airlangga. This research has been approved by the ethics committee of the Faculty of Dentistry, Universitas Airlangga with a certificate number 606/HRECC.FODM/IX/2019.

Polymorphism of the BMP2 gene rs235768 A>T was analyzed with PCR-RFLP method. The primers used were produced by Integrated DNA Technologies, namely 5’ GAAACGAGTGGAAGAACAC-3’ and 5’GAGACACCTGGTCTTCTCTCAA-3. The reaction among PCR 25 µl 2.5 µl Promega Go Taq™ Master Mixes, primary forward 2.5 µl (10 µmol), reverse primer 2.5 µl (10 µmol), template 7.5 µl was amplified with the BioRadCycler PCR machine. The PCR machine procedure is at 58°C for 00:30 seconds and 34 cycles followed by elongation at 72°C for 00:40 seconds. Electrophoresis was conducted using 2% of agarose gel (Promega) at 100 V for 30 minutes and a PCR product was observed at 353 bp length. Furthermore, the RFLP method was carried out using BsrI restriction enzyme from Thermo Scientific. Afterwards, the enzyme mixture and PCR products were incubated at 65°C for 1 hour and inactivated at 80°C for 20 minutes. Visualization of the RFLP band was electrophoretic 100 V for 35 minutes and the cut tape could be seen in a UV light to read the results.

There are three variations of the genotype results, namely 353 bp for wildtype homozygote (AA); 353 bp, 200 bp, and 153 bp for mutant heterozygote (TT); 200 bp and 153 bp for mutant homozygote (AT). All statistical analysis was performed using SPSS Inc., IBM Corporation, NY, and USA Statistics Version 16. The Fisher’s exact test was used to analyze the distribution of genotypes between two groups. Genotype and allele frequencies were calculated and assessed by the Hardy Weinberg Equilibrium where a p-value <0.05 was considered to be statistically significant in all groups.

**Results and Discussion**

This study consists of 70 samples from the
Indonesian population with the 34 of whom were the CL/P group and 36 were the control group. Genotype distribution between CL/P groups and control for BMP2 gene polymorphisms 235768 A>T is significantly different (Table 1).

Table 1. Genotypes distribution of the BMP2 gene rs235768 A>T polymorphism in the CL/P and control group

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AT</td>
</tr>
<tr>
<td>CL/P</td>
<td>2 (5.9%)</td>
<td>4 (11.8%)</td>
</tr>
<tr>
<td>Control</td>
<td>2 (5.6%)</td>
<td>13 (36.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>4 (5.7%)</td>
<td>17 (24.3%)</td>
</tr>
</tbody>
</table>

*Significant at P < 0.05

Allele frequency distribution BMP2 gene rs235768 A>T in the CL/P and control groups is not significantly different (p>0.05, Fisher’s exact test) (Table 2).

Table 2. Distribution of the alleles of BMP2 gene rs235768 A>T polymorphism in the CL/P and control group

<table>
<thead>
<tr>
<th>Group</th>
<th>Allele</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>T</td>
</tr>
<tr>
<td>CL/P</td>
<td>8 (12%)</td>
<td>60 (88%)</td>
</tr>
<tr>
<td>Control</td>
<td>17 (24%)</td>
<td>55 (76%)</td>
</tr>
</tbody>
</table>

*Significant at P < 0.05

PCR products and RFLP results with the restriction enzyme BsrI on BMP2 gene rs235768 A>T are presented in Figures 1 and 2.
Figure 1. Visualization of the PCR BMP2 gene rs235768 A>T product.

Lanes 1, 2, 3, show bands at 353 bp, and lane 4 shows a 50-bp DNA ladder marker.

Figure 2. Results of PCR products carried out by RFLP with the enzyme BsrI.

Lane 1, 4, and 5 indicate TT; lane 2 indicates AA; and lane 3 indicates AT genotype.
Lane M is 50 bp ladder marker.

The conformation of PCR results using sequencing read with Finch TV and Bioedit programs from three samples that were examined and obtained three SNP genotypes on the BMP2 gene rs235768 A>T, namely AA (homozygous wild type), AT (heterozygous), and TT (homozygous mutant).

![Figure 3. (A) AA genotype (wild type), (B) AT genotype (heterozygous), (C) TT genotype (homozygous mutant), (D) Finch TV application, (E) Bioedit application](image)

The BMP2 gene encodes a ligand that is secreted from the TGF-β superfamily. The ligands of this superfamily bind to various TGF-β receptors that play a role in the activation of transcription factors, especially the SMAD protein in regulating gene expression. The BMP2 protein plays a role in bone and cartilage development. In addition, BMP2 stimulates myoblast differentiation into osteoblasts via the EIF2AK3-EIF2A-ATF4 pathway. Activation of BMP2 EIF2AK3 stimulates phosphorylation of EIF2A which leads to increased expression of ATF4 which plays a central role in osteoblast differentiation. BMP2 also stimulates TMEM119 which can increase the regulation of ATF-4 expression.14

The BMP2 gene is located on chromosome 20p12.3 (short arm of chromosome 20 area 1, band 2, and sub band 3). The length of the BMP2 sequence in the NCBI annotation (GRCh38.p12) starts from 6,768,098 to 6,780,280 along 10.6 kb (Homo Sapiens Annotation Release). Consisting of three exons, the focus of this study was Exon 3 with reference to SNP (rs235768), a change in the amino acid Serine to arginine at position 190 in the BMP2 gene. Other names for the BMP2 gene are BDA2, BMP2A, and SSFSC.14

Single Nucleotide Polymorphism are is a small change to genetics or variations that can occur in a person’s DNA sequence. The genetic code is determined by the four nucleotides A (adenine), C (cytosine), T
SNP variation occurs when one nucleotide, such as A replaces one of the three other nucleotides, such as C, G, or T \cite{11}. Based on molecular analysis using restriction enzymes and bioinformatics analysis of Finch TV and Bioedit programs, it was identified that there was a change from nucleotide A to nucleotide T which was a mutation missense (Serine - Arginine/TCA-TCT). Missense mutase is a change in the composition of nitrogen bases which causes changes in amino acids in a polypeptide chain. Changes in amino acids can produce a mutant phenotype if the changed amino acid is an essential amino acid for the protein. This type of mutation can be caused by transitional and transformation events. \cite{15}

The genotype frequency showed that the highest percentage of 82.4% in the CL/P group was the TT genotype (homozygous mutant). The results of the Fisher’s exact genotype test in the two groups were significantly different. The allele frequency in the CL/P group was the highest in the T allele which was a polymorphic allele, namely 88% and the Fisher’s exact test results were not significant between the alleles and the two groups. There are differences in the research results obtained by researchers from previous research, in Iran which indicate there was an association between BMP2 and the incidence of CL/P, but this difference can be due to the different number of samples even though the procedural laboratories were the same from the primary arrangement, the annealing temperature of the PCR, and the type of restriction enzymes used. The difference in results was also seen in the PCR product and the resulting RFLP cut results between the Iranian and Indonesian samples. However, the differences between PCR and RFLP products have been confirmed by sequencing the PCR results and studying the nucleotide bases one by one with the Finch TV and Bioedit programs. The difference in each place is influenced by the number of samples in the study as well as differences in ethnicity and sex. \cite{16}

From this study, the SNP genotype results can be ascertained that the changes are the same, namely AA (wild type), AT (heterozygous), TT (homozygous mutant) which differ only in their position. Thus, the BMP 2 gene still needs to be examined more deeply in this study, one of which is the addition of research samples and analysis studies at certain points in the BMP 2 gene. The high percentage yield for the mutant genotype (TT) and T allotype could potentially suggest a possible etiological link between CL/P and mutations in the BMP 2 gene.

**Conclusion**

There is a change from nucleotide A to nucleotide T on BMP2 gene rs23576 which is a missense mutation (Serine - Arginine/TCA-TCT). There was significant relationship between the rs235768 A>T polymorphisms of the BMP2 gene in CL/P patients. The allele frequency T (Polymorphic) has a higher value than the frequency of the A allele (non-polymorphic).

**Ethical Clearance**

This study was approved by the ethical committee of the Faculty of Dentistry, Universitas Airlangga number 606/HRECC.FODM/IX/2019.

**Conflict of Interest**

There was no conflict of interests regarding the publication of this study.

**Source of Funding**

This research was funded by the Grant Doctoral Dissertation Research for Fiscal Year 2020 No: 592/UN3.14/PT/2020 Directorate of Research and Community Services, Deputy of Research and Development Reinforcement, Ministry of Research and Technology/National Agency for Research and Innovation.

**Acknowledgments:** I would like to deliver my gratitude to the Ministry of Research and Technology/ National Agency for Research and Innovation for supporting this research. I also express my gratitude to the laboratory staffs of Human Genetic and Forensic Laboratory, the Institute of Tropical Disease, Universitas Airlangga; Bima Regional Hospital, West
Nusa Tenggara; and Nahdlatul Ulama Hospital of Tuban, East Java for the support in sample collection. Lastly, I appreciate the teaching staffs of the Department of Oral and Maxillofacial Surgery, the Faculty of Dental Medicine, Universitas Airlangga, Indonesia and those who helped in this research.

References

Inhibitors in Hemophilia A with Posterior Dextra Femoral Region Hematoma

Malfira Iswary\textsuperscript{1}, Hartono Kahar\textsuperscript{2}

\textsuperscript{1}Resident, Clinical Pathology Specialization Program, Department of Clinical Pathology Medicine, \textsuperscript{2}Lecturer, Department of Clinical Pathology, Faculty of Medicine, Airlangga University - Dr. Soetomo Hospital, Surabaya, Indonesia

Abstract

Hemophilia A is a hereditary coagulation disorder caused by deficiency or dysfunction of clotting factor (F) VIII. FVIII inhibitors are antibodies that functionally have the ability to inhibit FVIII activity. Patients are suspected of having FVIII inhibitors if the response to FVIII replacement therapy is inadequate. In this research, we observed a case of a 60-year-old woman with complaints of pain and swelling of her right thigh for the past 3 days. Physical examination revealed anemic conjunctiva and hematoma in the right posterior femur region. Laboratory results showed Hb 9.1 g / dL, WBC 10.96 / \(\mu\)L, PLT 351.000 / \(\mu\)L, APTT 74 seconds, CT 40 minutes, FVIII 1% and inhibitor assay 928 BU. Patient received Koate therapy, tranexamic acid and RICE (Rest, Ice, Compression, Elevation). Hemophilia A cases are generally seen in males with history of bleeding in the family and most often characterized by repeated bleeding in the joints. In this case, we found a female patient with a hematoma in the right posterior femur region. The result of FVIII examination was 1% and inhibitor assay was 928 BU. It can be concluded that the patient in this case suffered from hemophilia A with inhibitor.

Keywords: Hemophilia A, inhibitor, hematoma.

Introduction

Hemophilia A is a hereditary coagulation disorder caused by F VIII deficiency. The severity of bleeding in hemophilia A correlates with FVIII activity. In severe hemophilia A cases with FVIII activity <1%, there can be spontaneous bleeding in joints, muscles and internal organs. Meanwhile, in moderate hemophilia A cases with FVIII activity 1-5%, bleeding can occur due to minor trauma, and in mild hemophilia A cases with FVIII activity> 5- 40%, bleeding may occur due to severe trauma or surgery. Patients are suspected of having FVIII inhibitors if the response to FVIII replacement therapy is inadequate\textsuperscript{1}.

Inhibitors against F VIII can occur in 20-30% of cases of hemophilia A. The incidence of inhibitors in severe hemophilia is 25-50%, whereas in mild and moderate hemophilia 3-13%. Factors associated with inhibitor formation include patient factors and therapeutic factors. Patient factors include type and severity of hemophilia, race, genotype of hemophilia and age at first F VIII replacement therapy. Therapeutic factors include the type of FVIII product, exposure day, and intensity of therapy\textsuperscript{1,2}.

FVIII inhibitors can be identified by examining the inhibitors according to Bethesda. ISTH (International Society of Thrombosis and Haemostasis) classifies hemophilia A patients as high responders if the inhibitor titer is >5 BU/mL and low responders if the inhibitor titer is ≤5 BU/mL\textsuperscript{3}.

Based on the case report by Malfira Iswary, the results of the hematology examination were F VIII of 1% and Inhibitor Assay of 928 BU.

Case

Patient identity: Woman, 60 years old
Main complaint: Pain and swelling of the right thigh

Current medical history: The patient presented with complaints of pain and swelling of the right thigh for 3 days of SMRS, fever (-), bleeding gums (-), nosebleed (-). Defecation and bowel movement within normal limits.

Past medical history: The patient was aware that she suffered from hemophilia since the previous year and was treated 3 weeks earlier with the same complaint. The patient has routinely received 1000 units of factor VIII transfusion every 2 weeks since the last 8 months.

Physical Examination

General condition: Moderate, composmentis, GCS 456

Vital signs: Pulse: 115x/minute, Temperature: 36.5°C, Respiration rate: 20x/minute, Blood pressure: 120/70mmHg

Head / neck: Anemic conjunctiva

Thorax: Cor and Pulmo within normal limits

Abdomen: Supple, bowel sounds (+), liver and spleen impalpable

Extremities: Warm acral, posterior dextra femoral hematoma (+)

Figure 1. Posterior Dextra Femoral Hematoma

Supporting Examination

Table 1. Routine Blood Test Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>21/01/20</th>
<th>27/01/20</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (103/uL)</td>
<td>10.96</td>
<td>7.41</td>
<td>4.5-13.5</td>
</tr>
<tr>
<td>% Neu (%)</td>
<td>76</td>
<td>62.4</td>
<td>39.8-70.5</td>
</tr>
<tr>
<td>% Eos (%)</td>
<td>1.1</td>
<td>2</td>
<td>0.6-5.4</td>
</tr>
<tr>
<td>% Baso (%)</td>
<td>0.5</td>
<td>0.1</td>
<td>0.3-1.4</td>
</tr>
<tr>
<td>% Mo</td>
<td>10.7</td>
<td>11.7</td>
<td>4.3-10</td>
</tr>
<tr>
<td>% Lym</td>
<td>11.7</td>
<td>23.8</td>
<td>23.1-49.9</td>
</tr>
<tr>
<td>RBC (106/uL)</td>
<td>3.08</td>
<td>2.3</td>
<td>3.4-5.0</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>9.1</td>
<td>6.8</td>
<td>12.0-15.0</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>27.2</td>
<td>21.5</td>
<td>35-49</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>88.3</td>
<td>93.5</td>
<td>80-94</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>29.5</td>
<td>29.6</td>
<td>26-32</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>33.5</td>
<td>31.6</td>
<td>32-36</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>15</td>
<td>15.6</td>
<td>11.5-14.5</td>
</tr>
<tr>
<td>Plt (103/uL)</td>
<td>351</td>
<td>342</td>
<td>150-450</td>
</tr>
</tbody>
</table>
HDT Results (16/01/2020)

Erythrocyte: Normochromic Normocytic poikilocytosis (ovalocyte), polychromasia cells (+), normoblast (-)

Leukocyte: The impression of normal number is dominated by segmented neutrophils, atypical lymphocytes (+), immature granulocytes (+) (myelocytes), blast (-)

Platelets: Impression of normal amount, giant platelet (-)

Impression: Poikilocytosis normochromic anemia

Figure 2. Capture of Patient’s HDT.

Table 2. Coagulation Test Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>21/1/2020</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPT</td>
<td>9.8</td>
<td>9 – 12 detik</td>
</tr>
<tr>
<td>APTT</td>
<td>74</td>
<td>23 – 33 detik</td>
</tr>
</tbody>
</table>

Table 3. Hematology Test Results (15/06/19)

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleed Time (BT)</td>
<td>2 minutes</td>
<td>1 – 3 minute(s)</td>
</tr>
<tr>
<td>Clotting Time (CT)</td>
<td>40 minutes</td>
<td>9 – 15 minutes</td>
</tr>
<tr>
<td>Von Willebrand Factor</td>
<td>242</td>
<td>50 – 160 %</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>1</td>
<td>60 – 150 %</td>
</tr>
<tr>
<td>Factor IX</td>
<td>80</td>
<td>60 – 150 %</td>
</tr>
</tbody>
</table>
Clinical Diagnosis and Procedure

The patient was diagnosed with hemophilia A with posterior dextra femoral hematoma by the clinician. The patient received tranexamic acid, ketorolac and koate injection therapy.

Discussion

Cases of hemophilia A with posterior dextra femoral hematoma in women is a very rare case. Hemophilia A generally occurs in men. Siddiqi et al. (2010) reported 19.92% cases of hemophilia A from the total male population in America. Karim & Jamal, 2013 reported that 1 in 10,000 male births suffer from hemophilia A. The most recent data on cases of hemophilia A in Indonesia were 314 cases in 2001. The United States Universal Data Collection project (UDC) describes the ratio of women to men with hemophilia A of 1:32. The incidence of hemophilia A cases in women is associated with lyonization phenomena, the lack of X chromosomes and genetics of both parents of the patient4–7.

In this patient, a hematoma was found in the right posterior femur region. Repeated episodes of bleeding can result in joint deformities in the patient’s knee, known as Haemophilic Arthropathy. Hemarthrosis is the most common clinical characteristic, especially in cases of severe hemophilia A. The AUC value of joint involvement in hemophilia A is 63% (p = 0.099), when combined with the AUC value of the clinical degree of hemophilia 73% (p = 0.004) it becomes an important diagnostic value in describing the quality of life of hemophilia A patients, where AUC increases to 76.6. % (p = 0.001)8,9.

The result of hematological examination in this patient showed FVIII of 1%, therefore the patient was classified as ‘severe hemophilia A’ and the patient routinely received FVIII infusion therapy for the last 8 months. Patients are suspected of having an FVIII inhibitor if they do not respond after receiving adequate FVIII infusion at the time of bleeding or if FVIII levels are lower than prediction after receiving infusion or if there is no increase in FVIII after infusion. The stagnant levels of FVIII indicates the presence of FVIII inhibitors. Rikarni et al. (2013) found that patients who first received FVIII replacement therapy at <12 months of age had 2.66 times the risk of forming FVIII inhibitors compared to patients who first received FVIII replacement therapy at ≥12 months of age with p value <0.032,3.
ISTH (International Society of Thrombosis and Haemostasis) classifies hemophilia A patients as high responders if the inhibitor titer is >5 BU/mL and as low responders if the inhibitor titer is ≤ 5 BU/mL. In this case, the FVIII Inhibitor Assay examination result was 928 BU. Miller et al. (2015), reported 23 (2.8%) hemophilia A patients with inhibitors of which 9 were high responders. High responders tended to be younger than low responders (median value 2 years vs 11 years, p value <0.016). There were significant differences in the severity of hemophilia in the high responder group, namely severe, moderate, and mild with percentage of 78%, 22%, and 0% respectively, and with p value <0.05\(^{10,11}\).

There were limitations in this case, namely other antibody tests and genetic testing was not performed. Aside from hemophilia patients treated with FVIII (alloantigen), antibodies against FVIII can also occur in healthy individuals and patients with autoimmune diseases (autoantigen)\(^1\).

**Conclusion**

The diagnosis was made based on the results of hematological examination of factor VIII and its inhibitors, so it could be concluded that the patient was diagnosed with hemophilia A with inhibitors.

**Conflict of Interest:** The author declare that they have no conflict of interest.

**Source of Funding:** None.

**Acknowledgements:** We thank Rr. Putri Amaristya Purwono and Arif Nur Muhammad Ansori for editing the manuscript.

**Ethical Approval:** This study approved by the ethical committee of the Faculty of Medicine, Airlangga University - Dr. Soetomo Hospital, Surabaya, Indonesia.

**References**

Topical Application of Povidone Iodine to Minimize Post Appendectomy Wound Infection

Mamoon Othman Khalid Alabdulla¹, Asaad Mohammed Kadhim², Hussein Abduljabbar Shihab Al-Katrani³

¹Surgent, Researcher, Department of Surgery, AL-Mawani Teaching Hospital, Basrah Health Directorate, Ministry of Health and Environment, Basrah, Iraq, ²Assistant Professor, College of Medicine, University of Basrah, Department of Surgery, AL-Mawani Teaching Hospital, Basrah Health Directorate, Ministry of Health and Environment, Basrah, Iraq, ³Surgent, Researcher, Department of Surgery, AL-Mawani Teaching Hospital, Basrah Health Directorate, Ministry of Health and Environment, Basrah, Iraq

Abstract

Background: Despite the use of prophylactic antibiotic and sterilization techniques and nemours methods of wound care, post appendectomy wound infection is still high.

Methods: A randomised control study (RCS) has been implied to prove, whether uses of povidone iodine just prior to wound suturing could decrease surgical wound infection rates after surgery for appendectomy. A total number of 120 patients operated for acute appendicitis with open appendectomy at AL-Mawani Teaching Hospital during the period from March 2016 to October 2020.

Results: After appendectomy patients were randomised into three groups, group A which include 40 patients the subcutaneous tissue was irrigated with 1% diluted povidone-iodine solution before skin closure. Group B which contained other 40 patients the wound has been washed with normal saline, and in group C (control group) no irrigation was done. All patients were followed for surgical site infection according to Southampton wound grade system for ten days after surgery. In this study we notice that a reduction happened in the number of wound infection in group A compare with group B and C.

Conclusion: The uses of povidone iodine 1% before skin closure is an effective method in reducing the rate of infection of the wound after surgery for acute appendicitis.

Keyword: Povidone iodine, wound infection, Acute appendectomy

Introduction

Acute appendectomy is a common surgical emergency in a wide range of ages and sexes. Post appendectomy wound infection is a frequent complication after appendectomy even by the use of antibiotic before surgery as prophylaxes and proper sterilization techniques, percentage of post appendectomy wound infection are quite high reaching to 18% - 20% (1,2). Povidone iodine is composed of iodine, iodide and polyvinyl pyrrolidone dissolved in sterilized water. Data had been shown that its action against bacteria will be increased by dilution in a range between 0.1 – 1% and its effect as bactericidal will be more than 10% strength (3), at this concentration it is neither toxic to the tissue cells or interfere with healing of the wound and has been FDA approved for short course therapy to prevent superficial wounds infection (3-6). The present study was designed to compare the efficacy of povidone – iodine 1% irrigation versus normal saline irrigation and no irrigation applied.

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to the wound before closure. Importance of this study as if 1% povidone-iodine decreases. The surgical site infection, then it will be a simple and inexpensive remedy for prevention of surgical site infection.

Methods

A prospective randomized controlled study was conducted in the surgical units of AL–Mawani Teaching Hospital, Basrah, Iraq, from March 2016 to October 2020. It included patients from the two sexes who were brought to the hospital with a diagnosis of acute appendicitis with the aid of clinical, laboratory and radiological investigations and confirm during operation, later by histopathological studies of the removed appendices. A total number of 120 patients above 13 years of age (55 female and 65 male) who underwent open appendectomy via a grid iron incision. We exclude patients who were sensitive to prophylactic antibiotic use in the study (third generation cephalosporin), patient with immune deficiency (diabetic mellitus, chronic renal failure, chemotherapy, radiotherapy or corticosteroids therapy).

All patients were given 1 gram of third generation cephalosporin intravenously as prophylaxis against infection at induction of anesthesia.

In this study group A, before skin closure, subcutaneous tissue was irrigated by 1% povidone-iodine using 10cc syringe, kept there for 2 to 3 minute and then aspirated. Group B the subcutaneous tissue was washed by normal saline using 10cc syringe. Group C no irrigation was done.

Skin closure was done by interrupted sutures and then aseptically dressed. Other two doses of ceftriaxone 1gm given postoperatively intravenously. Patient examined for surgical site infection after 10 days in the outpatient clinic.

Results

In this study we included a total number of 120 patients [65 male (54.16%) and 55 female (45.84%)]. All patients ages were from 13-45 years. The wound of the patients was examined post operatively after tendays, and had been graded by using Southampton grading system into 5 grades (0 – 4) as in figure 1, 2, 3. Surgical site infection in Southampton grading system was from grade II and above. It was presented in overall 24 (20%) of patients. In group A it presented in six patients (15%) and in group B eight patients (20%) and in group C ten patients (25%).
Table 1. Comparison of wound infection between the three groups following appendectomy.

<table>
<thead>
<tr>
<th>Wound grade by Southampton</th>
<th>No. N = 120</th>
<th>Group A Povidone irrigation N = 40</th>
<th>Group B NS wash N = 40</th>
<th>Group C No irrigation N = 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0: healing is normal</td>
<td>89 (74.167%)</td>
<td>31 (77.5%)</td>
<td>30 (75%)</td>
<td>28 (70%)</td>
</tr>
<tr>
<td>Grade I: normal healing + mild bruising</td>
<td>7 (5.834%)</td>
<td>3 (7.5%)</td>
<td>2 (5%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Grade II: erythema / tenderness / heat</td>
<td>8 (6.6%)</td>
<td>3 (7.5%)</td>
<td>2 (5%)</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>Grade III: serous discharge</td>
<td>8 (6.6%)</td>
<td>2 (5%)</td>
<td>3 (7.5%)</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>Grade IV: purulent discharge</td>
<td>8 (6.6%)</td>
<td>1 (2.5%)</td>
<td>3 (7.5%)</td>
<td>4 (10%)</td>
</tr>
</tbody>
</table>
Discussion

In this study we try to compare the three methods and substances. That may assist in decreasing the rate of the infection in the surgical wound. After appendectomy we notice that the rate of wound infection was decrease.

In group A When we use syringe irrigation with 1% povidone – iodine (15%) In comparison with group B in which we use syringe irrigation with normal saline (20%). And in group C in which no irrigation was used to (25%).

The wound infection is due to operative contamination and many ways are used to reduce such contamination\(^7\), many factors play a role in postoperative wound infection, including perioperative and intraoperative care and management with evaluation of any surgery to reduce infection rate\(^8\); wound wash and debridement are important for wound healing as shown by many experimental trauma cases\(^9\).

In surgical wound infection pus discharge from the wound indicate infective process. This pus discharge is believed to be due to production of inflammatory mediators.

Antiseptics are substances that kills or inhibit the microorganisms growth, so it will reduce the formation of pus in the wound cavity\(^3^,\(^4\). Sindelar and Mason study show that wash of abdominal and urological wound with 10% povidone-iodine solution will lead to reduce pus formation from the wound\(^10\). The study done by Hiramatsu and colleagues show that there is a benefit from use of povidone-iodine applying to the wound in reducing wound infection postoperatively\(^11\).

Conclusion

The present study we conclude that wound syringe irrigation with diluted 1% povidone-iodine is a safe way, low cost and currently available in any theater room which does not interfere with healing process and it will significantly reduced the surgical site infection and pus formation.

Conflict of Interest: The authors declare no conflicts of interest.

Funding: This study was unfunded.

Ethical Clearance: Taken from AL– Mawani Teaching Hospital committee

References

10. Sindelar WF, Mason GR. Irrigation of subcutaneous tissue with povidone-iodine solution for prevention

Factors Associated with the First Sexual Intercourse among Students: Population-Based Study

Manasanun Limpavithayakul¹, Chuthamat Nopparat²

¹Lecturer at Primary Health Care Management Program, Faculty of Science, The Eastern University of Management and Technology, Ubon Ratchathani, Thailand, ²Assistant Professor at Department of Research and Medicine Innovation, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand

Abstract

Background: According to the current situation, Thai adolescents have premature sex and unprotected sex, resulting in pregnancy problems and sexually transmitted diseases in students. This study aims to study the factors affecting to first sexual intercourse among students: a population-based study. Methods: A cross-sectional analytical study was conducted between January 1 and June 30, 2018 among 328 students. That using data from the surveillance participant characteristics for first sexual intercourse among students between the ages of 12-18 years old in the study area, Ubon Ratchathani Province. The instrument used was a self-administered questionnaire. Data analysis was used by EPI Info 7 program and SPSS version 20. Logistic regression analysis was performed to identify the factors associated with first sexual intercourse among students by multivariate analysis (95%CI).

Results: The results findings revealed that respondents were females 53.0% with an average age of 16.5 ± 2.4 years old. Around 64.6% had experienced sexual intercourse, their first sexual intercourse was found at the average age of 14.1 ± 1.6 years old and did not use a condom when having the first sexual intercourse 44.4%. The multivariate analysis found significantly greater for males 2.15 times (ORadj = 2.15, 95%CI= 1.36-3.22), significantly greater for students income >5,000 Bath/month compared to income ≤ 5,001 Bath/month, 3.35 times (ORadj = 3.35, 95%CI= 2.82-7.67), experience alcohol consumption were 6.42 times (OR adj = 6.42, 95%CI= 3.75-9.97) and low-medium of the attitude sexually transmitted diseases were 4.88 times (OR adj = 4.88, 95%CI= 2.89-6.98). Conclusion: The modernized training program in the accurate attitude, first sexual intercourse and sexually transmitted diseases, values, and self-esteem to prevent sexual risk behaviors with appropriate gender norms and the recent era among students need to be considered.

Keywords: First sexual intercourse, Students, Adolescents, Sexually transmitted diseases

Introduction

Adolescents are the ages that go from childhood to adulthood. They are between the ages of 12 and 20, with females entering the adolescence faster. Males are about 1-2 years old. According to the current situation, Thai adolescents have premature sex and unprotected sex, resulting in pregnancy problems in adolescents. Abortion and sexually transmitted diseases are more likely. As adolescents are of physical, mental, and social, changes in all dimensions are at an age when the need for self-reliance increases. Adolescents are sensitive, easy to change due to the complete sexual development of the hypothalamus, pituitary gland, and gonads, and women have the characteristics of femininity and masculinity. The results of sexual changes bring adolescents to a young age. The sex glands are responsible for the

Corresponding Author:
Chuthamat Nopparat (Dr.P.H).
Department of Research and Medicine Innovation, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand, 10300
Tel: +6661-165-5397
E-mail: Chuthamat.no@nmu.ac.th

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production of estrogen and testosterone, both of which cause the body to become young and influential as sexual drivers. Teenagers are the age of wanting to try it. Early adolescents begin to have sexual desire, interest in the opposite sex, and express sexual behaviors, such as being interested and trying to build close relationships. Talking holding your arm, arranging to be alone, two-on-two. Which makes it possible for there has a sense of satisfaction between each other. Confusion in the role itself between being a child and an adult and a friend has a high influence on the idea. Some teenagers have an emotional imbalance. Cannot adapt to the changes made. Nowadays, Thai society has changed the way of life and culture that embraces western culture values more everyday life, such as dressing. The embrace and handshake has resulted in a decrease in the value of love reserved for Thai culture. Today’s teenagers are teenagers in globalization with modern technologies such as internet, mobile phones, and other technologies. It has a social network like Facebook. Line makes communication easy, convenient to meet up, and talk between teenagers. Other environmental influences are that sexual media, alcohol consumption are easily accessible, and this age has changed psychosocial, with emotional expression, swaying, variability, and lack of mental inhibition. When there is sexual driving. As a result, sexual mismanagement is diminished. The students in school and aged between 12-15 years old are the unique sample group that have the transition between children and adolescents. Their social role and environment including self-esteem and sexual values changes. As a result, it is considered the beginning of sexual intercourse at the same time. Despite everything, Preventive intervention for adolescents because it is evident that sexual risky behaviors such as having sexual relationships with multiple partners and not using condoms act as factors that increase the STD infection rate. Therefore, considering the social lifting style and culture aspect in which sexual behaviors and norms are developed, a sexual risky group with a high rate of sexually transmitted disease, infection, and having sexual can be called a vulnerable group to sexual diseases rather than a socially hazardous group. There is a rarity on the study based on the first sexual intercourse among student group which is specific.

**Objective**

This research aimed to determine the factors associated with the first sexual intercourse among students in school, which provide useful information for the planning of counseling on premature sex in the school and parents.

**Material and Methods**

**Research model and sample**

A cross-sectional study was conducted between January 1 and June 30, 2018, among students in Ubon Ratchathani province of Thailand. Students aged between 12-18 years old were selected by multistage random sampling. After sample size calculation by using the estimated population mean equation, a total 328 students (including 5% missing cases) with males and females were recruited from schools.

**Research instruments**

The tool used for this research was a questionnaire, asking students’ demographic, had sexual intercourse, attitude ever have sexual intercourse, attitude sexually transmitted diseases and knowledge sexually transmitted diseases.

**Human research ethics**

In this research, research ethics were considered by the Human Research Ethics Committee, Ubon Ratchathani Provincial Public Health Office, No. SSJ. UB 2563-098 given on 20 October 2020.

**Data Analysis**

In this study, data were analyzed using frequency, percentage, mean, standard deviation to characterize and risk factors were used to determine the behaviors of the first sexual intercourse among students by univariate analysis and multivariate analysis at the 95% confident interval (95%CI).
Results

Total 328 participants were 154 (47.0%) males and 174 (53.0%) females, average age 16.5 ± 2.4 years old. The results from self-administered questionnaire about risk of characterize behavior and who ever have sexual intercourse among students who ever have sexual intercourse were 212 (64.6%) (Table 1). The mean age of their first sex was 14.1 ± 1.6 years old. The average scores of the attitude ever had sexual intercourse among students were in low and levels. The result shows the participants who ever had sexual intercourse did not use a condom when having the first sexual intercourse 92 (44.4%) participants. The results from univariate analysis found the factor associations between participant characteristics associated with the first sexual intercourse among students were significantly greater for males when as compared with females, 1.97 times (OR =1.97, 95%CI = 1.24-3.14) significantly greater for student age between 16-18 years old as compared to who age between 12-15 years old, 2.30 times (OR = 2.30, 95%CI= 1.45-3.67) significantly greater for students living alone/lover/friend compared to who were living with parent, 4.48 times (OR = 4.48, 95%CI= 2.71-7.40) significantly greater for students experience substance and experience alcohol when compared to those who had no experience. Participants who had Low - medium of the attitude sexually transmitted diseases were 4.14 times (OR = 4.14, 95%CI = 2.56-6.68) (Table 2).

Table-1: Participants who ever had sexual intercourse among students in Ubon Ratchathani province, Thailand (N=328)

<table>
<thead>
<tr>
<th>Participants who ever have sexual intercourse</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>212</td>
<td>64.6</td>
</tr>
<tr>
<td>No</td>
<td>116</td>
<td>35.4</td>
</tr>
</tbody>
</table>

Table-2: The univariate analysis factors associated with the first sexual intercourse among students

<table>
<thead>
<tr>
<th>Factors</th>
<th>Participants who ever have sexual intercourse</th>
<th>OR</th>
<th>95%CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n=212)</td>
<td>No (n=116)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>112</td>
<td>42</td>
<td>1.97</td>
<td>1.24-3.14</td>
</tr>
<tr>
<td>Female</td>
<td>100</td>
<td>74</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-18</td>
<td>124</td>
<td>44</td>
<td>2.30</td>
<td>1.45-3.67</td>
</tr>
<tr>
<td>12-15</td>
<td>88</td>
<td>72</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Living</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>living alone/lover/friend</td>
<td>127</td>
<td>29</td>
<td>4.48</td>
<td>2.71-7.40</td>
</tr>
<tr>
<td>living with parents</td>
<td>85</td>
<td>87</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>
Table-2: The univariate analysis factors associated with the first sexual intercourse among students

| Income (Baht) | | | | | |
|--------------|---|---|---|---|
| >5,000       | 118 | 37 | 2.86 | 1.67-4.31 | <0.001* |
| ≤ 5,001      | 94  | 79 | 1.00 |       |       |

| Relationships (girlfriend/boyfriend) | | | | | |
|--------------------------------------|---|---|---|---|
| Relationships                       | 168 | 35 | 8.84 | 5.27-14.82 | <0.001* |
| Not in relationships                | 44  | 81 | 1.00 |       |       |

| Experience substance | | | | | |
|----------------------|---|---|---|---|
| Yes                  | 63 | 15 | 2.85 | 1.54-5.28 | 0.001* |
| No                   | 149 | 101 | 1.00 |       |       |

| Experience alcohol | | | | | |
|--------------------|---|---|---|---|
| Yes                | 167 | 45 | 5.86 | 3.56-9.63 | <0.001* |
| No                 | 45  | 71 | 1.00 |       |       |

<table>
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<tr>
<th>Level of the attitude ever have sexual intercourse among students</th>
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<td>89</td>
<td>42</td>
<td>1.27</td>
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<td>Good – very good</td>
<td>123</td>
<td>74</td>
<td>1.00</td>
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<tr>
<td>Low - medium</td>
<td>147</td>
<td>41</td>
<td>4.14</td>
<td>2.56-6.68</td>
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<td>Good – very good</td>
<td>65</td>
<td>75</td>
<td>1.00</td>
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<tbody>
<tr>
<td>Low level (&lt;80% of corrected answer)</td>
<td>111</td>
<td>54</td>
<td>1.26</td>
<td>0.80-1.99</td>
</tr>
<tr>
<td>High level (≥80% of corrected answer)</td>
<td>101</td>
<td>62</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

*p-value <0.05

In the multivariate analysis (multiple logistic regressions adjusted for relationships (girlfriend/boyfriend) and experience with alcohol) we found the factor associations between participant characteristics associated with the first sexual intercourse among students were significantly greater for males compared with females, 2.15 times (OR adj = 2.15, 95%CI= 1.36-3.22) significantly greater for students age between 16-18 years old as compared to who age between 12-15 years old, 2.68 times (OR adj = 2.68, 95%CI= 1.65-3.89) significantly greater for students income >5,000 Bath/ mount compared to income ≤ 5,001 Bath/mount, 3.35 times (OR adj = 3.35, 95%CI= 2.82-7.67) significantly greater for students experience alcohol consumption compared to those had no experience were 6.42 times (OR adj = 6.42, 95%CI= 3.75-9.97) and participants who had Low - medium of the attitude sexually transmitted diseases were 4.88 times (OR adj = 4.88, 95%CI= 2.89-6.98). The result show the level of attitude ever had sexual intercourse among students and the level of
knowledge that sexually transmitted diseases not significantly with the first sexual intercourse among students (Table 3).

Table-3: Multivariate analysis factors associated with the first sexual intercourse among students

<table>
<thead>
<tr>
<th>Factors</th>
<th>Participants who ever have sexual intercourse (n=212)</th>
<th>ORa</th>
<th>ORadj</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>112</td>
<td>1.97</td>
<td>2.15</td>
<td>1.36-3.22</td>
<td>0.004*</td>
</tr>
<tr>
<td>Female</td>
<td>100</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-18</td>
<td>124</td>
<td>2.30</td>
<td>2.68</td>
<td>1.65-3.89</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>12-15</td>
<td>88</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Income (Baht)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 5,000</td>
<td>118</td>
<td>2.86</td>
<td>3.35</td>
<td>2.82-7.67</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>&gt; 5,001</td>
<td>94</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Relationships (girlfriend/boyfriend)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relationships</td>
<td>168</td>
<td>8.84</td>
<td>10.22</td>
<td>5.89-15.32</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Not in relationships</td>
<td>44</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experience substance</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>63</td>
<td>2.85</td>
<td>3.12</td>
<td>1.67-5.38</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>No</td>
<td>149</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experience alcohol</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>167</td>
<td>5.86</td>
<td>6.42</td>
<td>3.75-9.97</td>
<td>0.001*</td>
</tr>
<tr>
<td>No</td>
<td>45</td>
<td>1.00</td>
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<tr>
<td><strong>Level of the attitude ever have sexual intercourse among students</strong></td>
<td></td>
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<tr>
<td>Low - medium</td>
<td>89</td>
<td>1.27</td>
<td>1.34</td>
<td>0.96-2.14</td>
<td>0.344</td>
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<tr>
<td>Good – very good</td>
<td>123</td>
<td>1.00</td>
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<td><strong>Level of the attitude sexually transmitted diseases</strong></td>
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<tr>
<td>Low - medium</td>
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<td>4.14</td>
<td>4.88</td>
<td>2.89-6.98</td>
<td>0.003*</td>
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<td>Good – very good</td>
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<td>1.00</td>
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<tr>
<td><strong>Level of the knowledge sexually transmitted diseases</strong></td>
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<tr>
<td>Low level (&lt;80% of corrected answer)</td>
<td>111</td>
<td>1.26</td>
<td>1.38</td>
<td>0.93-2.09</td>
<td>0.336</td>
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<tr>
<td>High level (≥80% of corrected answer)</td>
<td>101</td>
<td>1.00</td>
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<td></td>
</tr>
</tbody>
</table>
$p$-value $< 0.05$, Goodness of fit $= 0.739$, Adjusted for Relationship (girlfriend/boyfriend) and Experience alcohol

a Univariate analysis, Chi-Square test

b Multivariate analysis, Multiple logistic regression

* $p$-value $< 0.05$

**Discussion**

According to the study, almost half of the students have ever had a sex with an average age of 14.1 years old for the first time. The rate exceeded national estimates among adolescent samples. This study presented an earlier age than the previous study by the Ministry of Public Health, Thailand, that found adolescences aged 15-16 years for the first sexual intercourse.9 This is likely to continue to decline. Since Thai society is influenced by Western culture, Thai adolescents are imitated and attitudes towards premature sex are common.8 And it has not a bad word to follow. Nowadays, Thai teenagers have changed attitudes and behaviors in their sexuality. Teenagers are getting in love, having girlfriends. The adolescents had first sex from an early age. Some couples have been in a relationship for a while, and there may be more than one sexual partner.9 The result shows the participants who ever had sexual intercourse did not use a condom when having the first sexual intercourse 92 (44.4%) participants. The first sexual intercourse of adolescents in Thailand does not use condoms up to 50 percent, which is at risk of HIV/aids, sexually transmitted diseases, and pregnancy. Because most teens drink alcohol before the first sex causing forgetting or failing to supply condoms. This study finding was supported by Baokhumkong, et al, that reported causes of the first sexual activity in early adolescence were due to having sexual intercourse but did not use a condom.10 And most have experienced sex with a boyfriend or a couple without protection. There have been reports on sexually risky behavior in the general population.9 Because teenagers believe that sex is a show of affection for each other. Most female students are voluntarily. Most of them were living alone/lovers/friends. These behaviors could increase the first sexual intercourse among students in school. The result found that the risk behaviors of HIV and sexually transmitted diseases among students 132 (62.3%) and 40.3% reported among undergraduate students in Canada, respectively.11-12 The earlier young women in Northeast Brazil started sexually, the more pregnancies she had until the time of her reproductive life, as well as the greater number of sexual partners.13 The multivariate analysis in this study showed that sex, age, income, relationships (girlfriend/boyfriend), experience substance, and experience alcohol were related to having sexual intercourse. All socio-demographic characteristics were related to sexual intercourse and HIV knowledge among Indonesian women ($p<0.05$).14 The associations between participants characteristics associated with the first sexual intercourse among students were significantly greater for males compared with females, 2.15 times because in Thai society the male was assumed to be the significant person for the decisions about sexual and reproductive health regarding the role of condom use, this effort and decision have to be empowered in both male and female to promote safe sex.15,16,17,18 The attitudes and behavior of rural Thai adolescent students aged 16 to 20 years from northern Thailand regarding sexual intercourse were significant differences by gender, religion, ethnicity, and household income between those who had previously had sex.8 The level of attitude ever having sexual intercourse among students and the level of knowledge about sexually transmitted diseases had no significant relationship with the first sexual intercourse among students.15 However, during this study and for many young people who start a sexual life in adolescence, this event is perceived as an opportunity to express the exercise of sexual autonomy and freedom, sensations that express a more emotional than rational meaning.19 Thai culture parents do not talk openly about sexual, and the condom used for first sexual intercourse is uncommon to carry. It is believed that people who carry condoms are sex workers and obsessed with sex. Therefore, friends, parents, and teachers should be suggested to be a considerable role for promoting the prevention of sexual risk behavior, the effect of not wearing a condom, in addition to HIV/
sexually transmitted diseases, and premature pregnancy and encouraging to understand and talk openly regarding sexual issues. Although Thai culture and society are conservative about sexual issues, they currently have been changed dramatically among students.20-21 The transition from childhood to adolescence affects the social role to be more freedom and foreign cultures have also influenced the students’ lifestyle and show intimacy with public expression of love with their partners.

**Conclusions**

The adolescents that are those between 12 and 18 years old, experience a stage of life characterized by late childhood to adolescence, with all their biological and psychosocial changes, being a period that usually coincides with entering into high school. Sexual behavior of youth could have reproductive health outcomes with an impact on individual and general population health as a result of the new social and subjective relationships that they should live, making it a vulnerable period of exposure to situations with health risk behaviors, especially those related to lifestyle, such as the use of toxic substances, alcohol and unsafe sexual practices.23-24 Most participants of the study had already begun their sexual life before 15 years old, especially men. There have relationships (girlfriend/boyfriend). Just over half of them were seeking information about sex with friends. Condom use was for the prevention of sexually transmitted infections. Condom was the most commonly known contraceptive and more frequently used while other methods like intra-uterine device, location, amenorrhea, and norplant were rarely mentioned.25 The high school is highlighted as a place of long term for youth, favorable to the construction and reconstruction of knowledge and values. However, due to the plurality of groups that compose it, it favors student’s vulnerability to health risk lifestyles such as alcohol and other drugs, unsafe practices in traffic, and risky sexual practices, among others. High rate of hazardous or harmful use are found and various factors were identified that can be used to guide interventions to reduce problem drinking among university students.26 Thus, it is extremely important to build supportive environments within the high school space for discussion of issues with their health, with emphasis on sexuality as away to manipulate the student, good attitudes ever have sexual intercourse among students are the tools for healthy sexuality issues can reducing their exposure to sexually transmitted infections, unwanted pregnancies and other risk factors.27 The modernized and innovative training program in the accurate attitude, first sexual intercourse and sexually transmitted diseases, values, and self-esteem to prevent sexual risk behaviors with appropriate gender norms and the recent era among students need to be considered. Therefore, early sexual initiation had a negative impact on these variables. Results indicate the need of intervention and continuous education on reproductive health among school students in Ubon Ratchathani province, Thailand.

**Acknowledgment:** We would like to express our special thanks to the Ubon Ratchathani Provincial Health Office. This study was supported by the BSS program of Bureau of Epidemiology, Department of Diseases control, Ministry of Public Health, Thailand. And Faculty of Medicine Vajira Hospital, Navamindradhiraj University and all respondents who cooperated well in this study.

**Ethical Clearance:** In this research, research ethics were considered by the Human Research Ethics Committee, Ubon Ratchathani Provincial Public Health Office, No. SSJ.UB 2563-098 given on 20 October 2020. Participants could refuse and/or leave this research at any time. The data in the evaluation forms was kept confidentially without specifying the participants’ names in the document.

**Conflict of Interests:** The authors have no conflicts of interest with the material presented in this study and research tool was self-assessment questionnaire consisting of questions related to sexual activities among students in school Ubon Ratchathani province. Participants received both written and verbal information before they agreed to participate in the study.

**Source of Funding:** This study was supported by Ubon Ratchathani Provincial Public Health Office,
School in Ubon Ratchathani province, Thailand.

References


Effect of Mannitol Hydration as Renoprotective on Cisplatin Induced Nephrotoxicity (CIN) in Head and Neck Cancer Patients

Mareta Rindang, Andarsari 1, Yunita Dyah, Kusumaningrum 2, Rosy Nurlita, Hapsari 3, Dwi Hari, Susilo 4, Dewi Wara, Shinta 1

1Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia, 2Postgraduate Student of Magister of Clinical Pharmacy, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia, 3Department of Pharmacy, Dr. Soetomo Hospital, Surabaya, Indonesia, 4Division Head and Neck Surgery, Department of Surgery, Faculty of Medicine, Universitas Airlangga / Dr. Soetomo Hospital, Surabaya, Indonesia

Abstract

Background: Cisplatin is a cytostatic agent used as treatments in head and neck cancer patients. Nephrotoxicity is one of cisplatin major side effects. This study aimed to evaluate the renoprotective effect of mannitol-hydration administration in head and neck cancer patients who receive cisplatin chemotherapy.

Methods: This was a cohort observational study to analyze the renal function of head and neck cancer patients who receives cisplatin before and after mannitol-hydration administration in IRNA Surgery and Soekardja Room of Chemotherapy at RSUD Dr. Soetomo Surabaya during June – September 2018. The data obtained were BUN, SCr, and eClCr Cockroft-Gault of each cycle. Result: A total of 52 patients were evaluated. The average value of creatinine serum, creatinine clearance, and BUN at each cycle were fluctuate. However, the average value of creatinine serum and BUN were within the normal limit. The result of statistical analysis using paired t-test in those parameters at each cycle were not statistically significant (p > 0.05) after mannitol administration except during first cycle to second cycle the SCr increased significantly and eClCr was decreased significantly (p-value 0.024; 0.006, CI 95% respectively ). At fourth cycle the eClCr also decreased significantly (p value 0.008, CI 95%). Conclusion: The renoprotective effect of mannitol in head and neck cancer patients who receive cisplatin chemotherapy is sufficiently adequate which can maintain the value of BUN and creatinine serum of patients at each cycle within the normal limit.

Key words: cisplatin, mannitol, nephrotoxicity, prevention

Introduction

In 2018, the prevalence of head and neck cancer around the world was ranked at seventh of most common cancer with new cases more than 850,000 and deaths around 450,0001. Management therapy of head and neck cancer based on NCCN Guideline can be surgery, radiotherapy, and/or chemotherapy. Cisplatin is the preferred chemotherapy agent which can be used along with radiation as a chemoradiotherapy regimen usually given in high dose (100 mg/m2 every 21 days for three cycles)2,3. Nephrotoxicity is the renowned major side effect (around 28-36%) in the form of both acute or chronic renal insufficiency which also becomes major consideration in giving cisplatin therapy. Cisplatin is mainly eliminated through kidneys (> 90%) approximately 25% is excreted in urine within the first 24 hours after administration4,5. Patients treated with cisplatin about 20% - 30% will experience nephrotoxicity after 10 days administration6,7.

A study by Arunkumar et al8 evaluated the renal injury in several patients at stage IIA to stage IVA with various types of cancer who received a 40-50 mg/m2 cisplatin regimen and reported the incident of renal injury in patients like hypomagnesemia, hypokalemia,
hypocalcemia, hypophosphatemia, increased of BUN and creatinine serum. Kidera et al\(^9\) also assessed the effect of cisplatin on renal who received cisplatin with doses > 60 mg/m\(^2\) for the first time and this study reported that 32% of patients experience nephrotoxicity which characterized by increase value of creatinine serum. Cisplatin nephrotoxicity is dose-dependent toxicity, it’s maybe prevented by hydration and other supplementation strategies like magnesium and mannitol. Mannitol as an osmotic diuretic agent has been widely used for renal protection because it can reduce the contact time between fluid and the tubular epithelium, this way is believed to protect against renal injury by accelerating the removal of renal toxin\(^{10,11}\).

A study by Morgan et al, reported the outcomes in patients who receive mannitol improved and a 2.6 increased probability of developing acute kidney injury among adults who did not receive mannitol\(^{12}\). Another study by McKibbin et al, in head and neck cancer patients who are using cisplatin 100 mg/m\(^2\) showed that the addition of mannitol decreased the incidence of grade 3 acute kidney injury compared to saline only group\(^{13}\). Williams et al\(^{14}\) also assessed the impact of mannitol administration, patients who acquired mannitol administration has lower average increase of creatinine serum (mg/dl) and experienced less frequent grade 2 or higher nephrotoxicity compared with patients who did not acquire it. Meanwhile, study by Beeler et al reported that the addition of mannitol to saline pre-hydration in patients who receive cisplatin therapy did not change the outcome of renal function decline measured by BUN/Cr ratio, GFR, creatinine, and BUN and the decline of renal function is limited to grade 1 and most patients recover\(^{15}\).

Based on the description above, we undertook a study to evaluate the renoprotective effect of mannitol 20% 500 ml administration with saline hydration in head and neck cancer patients who receive cisplatin chemotherapy by observing the renal function (seen from laboratory data BUN and creatinine serum value).

**Material and Methods**

This was a cohort observational study that evaluated the renal function of head and neck cancer patients before and after cisplatin combination chemotherapy administration. The patients were given the combination of mannitol (20% 500 ml) and saline hydration one time after cisplatin combination chemotherapy at each cycle. Laboratory examination according procedure established by RSUD DR. Soetomo including BUN, creatinine serum and estimated creatinine clearance with Cockcroft-Gault formula were carried out before patients received cisplatin combination chemotherapy as the baseline data (pre-data). The change of renal function was observed at the start of the next cycle of chemotherapy.

The study subject were patients diagnosed with head and neck cancer who received cisplatin combination chemotherapy in IRNA Surgery and Soekardja Room of Chemotherapy at RSUD Dr. Soetomo Surabaya during June – September 2018. Patients were included if they aged between 21 years old up to 80 years old and had normal function of liver, heart, kidney and lungs. Another important inclusion criteria was patients voluntarily to be involved in this study by signing the informed consent. Meanwhile, patients were excluded in this study if they were allergic to cisplatin and received another therapy that can also induced nephrotoxicity such as aminoglycoside, cyclophosphamide, and others.

The normality of the data distributions was assessed using the One Sample Kolmogorov-Smirnov test before analysis. Comparison of the patient renal function before and after mannitol-hydration administration was performed using the paired t-test if the data were normally distributed and Wilcoxon signed-rank test if the data were not. The differences of renal function between patients with cisplatin-5FU regimentation and cisplatin-paclitaxel regimentation were also evaluated using independent t-test if the data were normally distributed or Mann-Whitney test if the data were not. P-values ≤ 0.05 were considered statistically significant. The obtained data were recorded in a special form and then processed through SPSS version 24.0 for Windows.
with confidence interval 95%.

**Result and Discussion**

**Patients characteristic**

A total of 52 patients were included in this study between June - September 2018 with sample characteristics shown in Table 1. The percentage of male patients (67%) was higher than female patients (37%). Head and neck cancer is more common and affected significantly in males rather than females with a ratio 2:1 to 4.11,16. In this study, the patient’s mean age range is between 41-64 years (mean 51.92 years), several studies reported that patients with age ≥ 50 years had a higher incidence of cisplatin-related nephrotoxicity17,18. Most of the patients suffering from nasopharyngeal cancer (33%) and then followed by squamous cell carcinoma (23%) and tongue cancer (18%). Nasopharyngeal cancer is the most common site type of head and neck cancer.

All patients in this study receive chemotherapy minimally for three cycles and up to six cycles for several patients with chemotherapy regimentation were combination of cisplatin-5fluorouracil in 42 patients and combination of cisplatin-paclitaxel in 10 patients. Both of them are included as recommended regimens or first-line systemic therapy for advanced head and neck cancer patients2. However, a study by Gibson et al19 showed that there were no statistically significant difference of overall survival or response rate and toxicity between cisplatin-5FU regimen compared with cisplatin-paclitaxel in head and neck cancer patients.

The dose of cisplatin received by the patient varies from 35 mg/m2 up to 100 mg/m2 and the most frequently used was 100 mg/m2. A study by Kidera et al and Prasaja et al showed that cisplatin-induced nephrotoxicity occurred in patients who received doses > 60 mg/m2 in around 32% and 34.1% respectively9,17. Therefore, the dose of cisplatin is one of risk factors that affect cisplatin nephrotoxicity. Hydration frequency in cisplatin-5 fluorouracil regimen was 166 times and in cisplatin-paclitaxel regimen was 47 times, so hydration frequency in this study overall was 213 times.

<table>
<thead>
<tr>
<th>Characteristic of Sample</th>
<th>Total Patients (n = 52)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>33</td>
<td>63</td>
</tr>
<tr>
<td>Females</td>
<td>19</td>
<td>37</td>
</tr>
<tr>
<td>Age (years)</td>
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<td></td>
</tr>
<tr>
<td>21-40</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>41-60</td>
<td>28</td>
<td>54</td>
</tr>
<tr>
<td>61-80</td>
<td>14</td>
<td>27</td>
</tr>
<tr>
<td>Mean</td>
<td>51.92 ± 11.32 (41 – 64)</td>
<td></td>
</tr>
<tr>
<td>Types of Head and Neck Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasopharyngeal cancer</td>
<td>17</td>
<td>33</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>Tongue Cancer</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Group of Other Types of Head and Neck Cancer</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Maxilla Cancer</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Mandibula Cancer</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
The change of creatinine serum, creatinine clearance, and BUN value in all patients on each chemotherapy cycle were shown in figure 1 until figure 3 respectively. The average value of those three parameters were fluctuating. Based on analysis using independent t-test, there were no statistically significance difference on patient renal function (SCr p-value 0.223; eGFR p-value 0.418; and BUN p-value 0.677, CI 95 %) between cisplatin-5 fluorouracil group and cisplatin-paclitaxel group.

Several previous studies evaluated the effect of mannitol on cisplatin-related nephrotoxicity. Three studies by Morgan et al, McKibbin et al, and Williams et al revealed that mannitol administration with cisplatin concomitantly has a significant protective effect that allows patients to receive cisplatin therapy with low risk of nephrotoxicity,12–14 . Even though in this study patients had already received saline-mannitol 20% 500 ml hydration combination one time after each cycle, the average value of patient renal function like SCr, eClCr, and BUN were fluctuating. However, the average value of creatinine serum and BUN were within the normal limit.
Figure 1: The average creatinine serum (SCr) value of patients from 1st to 6th cycles

Figure 2: The average creatinine clearance (ClCr) value of patients from 1st to 6th cycles

Figure 3: The average BUN value of patients from 1st to 6th cycles
Table 2: Statistical analysis result of renal function in all 52 patients who receive cisplatin combination chemotherapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Patients (n)</th>
<th>Creatinine Serum (mg/dl) Mean ± SD</th>
<th>Creatinin Clearance (ml/min) Mean ± SD</th>
<th>BUN (mg/dl) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>p-value</td>
</tr>
<tr>
<td>Cycle I</td>
<td>Cycle II</td>
<td>52</td>
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<tr>
<td></td>
<td></td>
<td>0.89 ± 0.26</td>
<td>1.01 ± 0.40</td>
<td>0.024</td>
</tr>
<tr>
<td>Cycle II</td>
<td>Cycle III</td>
<td>52</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>1.01 ± 0.40</td>
<td>0.99 ± 0.29</td>
<td>0.745</td>
</tr>
<tr>
<td>Cycle III</td>
<td>Cycle IV</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.98 ± 0.29</td>
<td>0.95 ± 0.25</td>
<td>0.660</td>
</tr>
<tr>
<td>Cycle IV</td>
<td>Cycle V</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.94 ± 0.26</td>
<td>1.05 ± 0.29</td>
<td>0.082</td>
</tr>
<tr>
<td>Cycle V</td>
<td>Cycle VI</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.97 ± 0.12</td>
<td>1.02 ± 0.22</td>
<td>0.253</td>
</tr>
</tbody>
</table>

Table 3: Statistical analysis result of renal function patients who receive combination cisplatin-5-fluorouracil regimen

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Patients (n)</th>
<th>Creatinine Serum (mg/dl) Mean ± SD</th>
<th>Creatinin Clearance (ml/min) Mean ± SD</th>
<th>BUN (mg/dl) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>p-value</td>
</tr>
<tr>
<td>Cycle I</td>
<td>Cycle II</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.85 ± 0.23</td>
<td>1.01 ± 0.42</td>
<td>0.008</td>
</tr>
<tr>
<td>Cycle II</td>
<td>Cycle III</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.01 ± 0.42</td>
<td>0.98 ± 0.31</td>
<td>0.429</td>
</tr>
<tr>
<td>Cycle III</td>
<td>Cycle IV</td>
<td>19</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0.96 ± 0.31</td>
<td>0.97 ± 0.28</td>
<td>0.945</td>
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<tr>
<td>Cycle IV</td>
<td>Cycle V</td>
<td>14</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0.94 ± 0.31</td>
<td>1.07 ± 0.35</td>
<td>0.212</td>
</tr>
<tr>
<td>Cycle V</td>
<td>Cycle VI</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.93 ± 0.09</td>
<td>0.98 ± 0.21</td>
<td>0.407</td>
</tr>
</tbody>
</table>
Table 4: Statistical analysis result of renal function patients who receive combination cisplatin-paclitaxel regimen

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Patients (n)</th>
<th>Creatinine Serum (mg/dl)</th>
<th>Creatinin Clearance (ml/min)</th>
<th>BUN (mg/dl)</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Cycle I</td>
<td>Cycle II</td>
<td>10</td>
<td>1.05 ± 0.33</td>
<td>1.00 ± 0.32</td>
</tr>
<tr>
<td>Cycle II</td>
<td>Cycle III</td>
<td>10</td>
<td>1.00 ± 0.32</td>
<td>1.07 ± 0.21</td>
</tr>
<tr>
<td>Cycle III</td>
<td>Cycle IV</td>
<td>7</td>
<td>1.04 ± 0.22</td>
<td>0.92 ± 0.17</td>
</tr>
<tr>
<td>Cycle IV</td>
<td>Cycle V</td>
<td>7</td>
<td>0.92 ± 0.17</td>
<td>1.02 ± 0.12</td>
</tr>
<tr>
<td>Cycle V</td>
<td>Cycle VI</td>
<td>3</td>
<td>1.05 ± 0.17</td>
<td>1.11 ± 0.27</td>
</tr>
</tbody>
</table>

Effect of mannitol-saline administration in the change of SCr, eClCr and BUN of patients

The differences in patients renal function value (SCr, eClCr, and BUN) in each cycle before and after mannitol-hydration administration were shown in Table 2. Furthermore, the differences in patients renal function during each cycle of chemotherapy before and after mannitol-hydration administration in both cisplatin-5fluorouracil group and cisplatin-paclitaxel group listed in Table 3 and Table 4. In cisplatin-paclitaxel regimen, group, the number of samples that receive chemotherapy up to fifth cycle and sixth cycle only 3 patients so it did not meet the requirements for differential statistical analysis using paired t-test. Based on the statistical analysis result in all 52 patients, it seems renal function impairment had occurred after first cycle cisplatin combination chemotherapy administration which is characterized by increased creatinine serum and decreased creatinine clearance significantly (p-value 0.024; 0.006, CI 95% respectively). It’s only occurred in group of patients with cisplatin-5FU regimentation therapy (p-value <0.05) but not in group of patients with cisplatin-paclitaxel regimenation (p-value >0.05).

Furthermore, the average value of creatinine clearance also decreased significantly after the fourth cycle of chemotherapy (p-value 0.008, CI 95%). It’s only occurred in patients with cisplatin-paclitaxel regimenation which was also accompanied by a significant increase in creatinine serum otherwise it did not occur in the group of patients with cisplatin-5FU regimenation. This result was consistent with the study by Tezcan et al and Prasaja et al. that state renal function reduction occurred after first cycle of cisplatin administration17,20. Based on those two studies, renal impairment function will be worsened along with increasing numbers of chemotherapy which characterized by creatinine clearance decline after each cycle and it might be permanent.
The average BUN value of all 52 patients who received cisplatin combination chemotherapy was increase after each cycle but not statistically significant (p-value > 0.05) and remained within the limit of the reference value. However, after fifth cycle those value was decline but also not statistically significant. It’s happened because in the group patients with cisplatin-5FU regimination had statistically significant decrease of average BUN value after fifth cycle (p-value 0.042, CI 95%). A study by Arunkumar et al and Teczan et al, reported that BUN value parameter will increase after cisplatin chemotherapy treatment, it’s just that in the study by Arunkumar et al the increase was not statistically significant as well as in this study\textsuperscript{8,20}.

This study result was consistent with the study by Leu and Baribeault which performed in patients using 40 mg/m2 of cisplatin (n= 92) showed that there were no statistically significant differences in average eCICr decrease (p = 0.09) in patients received either mannitol-hydration (n=46) or hydration alone (n=46)\textsuperscript{21}. A similar result also showed in a study by Beeler et al, that there were no statistically significant difference (p > 0,05) of BUN/Cr ratio, GFR, creatinine, and BUN between patients received mannitol-saline (n=25) and saline alone (n=23) during 14 days during cisplatin therapy\textsuperscript{15}.

Conclusion

In conclusion, the renoprotective effect of mannitol in head and neck cancer patients who receive cisplatin chemotherapy is sufficiently demonstrated in this study. Mannitol administration can maintain the value of BUN and creatinine serum of patients at each cycle within the normal limit. Although the average value of the patient renal function (SCr,eCICr, and BUN) at each cycle were fluctuating and the result of statistical analysis using paired t-test in those three parameters were not statistically significant (p > 0,05) except on first cycle and on fourth cycle that eCICr decreased significantly. Several risk factors like cisplatin dosage and patient age might affect cisplatin-related nephrotoxicity which can also affect the effectiveness of mannitol therapy as renoprotector.

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Ethical Clearance: Taken from Dr. Soetomo Hospital’s Ethics Comitie

Statement conflict of Interest: The authors disclose no potential conflicts of interest.

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References


The Function of the State in Providing Health Services: Indonesia Perspective

Marif¹, Muhammad Yunus Idy¹, Nurhaedah², Handar Subhandi Bakhtiar³

¹Faculty of Law, Universitas Islam Makassar, Indonesia, ²Faculty of Law, Universitas Muslim Indonesia, Indonesia, ³Faculty of Law, Universitas Pembangunan Nasional Veteran Jakarta, Indonesia

Abstract

The function of the state in providing health services is divided into the regulatory function, the controlling function, the entrepreneurial function and the supervisory function. In the regulatory function, the state develops policies in the field of health services that ensure that all people can enjoy health services. The state control function is responsible for the availability of equipment and medicines as well as maintaining the stability of the flow of health service distribution. In the entrepreneurial function, the state in addition to providing health services can receive income from health services which can later be used to equalize the costs of health care dependents, especially for the poor and underprivileged. In the oversight function, the state is responsible for carrying out full supervision of health facilities in order to optimally provide health services to the people. Keywords: State functions, health services, the right to health.

Keywords: State Functions, Health Services, The Right to Health

Introduction

The responsibility of the state for its people specifically in the health sector is contained in the constitution, namely in the fourth paragraph Opening of the 1945 Constitution of the Republic of Indonesia, as one of the goals of the state which is protecting all the people of Indonesia and all of Indonesia’s blood spilled. This is in relation to the objectives of the rule of law as constitutional and philosophical foundations as a nation and state. In addition, the mandate of Article 43 paragraph (1) of the 1945 Constitution of the Republic of Indonesia (hereinafter referred to as the 1945 Constitution) expressly stipulates that The poor and neglected children are maintained by the state, further on Article 34 paragraph (2) stipulated that the State develops a social security system for all people and empowers people who are weak and unable to match human dignity. Furthermore, Paragraph (3) regulates that: The State is responsible for the provision of adequate health service facilities and public service facilities.

In addition to Article 34, the constitution provides for the protection of human rights. In protecting human rights in the health sector, it can be seen in Article 28H paragraph (1) which regulates that every person has the right to live in a physical and spiritual prosperity, to live and obtain the environment. good and healthy and have the right to get health services. Furthermore, paragraph (2) regulates that every person has the right to get special facilities and treatment to obtain equal opportunities and benefits in order to achieve equality and justice. Whereas paragraph (3) states that every person has the right to social security which enables the development of himself as a whole with dignified humanity.

Talking about health services, especially about the health service system in hospitals and health clinics, is inseparable with regard to consumer protection efforts.
in Indonesia today, because the health service delivery system concerns the relationship between health service providers and consumers (health service recipients), also very related with regulations and policies in the field of health, this has long been raised especially about the importance of legal protection for the parties, especially the efforts of the state in this case the government in improving the quality of health services that are dignified, humane and fair.

The problem of the health service system since the last few years has attracted a lot of attention, not only in the world of health (medicine), but also outside the health community (medicine), not only domestically, but also abroad. In the health service system there are 3 (three) groups of people who are at least involved, namely, first, the human health service provider (health providers, such as doctors, nurses and other medical personnel), the second is the group receiving health services (health consumers) and third, which is indirectly involved, for example administrators (both among companies and governments in this case the State).

The special nature of health care is that both health providers and consumers rarely consider cost aspects, as long as it involves the problem of healing a disease. Health providers will always be urged to use the latest capabilities, technology and medicines. This fact is also supported by the needs of consumers who want the best possible service, also in order to obtain a sense of security. This situation brings a tendency to ignore economic calculations, cost efficiency, and others. Therefore, it is not uncommon for health services to be considered merely consumptive in nature, regardless of the profit and loss aspects, including the legal aspects.

The above interests will be contrary to the interests of administrators (government, company leaders), who will more or less pay attention to aspects of the cost of health services. It is not uncommon for administrators to complain about the attitudes of consumers and health providers complain about the attitudes of administrators. Conflicts of interest stemming from human behavior will include a balanced solution, if an approach to the development of a balance between obligations and rights is adopted, as well as responsibility and legal protection to the public or health consumers. Besides that, negative behavior must also be eliminated.

The right to health services is obtained since humans are still in the womb. This right is a part of basic human rights known as human rights. Although this basic right has been recognized by various religions and follows the development of the world, the literature records the name of John Locke as its originator. In the fourth paragraph of the Preamble of the 1945 Constitution it is strictly stated that: The state protects all of the Indonesian people and all of Indonesia’s blood spilled (which has been redeemed) protection which is intended is no exception also the issue of guarantee of rights in the health sector. In 1960, the right to health was only recognized in Indonesian law. Article 1 of Law No. 9 of 1960 regulates that: Every citizen has the right to obtain the highest degree of health and needs to be included in government efforts. This provision is updated in Article 4 of Law No. 23 of 1992, that: everyone has the same right in obtaining optimal health status. Likewise in Law No. 36 of 2009 (hereinafter referred to as Health Law), in Article 5 paragraph (1) the Health Law regulates that: everyone has the same right in obtaining access or resources in the health sector. Paragraph (2) regulates that: everyone has the right to obtain safe, quality and affordable health services.

In addition, in Article 4 letter g of Law No. 8 of 1999 (hereinafter referred to as UUPK) regulates that: Consumers have the right to be treated or served properly and honestly and not discriminatory. What is regulated in Article 4 letter g of the UUPK, then becomes the obligation of business / service actors as regulated in Article 7 letter C of the UUPK that: The obligation of business / service actors is to treat or serve consumers properly and honestly, not discriminatory. Likewise in Article 44 of Law No. 29 of 2004 (hereinafter abbreviated as the Medical Practice Law), regulates that: Doctors or dentists in carrying out medical practices must follow medical or dental service standards. Article 52 letter c of the Medical Practice Law stipulates that:
patients, in receiving services in medical practice, have the right to receive services according to medical needs. Thus, all professions involved in the field of health are required to re-explore the foundation of Indonesia’s state philosophy regarding the basic values adhered to, including also in health services, so that they can be consistently described from the central to the regional level. The basic values stated in the legislation need to specify the rights, obligations and responsibilities. For health, as a reference can be used the declaration of the United Nations (UN) on Human Rights and the Health Law are as follows:

1. Right:
   a) Everyone has the right to an adequate standard of living for health, including health care, and is entitled to guarantees in times of suffering (United Nations Declaration on Human Rights in 1948).
   b) Everyone has the same rights in obtaining access or resources in the health sector, including having the right to obtain safe, quality and affordable health services. (Article 5 paragraph (1) and (2) Health Law).

2. Responsible:

   The government is responsible for improving the degree of public health (Article 9 of the Health Law).

   The existence of free health programs as a form of government alignments to the needs of the poor in the health sector, as well as the government’s response to the mandate of the Basic Law, the Health Law and regulations in other health fields. The free health care program is a positive solution for the underprivileged (poor) people who expect a health service system that is safe, quality, and affordable. So that various complicated problems faced by the public so far, especially in the field of health, such as the high price of drugs, as well as the inaccessibility of treatment costs in hospitals, and the inability of the public to go to the doctor because the costs are quite expensive. It is hoped that the free health service program can solve various problems that have been faced by the underprivileged (poor) people.

   Hopefully it turns out that not everything can be in accordance with reality, many problems are still felt and faced by the poor (in poor) in an effort to obtain rights and free health program services.

Research Methods

This research is a normative-legal research using a statute and conceptual approaches. Those legal material collected are analysed descriptively related to the problems and prescriptively.

Analysis and Discussion

The State Functions As Regulator (Sturende)

The implementation of the State’s function of state responsibility in free health services is equitable, which can be seen from various existing laws and regulations. The laws and regulations referred to, among others, are contained in the 1945 Constitution can be seen in Article 34 paragraph (2) that the State develops a social security system for all people and empowers weak and unable to match human dignity. Furthermore, in paragraph (3) it is regulated that: The State is responsible for the provision of adequate health service facilities and public service facilities.

In addition to Article 34 of the 1945 Constitution of the Republic of Indonesia NRI above, the constitution also provides protection of human rights in the field of health which can be seen in Article 28H paragraph (1) of the 1945 Constitution of the Republic of Indonesia which regulates that every person has the right to live in a physical and spiritual prosperity, located living and getting a good and healthy environment and the right to obtain health services. Furthermore, paragraph (2) regulates that every person has the right to get special facilities and treatment to obtain equal opportunities and benefits in order to achieve equality and justice. Whereas paragraph (3) states that every person has the right to social security which enables the development of himself as a whole with dignified humanity.

The right to a healthy life is a basic right that must be guaranteed, because health is part of the primary
needs of every human being. Healthy condition of body and soul will enable every human being to do his activities and works. Health is also part of the needs towards a prosperous life. This kind of right is one of the basic rights in health services. But for low-income people, especially the poor, they need to get free health services for survival in the community and state, so that in the constitution they also provide guarantees for state responsibility.

In this case, it can be seen in Article 34 paragraph (1) of the 1945 Constitution of the Republic of Indonesia that: The poor and neglected children are cared for by the state this provision was formulated to provide guarantees to people who are weak in income to be given health insurance that should be free. The provisions are then formulated further with a social security system for all people and empowering the weak and unable to comply with human dignity and the provision of adequate health care facilities and public service facilities, these are regulated in Article 34 paragraph (2) and paragraph (3) The 1945 Constitution of the Republic of Indonesia. This shows that the state’s responsibility in the health sector is closely related to the state’s guarantee of the poor and displaced children.

Development policy in the health sector which was originally in the form of efforts to cure patients, gradually develops towards the unity of public health development efforts with a comprehensive, integrated and sustainable community participation which includes efforts to promotive, preventive, curative and rehabilitative. Based on the comprehensive, integrated and sustainable health development efforts, every effort to improve the level of public health is carried out based on the principles of non-discriminatory, participatory, protective and sustainable.

Furthermore, according to Article 1 point 1 of the Law No. 36 of 2009, what is meant by health is a healthy state both physically, mentally, spiritually which enables everyone to be socially and economically productive. The right of people to live healthy is a basic right that must be guaranteed. Because health is part of every human’s primary needs. Healthy condition of body and soul will enable every human being to do his activities and works. Health is also part of the need for a prosperous life. Basic rights in general and rights in health services in particular can be distinguished in basic social rights and individual basic rights.

The right to health care is a basic right which includes basic social rights and basic individual rights, so Health Services are all efforts and activities to prevent and treat disease, all efforts and activities to improve and restore health carried out on the basis of individual relations between medical service experts and individuals in need. As according to Wiku Adisasmita that health services are any efforts carried out alone or together in an organization to maintain and improve health, prevent and treat illnesses and restore the health of individuals, groups or communities.

Everyone has the right to health as regulated in Article 4 of the Health Law. The explanation of this Article stipulates that, The right to health referred to in this article is the right to obtain health services from health service facilities in order to realize the highest health status. Furthermore, the provision on the right to health services is regulated in Article 5 of the Health Law that everyone has the same right in obtaining access to resources in the health sector, has the right to obtain safe, quality and affordable health services, and is entitled to independently and independently. responsible for determining the health services needed for themselves.

As for the provisions of Article 6 of the Health Law, it is regulated that, Every person has the right to a healthy environment for the achievement of health status. So the right to healthy living and the right to get health services is a right that everyone has. The provisions of the Health Law further stipulate that the Government is responsible for fulfilling and guaranteeing the realization of these rights. The government is obliged to maintain and improve quality, equitable and affordable health services for all levels of society.

The responsibility that must be shouldered by the government in the health sector is ensuring the
availability of health resources according to the needs and all forms of health service efforts to fulfill the people’s right to health. Health resources in question include: health workers, health facilities, medical devices and pharmaceutical supplies, as well as other resources. The provisions of Article 15 of the Health Law stipulate that, The government is responsible for the availability of the environment, order, health facilities both physical and social for the community to achieve the highest degree of health. As for the provisions of Article 16 of the Health Law, it is regulated that.

In order to realize the right to a healthy life for the community, a source of health funding or health financing is needed. The Health Law regulates Health Financing, under the provisions of Article 170 of the Health Law it is formulated that health financing aims to provide sustainable health funding in sufficient quantities, fairly allocated, and utilized effectively and efficiently to ensure the implementation of health development so that improve the degree of public health as high as possible. The elements of health financing consist of sources of funding, allocation, and utilization. The sources of health financing come from the Government, regional government, community, private sector and other sources.

Based on the provisions on health financing, it can be interpreted that it is not possible for the government to endure or carry out its own health financing which is one of the main elements for the realization of the highest degree of health for the community. Because of that, it is necessary to hold a health financing guarantee which is at the same time the implementation of one of the national social guarantees.

Specifically the Government’s responsibility in the implementation of health insurance is regulated in Article 20 of the Health Law which is formulated that the government is responsible for the implementation of public health insurance through the national social security system for individual health efforts. The government is obliged to fulfill the right of the people to obtain the highest degree of health, namely the fulfillment of the right to live physically and mentally healthy, and fulfill their basic needs. Because the right to health services is a right that comes from human rights. Human rights in question are a set of rights that are inherently inherent and because of human existence as God’s creatures, are His gifts that must be respected, upheld by the state law.⁶

**According to Richard that:**

It is a well recognized principle that it is one of the first duties of a state to take all necessary steps for the promotion and protection of the health and comfort of its inhabitants. The preservation of the public health is universally conceded to be one of the duties devolving upon the state as sovereignty, and whatever reasonably likely to preserve the public health is a subject upon which the legislature, within its police power, may take action.

However, in this case it is necessary to maintain a balance between the implementation of the obligations of the state towards the people and the implementation of the obligations of the people towards the state, because the State / government will not be able to carry out or bear it on their own. The state’s obligation to fulfill people’s rights, including in terms of health financing, must be realized by the state. Instead the community’s obligation is to participate in realizing health in the family environment and itself is one form of social justice in health services, including in health financing. So the responsibility of fulfilling the needs of a healthy life is also the responsibility of the community, the government also regulates community participation in health services as formulated in Article 9 through Article 11 of the Health Law.

In the provision of Article 9 of the Health Law it is formulated that every person is obliged to participate in realizing, maintaining, and increasing the highest degree of public health. The obligations include the implementation of individual health efforts, public health efforts, and health-oriented development. As for Article 10 of the Health Law formulates that Everyone is obliged to respect the rights of others in an effort to obtain
a healthy environment, both physical, biological, and social. Whereas Article 11 of the Health Law stipulates that, Every person is obliged to behave in a healthy life to realize, maintain, and promote the highest health. Furthermore, Article 12 of the Health Law stipulates that, Everyone is obliged to maintain and improve the health status of others who are his responsibility. Whereas community obligations specifically related to the social health insurance program are regulated in Article 13 of the Health Law, which states that everyone has an obligation to participate in a social health insurance program.

As described above, that community participation is required for the realization of the highest degree of health. This is regulated in the provisions on Community Participation formulated in Article 174 of the Health Law, that People participate, both individually and organized in all forms and stages of health development in order to help accelerate the achievement of the highest degree of public health. Participation is intended to include active and creative participation.

**The State Functions As Provider**

Health services can be seen as an important aspect of social policy. Health is a determining factor for social welfare. A prosperous person is not just someone who has adequate income or a home. But also healthy people, both physically and spiritually.

Of course, health care is not a government monopoly. However, like social security and housing, public health services are also largely intended for underprivileged citizens. Public health service schemes are usually closely related to social security systems, especially social insurance, because some of the services involve or take the form of health insurance. In addition, the role of the government in public health services also includes the ownership of hospitals and health centers, including the establishment of policies on providers and health care providers conducted by the private sector.

Control of health providers is very closely related to doctors and health workers in providing health services to consumers in this case the patients. Therefore, it is very important for the State to provide control over doctors and health workers, so that the first need to be given responsibility to doctors is especially related to the obligations of a doctor because doctors become the main center of health care for patients.

According to J. Guwandi that the patient’s relationship with a doctor, the law establishes obligations as follows:

a) The doctor’s obligation to have the knowledge and skills of his profession. If someone already holds a doctor’s degree and has obtained a license to practice, then he must be expected, at least he has the ability, intelligence and skills of a doctor. If he is a specialist, then the benchmarks are also from a specialist in his field. According to Bambang Wibowo, Director General of Health Services of the Ministry of Health of the Republic of Indonesia that it is an obligation and obligation for every doctor to have knowledge and professional skills of a doctor if someone already holds a degree and works as a doctor, especially if he has obtained a license to practice, then he is already responsible to his profession and is expected to be able to carry out the duties and responsibilities of his profession, at least he has the ability, intelligence and skills of a doctor. He further said that if he is a specialist then the standard measure of a specialist doctor in his field, specialists obtained through special education specialists, knowledge and skills. Sources of knowledge and medical skills obtained from:

1) Faculty of medicine while still in college and clinical practice.

2) The results follow the development of his professional midwife by conducting research and reading literature, attending seminars, queries and international conventions.

3) The results of discussions with friends, conduct observations of the activities of other doctors in hospitals, clinics, etc.
b) The obligation to use their knowledge and skills with care, fairness and conscience as practiced by other doctors in the same circumstances.

The State function in the form of Control which is an important part of the health service system. Serious control from the government, especially for the provision of health services in a national health system to the community, especially for the poor and disadvantaged.

The national health system in question is an order that reflects the efforts of the Indonesian people to improve the ability to achieve optimal health status as a manifestation of public welfare through health development programs as a comprehensive, directed integrated and sustainable as part of national development.8

The aims and basis of health development within the National Health System are described as follows:

a) All citizens are entitled to obtain optimal health degrees, so they can work and live properly according to human dignity.

b) The government and the community are responsible for maintaining and enhancing the people’s health status.

c) The implementation of health efforts is regulated by the government and is carried out in an integrated manner with efforts to heal and recover.

d) Every form of health effort must be based on humanity based on the Godhead by prioritizing national interests, the people at large, and not merely the interests of groups or individuals.

e) The attitude, family atmosphere, mutual cooperation and all existing potential are directed and utilized as far as possible for health development.

f) In accordance with the principle of fair and equitable, the results achieved in health development must be enjoyed equally by the entire population.

g) All citizens are equal in law and must uphold and obey all statutory provisions in the health sector.

h) National health development must be based on a belief in one’s own abilities and strengths and on the basis of the nation’s personality.

Long-term development in the health sector, which is part of the National Health System, is directed towards achieving the main objectives of the health sector. The main objectives of national health include increasing the ability of the community to help themselves in the field of health, improving the quality of the environment that can guarantee health, improving the nutritional status of the community, reducing morbidity and mortality (mortality), developing healthy and prosperous families with increasing accepted the norm of a happy and prosperous small family.

Health services are any efforts carried out independently or jointly in an organization to improve and maintain health, prevent and cure illnesses and restore the health of individuals, families, groups and or communities. Health services according to Benjamin Lumenta all efforts to prevent and treat disease, all efforts and activities to improve and maintain health carried out by social institutions or institutions with a certain population, community or community

Furthermore Hodgels and Casio, differentiate personal health services or medical services and environmental health services or public health services. Leavel and Clark describe the characteristics of the two forms of health care, as follows: individual health services aimed at curative and rehabilitative with the primary goal of individuals and families; while environmental health services are intended to promotive and preventive with the main target of community groups.9

In general, the characteristics of health services raised by Marius Widjajarta, include: consumer ignorance, the influence of large health service providers on consumers so that (consumers do not have bargaining power and select power (supply induced demand), non-health service products homogeneous concept, discussion of competition, uncertainty about illness and health as human rights.
According to Benyamin Lumenta, good health services can be provided, if they meet the following principles:

1) Limited to treatment based on medical science;
2) Stress prevention;
3) Requires fair collaboration between lay people (patients) and medical science implementers (doctors);
4) Treating someone completely;
5) Maintaining personal relationships between doctors and patients closely and continuously;
6) Coordinated with fostering social welfare;
7) Coordinate all types (specialization) of medical services;
8) Utilize all the services needed and that modern medical science can provide to people in need.

Quality health services according to Tabish: Health Services means providing a health service product according to individual and community needs. High-quality health services begin with high managerial ethical standards, including: systems for carrying out professional standards; both from the standpoint of behavior, organization and assessment of daily activities, the observation system so that services are always provided according to standards and detection if there are irregularities; and a system to always support the implementation of professional standards.

The quality of health services is related to the quality and level of patient satisfaction as consumers. Guarantee for quality health services is a process of meeting the quality standards of managing health services consistently and continuously so that consumers get satisfaction. The aim is to maintain and improve the quality of health services on an ongoing basis which is carried out by an internal health service advice to realize the vision and mission and meet the needs of consumers.

According to Somers, that for the implementation of good medical services, many conditions must be fulfilled, covering 8 (eight) main points, namely available, appropriate, continue, acceptable, accessible, and affordable, efficient, and quality.

Health service is one of the efforts that can be done to improve the health status of individuals, groups or the community as a whole. In addition to health services another term of medical services is medical services, which is a service that includes all efforts and activities in the form of prevention, curative, promotive, and rehabilitative of health carried out on the basis of individual relationships between the experts in medicine with individuals who need it.

The State Function As An Entrepreneur Or Interpreneur

The role of the government as an agent of economic activity means that the government carries out consumption, production and distribution activities. The government in carrying out its role as an economic actor, establishing a state company or often known as a State-Owned Enterprise (abbreviated as BUMN). In accordance with Law No. 19 of 2003, BUMN is a business entity whose entire or most of its capital is owned by the state through direct investment from separated state assets. BUMN can be in the form of Perjan (Bureau Company), Perum (Public Company), and Persero (Corporate Company). SOEs make a positive contribution to the Indonesian economy. In a populist economic system, SOEs play a role in producing the goods or services needed in order to realize the greatest prosperity of the people.

BUMN was established by the government to manage production branches and sources of natural resources that are strategic and involve the lives of many people. These companies were established to improve the welfare and prosperity of the people, as well as to control the strategic and less profitable sectors. The government also acts as a consumption agent. Governments also need goods and services to carry out their duties. As is the case when carrying out their duties in the context of serving the community, namely carrying out construction of school buildings, hospitals, or highways. Of course
the government will need building materials such as cement, sand, asphalt, and so on. All these items must be consumed by the government to carry out their duties.

In addition to consumption and production activities, the government also conducts distribution activities. Distribution activities undertaken by the government in order to distribute goods that have been produced by state companies to the public. For example the government distributed nine staples to poor communities through BULOG. Distribution of groceries to the community is intended to help the poor meet their needs. Distribution activities undertaken by the government should be smooth. If the distribution activities are not smooth, it will affect many factors such as the scarcity of goods, high prices of goods, and even distribution of development is less successful. Therefore, the role of distribution activities is very important.

In the framework of carrying out government, community service, and development, the government basically has three main functions, namely the allocation function which includes, among other things, economic resources in the form of goods and services, community services, distribution functions which include, among others, income and wealth society, equitable development, and stabilization functions which include, inter alia, defense-security, economy and monetary. The distribution function and the stabilization function are generally more effectively carried out by the Central Government while the allocation function is generally more effectively carried out by the Regional Government, because the Regions in general are more aware of the needs and standards of community services.

The first task of the state by forming a business entity is to meet all the needs of the community, when these sectors cannot be done by the private sector. Then such tasks are translated as a form of pioneering efforts by the State to make SOEs a development agent / agent of development. The essence was formed of a State-Owned Enterprise (SOE) because it was based on the provisions of Article 33 specifically paragraphs (2) and (3) of the 1945 Constitution of the Republic of Indonesia which meant that: Production branches were important for the State that controlled the livelihoods of the people controlled by the State. Then the earth, water, and natural resources contained therein are controlled by the State and used for the greatest prosperity of the people.

The objectives of establishing a BUMN are (1) Give out contribution on economy national and acceptance cash country; (2) Chasing and looking for profit; (3) Fulfillment intention people’s lives; (4) Pioneer of business activities; (5) Give out helpand protection of small and weak businesses. BUMN is one of the economic actors in the national economy that is based on the principles of economic democracy so that it has a very important role in the administration of the national economy in order to realize the welfare of society as mandated by the 1945 Constitution of the Republic of Indonesia. The function of SOEs as implementing policies in the national economy is (1) Contribute to the development of the national economy and state revenue; (2) Improving the implementation of public benefits, in the form of providing goods and services in sufficient quantity and quality for the fulfillment of the lives of many people.

Health Insurance Administering Agency (BPJS Kesehatan) is a State-owned enterprises specifically assigned by the government to organize health care insurance for all people Indonesia, especially for Government employees, Recipients of civil servants and TNI/POLRI, Veterans, Pioneer of Independence and their families and other business entities or ordinary people. BPJS Health is a government program in unity National health insurance (JKN) which was inaugurated on December 31st 2013. BPJS Kesehatan began operating since January 2014. BPJS Kesehatan was previously called Askes (Health Insurance), which is managed by PT Askes Indonesia (Persero), but according to Law No. 24 of 2011, PT. Askes Indonesia changed to BPJS Health since the date January 2014.

Every Indonesian citizen and foreign citizen who has lived in Indonesia for a minimum of six months must be a member of the BPJS, that is regulated in Article 14 of the BPJS Law. Every company is obliged to register its
workers as BPJS members. Whereas people or families who do not work at companies are required to register themselves and their family members with BPJS. Each BPJS participant will be drawn in the amount determined later. As for the poor, BPJS contributions are borne by the government through the Contributions Assistance program. Being a BPJS participant is not only mandatory for workers in the formal sector, but also informal workers. Informal workers are also required to become members of the Health BPJS. Workers are required to register themselves and pay contributions according to the desired level of benefits.

In addition to the State having a business through BPJS, the State also benefits through hospitals, both government and private hospitals. Based on the legislation, private hospitals are seen as legal entities (rechtspersoon) because in reality hospitals through health workers can enter into therapeutic agreements with hospital service users. For government hospitals all matters relating to management/ management are determined by the government as the party that gives authority to hospital directors.

The difference in status between a government hospital as a public legal entity and a private hospital as a private legal entity/ foundation, makes a difference with regard to the parties responsible and accountable in the efforts of health services in hospitals.

Forms of hospital services can be divided into general hospitals and specialized hospitals. General hospital is a hospital that provides health services for all types of diseases from basic to subspecialistic. Special hospital is a hospital that organizes health services based on certain types of diseases or disciplines.

**The State Functions As A Referee / Umpire Or Oversight Function**

According to Stoner and Wankel that *Supervision means that managers try to ensure that the organization moves in the direction or direction of the goal. If one part of the organization goes in the wrong direction, managers try to find the cause and then redirect it to the right direction.**

Meanwhile according to Mc Farland in Handayaningrat that:

*Control is the process by which an executive gets the performance of his subordinates to correspond as possible to chosen plans, orders, objectives, or policies.*

Next Smith in Soewartojo, states that: Controlling is often translated also with control, including the understanding of plans and norms that are based on managerial goals and objectives, where these norms can be in the form of quotas, targets and guidelines for measuring actual work against those set. Supervision is activities in which a system is carried out within the framework of established norms or in a state of balance that supervision provides an overview of things that can be accepted, trusted or possibly imposed, and the control limit is the upper or lower value level a system can accept as a tolerance limit and still provide satisfactory results. In management, controlling is an activity to match whether operational actuating in the field in accordance with the planning that has been determined in achieving the goals of the organization. Thus, the object of surveillance activities is regarding mistakes, irregularities, defects and negative things such as fraud, violations and corruption.

There are various types of supervision as a form of implementation of the State’s function of the responsibility for health services can be carried out by the government, namely based on Article 23 paragraph (5) of the 1945 Constitution of the Republic of Indonesia. Furthermore, the existence of the Supreme Audit Board is governed by the Law No. 15 of 2006 (hereinafter abbreviated to the BPK Law). Based on Article 1 of the BPK Law, the Supreme Audit Board, hereinafter referred to as BPK, is a state institution whose task is to examine the management and responsibilities of state finances as referred to in the 1945 Constitution of the Republic of Indonesia, so that the BPK has the responsibility for the task and obligation to examine the government’s responsibility regarding state finances and checking all the implementation of the State Budget (APBN).
results of the examination are notified to the House of Representatives. If an examination uses things that give rise to a suspicion of a criminal offense or an act that is detrimental to the country’s finances, the Supreme Audit Board provides input to the government.

Conclusion

The state’s function of the nature of state responsibility in equitable free health services, in the form of a function regulator has issued several regulations related to health service programs, including government regulations that specifically regulate health insurance for the poor and displaced children, but have not been fully implemented well in accordance with the mandate of the 1945 Constitution of the Republic of Indonesia NRI automatically protects and guarantees the right to health insurance for the poor and underprivileged, as well as the controlling function (provider) has not been optimally implemented, as seen distribution and procurement of drugs in hospitals still often have problems, health workers in some the area is not evenly distributed as needed, while the entrepreneurial function has basically benefited the country from the assets and premiums of participants received so far, but these funds have not been utilized optimally to improve the quality of service and satisfaction of some of the participants, especially for the poor who have not been included in the PBI program, and the function of supervision/referee (umpire) will give optimal results if it is truly implemented properly according to its basic principles.

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References

Nonsurgical Management of a Patient with Hypopituitarism Secondary to Nonfunctioning Pituitary Macroadenoma: A Case Report

Martino Handoyo¹, Hermina Novida²

¹Resident in Department of Internal Medicine, Faculty of Medicine, Airlangga University, Dr. Soetomo General Hospital, Surabaya, Indonesia, ²Lecturer and Consultant in Division of Endocrinology, Metabolic, and Diabetes, Department of Internal Medicine, Faculty of Medicine, Airlangga University, Dr. Soetomo General Hospital, Surabaya, Indonesia

Abstract

Pituitary adenoma is a benign neoplasm of pituitary gland. Pituitary adenoma, particularly a macroadenoma, may produce mass effect symptoms by compressing surrounding tissues. Due to pituitary function as a neuroendocrine organ, pituitary adenoma may also cause multiple endocrine disturbances. The authors report the case of a 39-year-old male patient presenting with chronic headache and history of seizure. MRI imaging revealed pituitary macroadenoma with optic chiasm compression. Further diagnostic workup indicated secondary hypogonadism and adrenal insufficiency. The patient was given hormone replacement therapy and was then advised for surgery, but the patient refused any surgical procedure. Subsequent follow-ups were somewhat difficult due to poor patient adherence. Nevertheless, appropriate management of pituitary adenoma is needed to achieve optimal result.

Keywords: pituitary adenoma, macroadenoma, clinically nonfunctioning, hypopituitarism

Introduction

Pituitary adenoma is a group of benign neoplasms arising from pituitary gland, comprising of 10-20% of all intracranial tumors (¹) and more than 90% of intrasellar tumors (²). Prevalence of pituitary adenoma is reportedly to be around 80-100 cases per 100,000 (³), but many studies found the actual number might be far greater, because many are asymptomatic and could only be detected from incidental findings on imaging or autopsy (⁴). Based on its size, pituitary adenoma can be classified into macroadenoma (≥10 mm) and microadenoma (<10 mm). Pituitary adenoma can also be categorized based on its hormonal activity as functioning or nonfunctioning. This differentiation is important because they have different management approach. We reported a case of a male patient, aged 39, with partial hypopituitarism secondary to clinically nonfunctioning macroadenoma.

Case Report

A 39-year-old male was referred from a rural general hospital to our outpatient clinic with a complaint of 1 year of worsening headache. He described the headache as continuous heavy sensation in the head bilaterally. The headache had varying frequency and duration in a nonspecific fashion and only partially responded to analgesics. The patient also had a history of a seizure in previous week which lasted only a few minutes and he fell unconscious during the seizure. It only happened...
once and there was no previous history of seizure before nor thereafter.

Head MRI scan (Figure 1) revealed a solid intrasellar lesion with regular clear boundaries, isointense on T1 and T2 with rim contrast enhancement, with approximately 19x22x29 mm in size, which extended into suprasellar region and abutted the optic chiasm, compressing the latter to anterior and superior, which was suggestive of a pituitary adenoma. No other structural abnormalities were found.

Figure 1. Contrast MRI of the brain on various planes and sequences. (top) T1-weighted coronal. (middle) T1-weighted axial. (bottom) T2-weighted axial.
Hormonal panel evaluation results were as follows: serum morning cortisol 0.8 mcg/dL (N: 4.3-22.4 mcg/dL), ACTH 28 pg/mL (N: 20-52 pg/mL), LH 0.12 mIU/mL (N: 1.5-9.3 mIU/mL), FSH 0.66 mIU/mL (N: 1.4-18.1 mIU/mL), testosterone 5.17 ng/dL (N: 241-827 ng/dL), prolactine 3.01 ng/mL (N: 2.1-17.7 ng/mL), TSH 1.62 mIU/mL (N: 0.55-4.78 mIU/mL), and FT4 0.94 ng/dL (N: 0.89-1.76 ng/dL). Other basic laboratory tests including complete blood count, serum electrolytes and glucose levels were within normal limit.

Further history taking and physical examination revealed diminished secondary male sexual characteristics and decreased libido within the past 6 months, although the patient was at first did not realize that his condition was interrelated. The patient was already married for 15 years and had 2 children from his marriage, aged 10 and 7 years old. Although the patient did not feel any visual disturbance that he might be aware of, the patient was referred to ophthalmology department for evaluation and was subsequently assessed with bitemporal hemianopia.

Based on the examination results, the patient was diagnosed with nonfunctioning pituitary macroadenoma with secondary hypopituitarism, manifesting as secondary hypogonadism and adrenal insufficiency. The patient was referred to neurosurgery department for further management. He was advised for surgery, but unfortunately he refused, asking for more time to discuss it with his family.

The patient was then given hormone replacement therapy to correct hormonal deficiency caused by hypopituitarism. Before initiating testosterone replacement, serum PSA level was 0.02 ng/dL and hematocrit was 45%. The patient was subsequently given 250 mg of testosterone injection (a mixture of 30 mg testosterone propionate, 60 mg testosterone phenylpropionate, 60 mg testosterone isocaproate, and 100 mg testosterone decanoate) intramuscularly every 4 weeks. For glucocorticoid substitution, the patient was given methylprednisolone 4 mg orally in the morning once daily. On subsequent laboratory evaluations, serum PSA levels and hematocrit were always within normal limit, while serum testosterone levels were fluctuating, ranging from 82.45 to 277.75 ng/dL. One major problem concerning the patient’s treatment was poor adherence. The patient was unable to attend our clinic regularly because he lived in rural area very far away from the city, making optimal treatment and follow-ups somewhat difficult.

Discussion

Diagnostic workup for pituitary macroadenoma involves imaging modalities of sellar region, assessment of pituitary hormones function, and optic pathway evaluation. CT and MRI of the sellar region are the preferred imaging modality in detecting pituitary adenoma, with the latter is considered to be more sensitive (4).

Clinical symptoms and signs in patients with pituitary adenoma can generally be classified into mass effect-related symptoms and endocrine abnormalities arising from hormonal overproduction leading to hyperpituitarism. Males were more associated with neurological symptoms such as visual disturbances and seizures, while females were more likely to present with symptoms related to sexual function disturbances such as menstrual disturbances, galactorrhea, infertility, and hirsutism (5). Still, many do not exhibit symptoms and discovered by chance (incidentaloma).

Based on its hormonal activity, a pituitary adenoma can be classified into functioning and nonfunctioning. Among the functioning pituitary adenomas, prolactin-producing tumor (prolactinoma) is the most frequent type (4,6). Other functioning pituitary adenomas producing GH, ACTH, TSH, or LH are also reported in studies but they are less common.

Clinically nonfunctioning pituitary adenoma (NFPA) is defined as pituitary adenoma which is not hormonically active, that is, not associated with clinical syndromes caused by overproduction of any pituitary gland hormones (7). Most pituitary adenomas are clinically nonfunctioning (8); approximately 50%
of microadenomas and 80% of macroadenomas belong to this group (2). NFPA could be categorized further based on transcription factor expression and immunohistochemistry (IHC) staining into silent pituitary adenoma (at least one positive transcription factor expression or IHC staining) and null-cell adenoma (negative transcription factor expression and IHC staining) (1).

Clinically nonfunctioning pituitary microadenomas usually do not cause signs or symptoms, while the main complaints in clinically nonfunctioning macroadenomas are largely related to mass effects including headache, visual field defects, and hypopituitarism. Approximately 40-60% of patients have headache as one of their main symptoms which might be caused by increased intracranial pressure or dura mater stretch. Visual field defect is present in 58% of macroadenoma cases, which has typical bitemporal pattern (9).

Hypopituitarism may occur due to pituitary stalk compression (and thus inhibits signals from hypothalamus) and also compression of the surrounding healthy pituitary tissue by the growing mass (2). In 60-85% patients with nonfunctioning pituitary macroadenoma, at least one pituitary hormone deficiency is present, while panhypopituitarism is less frequent. Among these hormones, gonadotropin deficiency is the most common and accounts for more than 80% of cases, followed by somatotropin deficiency in 70% of cases; while thyrotropin and corticotropin deficiency present in 20-50% of cases (10). Different literatures report somatotropin deficiency as the most common, followed by gonadotropin, corticotropin, and thyrotropin deficiency (9,11).

In our case, a tumor size of ≥10 mm on imaging leads the diagnosis of a macroadenoma, which was accompanied by optic chiasm compression. This explains mass effect symptoms in our patient, including headache, seizure, and visual field defect. A very low serum morning cortisol accompanied by a low-normal level of ACTH is indicative of secondary adrenal insufficiency. Low level of serum testosterone combined with low levels of both FSH and LH suggests secondary hypogonadism.

Management of pituitary adenoma generally consists of several main strategies: surgery, radiotherapy, and medical treatment (using dopamine agonists and somatostatin analogs). Existing hypopituitarism should also be managed accordingly by administering hormone replacement therapy.

The first line and the most effective treatment for clinically NFPA is surgery, particularly in symptomatic macroadenoma. Without surgery, 50% will progress within the next 5 years, while only 10% might regress spontaneously (2). Surgery is indicated if there is optic chiasm compression or visual field defects which ensue from the compression. In these cases, visual function preservation or recovery should be the main goal of treatment. Approximately 78% of patients are reported to have improvement in visual field after surgery. However, only 30% of patients undergoing surgery obtain improvement in pituitary functions (7). These possible outcomes should be carefully discussed with the patient before surgery along with other surgical risks. Other mass effects such as headache could also be a consideration for surgery, although in a more individual approach. In NFPA without optic chiasm compression, the decision regarding surgical treatment should be tailored individually (2).

Regarding surgical method, transsphenoidal approach is preferred because it has lower complications rate and perioperative mortality risk compared to transcranial approach. Some complications that might occur after surgery (less than 5% of cases) include new visual disturbance, cerebrospinal fluid leakage, fistula, meningitis, and diabetes insipidus (7). Newer endoscopic resection method has comparable efficacy and safety (12). While combination of surgery and radiotherapy is reported to have lower risk of tumor recurrences than surgery alone (7), it must be noted that radiotherapy is not recommended in all cases, but may be considered when there is large residual tumor postoperatively or tumor recurrence.
Medical treatment using dopamine agonists and somatostatin analogs are considered to be adjunct therapy in functioning pituitary adenoma \(^{(6)}\). However, the effectiveness of these medications in the treatment for NFPA has not been proven \(^{(3,13)}\). Therefore, currently there is no recommendation for using these drugs in NFPA.

Hypopituitarism due to pituitary adenoma might be permanent despite tumor removal; therefore, the patient may require hormone replacement for a lifetime. Complete assessment of pituitary function is needed to detect hypopituitarism in one or more axis, although panhypopituitarism is less frequent. Management of hypopituitarism should be done on an individual basis. In our case, the patient develops secondary adrenal insufficiency and hypogonadism.

For glucocorticoid substitution, hydrocortisone use is recommended, with daily dose of 15-25 mg in single or divided doses. In our patient, we used methylprednisolone in its equivalent doses because oral hydrocortisone was not available in our center. It should be noted that there is no universal consensus on appropriate treatment for glucocorticoid replacement. There is also no objective monitoring parameter available. Doses should be adjusted based on clinical grounds, which can be increased 2-3 times the usual dose during mild illness or surgery and 10 times in major illness or surgery for a short time \(^{(14)}\). Overcorrection for prolonged period should be avoided to prevent undesirable adverse effects.

Secondary hypogonadism in males can be treated either with testosterone or gonadotropin formulations. Gonadotropin administration is preferred if fertility is desired \(^{(14)}\). Our patient already had 2 children from his marriage and did not have a plan to have another child; therefore, fertility is not an issue. The patient was then given intramuscular testosterone injection as replacement therapy which should have been administered every 3-4 weeks but unfortunately our patient only managed to visit our clinic every 2-3 months. Nevertheless, this formulation was chosen because it was the most readily available in our center and more convenient for the patient considering patient’s poor adherence.

Serum PSA and hematocrit level must be monitored during testosterone replacement treatment. An increase > 1.4 ng/mL of serum PSA from baseline within the first 12 months of treatment or a level >4 ng/mL is an indication for further urologic evaluation. Testosterone treatment should be stopped if hematocrit level is >54% until it decreases to a safe level and then might be reinitiated in a reduced dose. Serum testosterone level should be maintained in mid-normal range (400-700 ng/dL) during treatment and evaluation should be made midway between injections \(^{(15,16)}\). In our patient, baseline serum PSA and hematocrit levels were normal and there was no significant increase throughout therapy. However, achieving treatment goal was challenging since his serum testosterone never reached recommended level due to suboptimal treatment which was influenced by social and economic difficulties.

**Conclusion**

Patient with pituitary adenoma should be evaluated for mass effects related symptoms and complete pituitary axis function in order to give appropriate treatment. Management of pituitary adenoma should be made on individual basis. For symptomatic clinically nonfunctioning macroadenoma, surgery is the first line treatment. Based on data from previous studies, visual disturbance will improve in almost 80%, while pituitary function will improve in only 30% of cases after surgery. Patients with hypopituitarism may need continuous long term hormonal replacement to maintain physiological function. This case is reported to highlight the importance of appropriate workup and management of a clinically nonfunctioning macroadenoma.

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**Source of Funding:** None.

**Ethical Clearance:** All procedures were in accordance with the ethical standards of the Ethics Committee of Dr. Soetomo General Hospital, Surabaya, Indonesia. Written consent was obtained from patient...
for publication purpose.

References


Assessment of Pesticide Biohazards in Neurodegenerative Diseases; Data Analysis Statistical Study

Marwan M. Merkhan1,2, Zeina A. Althanoon1, Ahmed A Mohammed3, Ibrahim M. Faisal4

1Researcher, Department of Pharmacology and Toxicology, College of Pharmacy, University of Mosul, Mosul, Iraq, 2Researcher, Department of Pharmaceutics, College of Pharmacy, Ninevah University, Mosul, Iraq, 3Researcher, Alkhansaa Teaching hospital, Ninevah Health Directorate, Mosul, Iraq, 4Researcher, Department of Pharmacology, College of Medicine, University of Mosul, Mosul, Iraq

Abstract

Background: The initiation of late onset neurodegenerative diseases remains unknown. New researches suggested that prolonged exposure to pesticides might lead to the initiation of neurodegenerative diseases, but the results were subject to controversy. Aims: The present study aimed to demonstrate an update data-mining to compare published data for studies comparing role of pesticide in induction of neurodegenerative diseases (Parkinson’s diseases, Alzheimer’s diseases, and Amyotrophic lateral sclerosis) Results: A clear association has been noticed across different studies regarding correlation between pesticide exposure and Parkinson’s disease or Amyotrophic Lateral Sclerosis (Odd Ratio >1), nevertheless, the association was weaker between exposure to pesticide and the development of Alzheimer’s disease (Odd Ratio close to 1) Conclusions: There is good quality evidence that the impact of pesticide in initiation of neurodegenerative disease is statistically approved. However, there is inconclusive evidence from randomised controlled trials to show significant induction of neurodegeneration. More research is needed particularly long-term controlled studies on laboratory animals.

Keywords: agriculture, pesticide, neurodegenerative, Alzheimer, Parkinson, epidemiology.

Introduction

Aging-related diseases are worldwide major health issue, Alzheimer’s disease (AD) distribution constitutes two-third form of dementia and is described as a continuous diminishing of memory and other intellectual capacity1. Alzheimer’s disease (AD), Amyotrophic Lateral Sclerosis (ALS), and Parkinson’s disease (PD) are described as major motor health problems and it has been demonstrated that there is a correlation between these neurodegenerative diseases and the pesticide exposure2. The organophosphate (OP) insecticides are discovered very early last century, however, started to find application worldwide due to their effectiveness as an insecticide with low human toxicity3.

OP can be easily absorbed through GIT, respiratory system, mucous membrane, and skin4. OP toxicity is apparently common in farmers resulting in great morbidity and mortality, particularly in developing countries with rigorous agriculture5. However, the available data provide no clear-cut idea about the prevalence of the disease and the reported acute toxicity cases are relatively high (approximately 3000000 cases worldwide plus 220000 death rates)6. Nevertheless, in absence of accurate statistical study, these records provide only limited information about the real estimate due to unavailability of medical records in all countries and/or undiagnosed cases7.
Inhibition of the enzyme acetylcholinesterase is the principle mode of action of the OP insecticide, reducing the availability of the neurotransmitters acetylcholine (Ach) at synaptic clefts resulting in peripheral and central stimulation of nerve and muscle. Toxicity with OP is associated with autonomic overstimulation, movement impairments, muscular rigidity, reduction of motor activity and respiratory failure. Recovery is usual and it takes 1-2 days until all the symptoms disappear, however, delayed effects are uncertain and continuous exposure results in worsening of the condition overtime leading to neurological impairment, such as, mild paralysis, sensorineural polyneuropathy, cognitive dysfunction and psychiatric disturbances. This systematic review provides statistical clue about possible correlation between pesticide exposure and the potential development of long-term morbidity.

Study design and methods

We searched Iraqi Virtual Science Library (IVSL), PubMed, Cochrane Library MEDLINE, EMBASE, and the Chinese Biomedical Database (CBM), for the 2 target words ‘pesticide, neurodegeneration’ and target English papers. To be included, papers should be original work and full text articles. All published manuscripts were screened by reading the title and abstract for potential relevance to this research topic; whenever the title and abstract did not obviously state the degree of relevance, the manuscript itself was reviewed. To overcome search bias, another researcher conducted the review procedure independently; only studies jointly accepted by the two independent reviewers were taken into considerations. To simplify tracking the results in the present study, the results were categorised into Alzheimer’s disease, Parkinson’s disease, and Amyotrophic Lateral Sclerosis.

The online literature investigation revealed 827 publications, of which 731 duplicate studies were identified and thereby excluded, unrelated or not epidemiological study. The 96 target articles were downloaded and investigated carefully by two independent colleagues. We further removed another 14 unrelated publication from the total (Mixing other disease with other neurodegenerative disease). Ultimately, 82 trials were comparable to our inclusion standards, and the summarised data listed in figure 1, 2, and 3; related to Alzheimer’s, Parkinson’s and Amyotrophic Lateral Sclerosis, respectively. Only 82 epidemiological studies trials included patients diagnosed with neurodegeneration by specific criterion.

Results and Discussion

The collected epidemiological studies confirm that there is a great association between pesticide exposure and the development of neurodegeneration, more than 90% of these collected studies were showed that the odd ratio (OR>1) indication that there is a risk of developing the neurodegeneration due to exposure to these organic substances for long-periods (see figure 1, 2, and 3). The detailed information about the year, author, type of studies, and sample size about the collected epidemiological studies were mentioned below in Figure 1, 2, and 3.

Figure 2. Odds ratio of different epidemiological study of pesticide exposure and development of Amyotrophic Lateral Sclerosis. [Deapen, Savettieri, Gunnarsson, McGuire, Morahan, Weisskopf, Bonvicini]

*Case-Control study
#Cohort study

<table>
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<th>No.</th>
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<td>1036</td>
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<tr>
<td>1991</td>
<td>Savettieri*</td>
<td>138</td>
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<tr>
<td>1992</td>
<td>Gunnarsson*</td>
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<tr>
<td>1993</td>
<td>Chancellor*</td>
<td>206</td>
<td>1.0</td>
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<tr>
<td>1997</td>
<td>McGuire*</td>
<td>284</td>
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<tr>
<td>2006</td>
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The causative agents involved in initiation and progression of neurodegenerative diseases is yet unknown. A great efforts has been paid by scientists in an attempt to identify the causative agent involved in these diseases. The impact of these disease imposed a great burden on the individual and society due to high rate of morbidity and mortality. The health system funding of the neurodegenerative diseases is relatively high per annum as compared to other diseases including cardiovascular ones. Most of these neurodegenerative diseases start at elderly age group (>60 years). Identifying the causative agents is of great importance in determination of the underlying pathology of the disease to reduce its prevalence; reducing the burden on health care provider and reduce health cost budgets of geriatric-living cost. In the present statistical study we tried to identify the link between pesticide exposure and neurodegeneration in elderly age group through reviewing other case control studies conducted at different timeline and different area in the world. Many variables has been identified in the present study which could be a limitation of our study, including types of pesticides used which are in most cases unidentified by the participant or they reported their exposure to different agents during their life-time. The age of the participant varies (65-90 years) and this needs to be counted as an important variables because the neurodegenerative disease by default is age linked. The duration of our study is extended over 4 decades (1980s-2010s) which is regarded as an additional variables to be considered because during this extended period of time; different new pesticides were potentially introduced in farming. Therefore, the outcome of our
study is questionable and should be explained in the shadow of these variables. The outcome confirm that exposure to pesticide in field area for pesticide-dealers has been clearly associated risk of developing NDD with approximately overall studies showing an odd ratio of greater than 1.

The highlighted studies collected presently confirm that age is important factor in these diseases. There are a clear link between the exposure to pesticide and increased risk of development of neurodegenerative disease and this association is reciprocal to increased age, however, there is no consent on the age or age range at which pesticide could induce or increase the risk of association. Clear association with age were noticed at elderly (after 70) for both PD and AD, however, ALS shown onset at different age ranges excluding the possibility of association between pesticide exposure and age of participant. However, no differences were reported to exist in relation to sex variation. The studies records shown equal involvement of male/female in the study with no reported variation between studied group. The timeline of exposure to the pesticide showed important consideration regarding earlier type of pesticide which were reported to be more neurotoxic than those newer agents. In 1990s the available agents has been shown to be more neurotoxic than those newer agents introduced in 2010s.

The present study aimed at identifying the correlation between the exposure to pesticide and the incident of neurodegenerative ailments. However, so far there is no idealagreement about the link between exposure to pesticide and neurodegeneration initiation, therefore, more investigation and data analysis is needed before a clear–cut conclusion is drawn. Age is a principle factor for neurodegeneration exaggerated by pesticide exposure. Four mechanisms exist for illustration of the pesticide-induced neurodegeneration linked with aging, these include; oxidative mechanism of action, reaction between amyloid and organophosphates, cognitive impairment, and neuronal or haemostatic damage.

The relationship between the rate of exposure to pesticide and the incident of Parkinson’s, Alzheimer’s disease, and Amyotrophic lateral sclerosis is theoretically acceptable due to structural similarities between neurotoxin 1-methyl-4-phenylpyridinium (MPP+), a metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); with some pesticides, insecticides, and herbicides. Researchers has recently found a link between the molecular mechanism of action of the agricultural compounds and the development of different disease. For instance, rotenone an enzymatic process in mitochondrial membrane complexes enzyme (Complex I) which are involved in oxidative phosphorylation reaction resulting in mitochondrial depolarisation leading to cellular necrosis. Simple defect in this Complex I-enzyme could results in pathophysiological features of neurodegenerative disease. Investigators have found that organochlorines generate reactive oxygen species, accumulation of a-synucleinbiomolecules, impairment of the ubiquitin-proteasome system and the disruption of mitochondrial membrane potential, and stimulation of dopamine production leading to intracellular dopamine vesicle exhaustion. Moreover, paraquat long-term dopamine overproduction and consequently reduction in dopamine synthesis due to activation of N-methyl-D-aspartate (NMDA) receptor and suppression of the complex I of the mitochondrial membrane potential transport chain resulting in cellular apoptosis or necrosis. Carbamate inhibits ubiquitin proteasome system (UPS) by preferential damage to TH+ neurons and elevated alpha-synuclein levels. Imidazoles (e.g. benomyl), dithiocarbamates (e.g. maneb, and ziram), organochlorine, and other pesticides can inhibit aldehyde dehydrogenase (ALDH) enzyme. Recent studies indicated the importance of ALDH in involvement in the pathology of neurodegenerative disease.

**Conclusion**

To sum up; oxidative stress and neuronal inflammation, microtubule modification together with compromised axonal transport, beta amyloid formation, calcium homeostasis and mitochondrial dysfunction are
all factors that at least are able to impact on intellectual or motor decline. The major challenge for future investigators is in demonstration of variables which required to be controlled in epidemiological studies and designing more focused and translational experimental studies.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Taken from College of Pharmacy Research Ethics Committee

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The Leptin Concentration Level in Progression of Hand Osteoarthritis Disease

Maryam Qais Ahmed1, Ghaith Sael Mohammed2

1Researcher, Medical Instrumentation Engineering Department - Al-Esraa University College – Baghdad – Iraq,
2Researcher, Surgery Department - College of Medicine – Al-Nahrain University – Baghdad – Iraq

Abstract

Subject: Hand Osteoarthritis (HOA) is known as degenerative cartilage cell disease in joint association with generation various inflammatory responses. This disease is more common in geriatric and affected by many factors such as obesity. Leptin is hormones secreted of fatty tissue involve as mediators in many pathophysiology process, and support inflammation at cartilage of hand joint.

Objective of the Study: Role of leptin concentration level in progression of hand Osteoarthritis.

Materials and Methods: This study was done on 60 patients with HOA disease and 60 healthy persons (control), the all subjects age within this study were more than 60 years of both genders. After obtained serum, immediately used quantity method (immunoassay) for measured level of leptin concentration.

Results: This study shows elevation of serum leptin concentration level in HOA group compare with healthy control group.

Conclusion: This study confirms that serum leptin concentration level can act as support HOA disease progression.

Keywords: Hand Osteoarthritis, Leptin and Osteoarthritis.

Introduction

Osteoarthritis (OA) is one of joint disorders that consider as chronic, associated with age due to changes in physiologic processes at geriatric, OA know as complex disease characterize degradation of cartilage cells (chondrocytes) that layering end bones in joints, with interaction recognized inflammatory factors. The OA disease has many pathologic factors classified into mechanical (that is effect on weight-bearing joints) and non mechanical (that is effect on non weight-bearing joints), one of the main cause of OA disease is obesity. The hand joints consider as non weight-bearing joints.

Adipokines are hormones secreted of fatty tissue involve as mediators in many pathophysiology process (such as rheumatic diseases), newly recognized family of compounds like leptin. The leptin has role in contribute of OA prevalence in obesity persons.

Many studies suggested the correlation between OA disease and leptin level in weight-bearing joints like knee joints, but few studies included the correlation with non weight-bearing joints like hand joints. Leptin level consider risk factor to degradation of cartilage cells in joints due to it’s has link HOA disease and obesity.

The study aim is evaluate leptin level in HOA group and healthy control group at comparison study.

Materials and Methods

The present study included 60 cases with HOA disease and 60 healthy persons (as control) groups, all individuals age was more 60 years of both sexes. The sampling process of all subjects was random manner.
blood samples separated immediately without any preservative factor to product serum as pure form. The serum used for measured leptin concentration level (as quantity measurement) by immunoassay method.

After measurement, used t-test method for statistic analysis to show different of leptin level by comparison between HOA patients and control groups via used mean ± standard deviation (SD) and p-value (p-value > 0.05 mean significant value).

**Results**

The present study explain a different of leptin concentration level between HOA patients and healthy control groups via used mean ± SD statistic method. The leptin level found in HOA group was 14.1+9.4, while in control group was 11.5+9.9 with P-value was 0.021. The present result confirms elevation of leptin concentration level in HOA group. Show table 1.

**Table 1: Comparison of serum leptin level between HOA and control groups according to mean ± standard deviation (SD)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HOA group (No. =60) Mean+SD</th>
<th>Control group (No. =60) Mean+SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin level (ng/ml)</td>
<td>14.1 + 9.4</td>
<td>11.5 + 9.9</td>
<td>0.021*</td>
</tr>
</tbody>
</table>

*Significant value

**Discussion**

The HOA is degenerative cartilage joint disease consider as more prevalence disease in geriatric, and it consider as complex disease due to interact of many pathological factors to generate this disease such as mechanical factor, non mechanical factor and cytokiens. The cytokines and hormones secreted of fatty tissue like leptin act to support of inflammatory process in joints (3).

There are many studies such as Stannus et al study showed correlation between general OA patients, leptin concentration level and joint pain, this studies showed elevated of leptin level with reduced cartilage thickness that product from cartilage degradation (5). Also there are studies showed correlation between leptin level with HOA at specially form, Veenbrin et al explained that concluded that leptin is partially involved in the relationship between BMI and HOA (6).

The hand joint not effect by mechanical factors such as knee joints, so this study remarkable to focus on involve of metabolic factors on generate of HOA.

This study demonstrated elevated of leptin level in HOA, and agree with previous studies. We recommend to other studies and take more HOA patients to confirm this study results.

**Conclusion**

This study concluded to confirms that serum leptin concentration level are elevate in HOA cases compare with healthy control, this support that leptin involve in pathological process that product cartilage degradation in hand joints.

**Conflict of Interest:** No

**Source of Funding:** Self or other source: self

**Ethical Clearance:** Yes

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Formulation Herbal Mouthwash Combination Extract of Ginger and Lemongrass as Antibacterial Causes of Halitosis in Diabetes Mellitus Patients

Masriadi¹, Sukmawati², Hasta Handayani Idrus³

¹Professor, Department of Epidemiology, Faculty of Public Health, ²Lecturer of Faculty of Pharmacy, ³Lecturer of Faculty of Medicine, Universitas Muslim Indonesia, Makassar

Abstract

Background. Ginger and lemongrass are plants known as the king of rhizomes with a healthy stance because empirically many have used them as halitosis treatment in people with diabetes mellitus. The research objective was to formulate herbal mouthwash from extracts of natural ingredients, namely a combination of ginger and lemongrass which has the potential as antibacterial causes of bad breath (halitosis) in people with Diabetes Mellitus (DM). Material and Methods. The extraction of the sample will be carried out by a modified extraction method using ethanol and water solvents, then biological activity testing will be carried out in-vitro with the diffusion method so that the antibacterial activity test against several bacteria that cause halitosis will be tested. Antibacterial sample is an active compound resulting from the extraction process. Bacteria that had been inoculated into the growth medium (NB) were put into sterile soft NA media (0.7%) with a concentration of 10,000, 8,000, 7,500 and 6,000 ppm, respectively. Results. There is an inhibitory effect, on p. gingivalis but the pattern of values is uncertain. A solution of formulation A with a concentration of 75% occurs inhibition with an inhibition diameter of 15.7%. This inhibitory effect is not an activity of formulation A, because acetic acid also has the ability to inhibit bacteria. In the sample solution with a concentration of 100% chitosan (w / v), the highest inhibition occurred with an inhibition diameter of 18.7 mm / mg of the sample extract. Formulation A with a concentration of 25% (w / v), the lowest inhibition occurred. The test solution of formulation A with a concentration of 25% has shown an inhibitory effect on the growth of Streptococcus mutans. This effect is stronger at concentrations of 100%, 75%, and 50%. The antimicrobial effect actually increased with an increase in the concentration of the test solution in succession of 25%, 50%, 75%, 100%. Formulation C with a concentration of 10000ppm, 8000ppm, 7500ppm, 6000ppm showed that the antimicrobial effect actually increased with an increase in the concentration of the test solution in succession. This shows that there is a strong positive relationship between concentration and inhibition zone. Conclusion. All tests for both formulation A and formulation C using several concentrations showed quite good results with the antibacterial activity being directly proportional to the concentration, the greater the concentration the greater the activity.

Keyword: Antibacterial, Diabetes Mellitus, Halitosis, Herbal mouthwash

Background

Assessment of health status can use several indicators that reflect the condition of mortality (death), nutritional status and morbidity (morbidity). The degree of public health in Maros Regency is illustrated through the Mortality Rate, Under-Five Mortality Rate, and Maternal Mortality Rate, Morbidity Rate (Morbidity Rate) such as several diseases including infectious diseases (pulmonary tuberculosis, dengue fever etc.), non-communicable diseases (hypertension, Diabetes Mellitus etc.), as well as oral dental diseases (gingivitis,
periodontitis, halitosis, etc.).

Halitosis is a crippling social problem with a common complaint of up to one-third of the general population.\[^{1}\] Halitosis is a lyrical term derived from the Latin word “halitus” (breath) and the Greek suffix “osis” (condition, action or pathological process). In simple words, it means “Bad Breath”. It is also called as fetor ex ore or fetor oris.\[^{2}\]

In most of the cases (90%), halitosis originates within the oral cavity. This is because the oral cavity harbours a large variety of microorganisms which include a large group of Gram-positive bacteria mainly *Streptococci* and a group of anaerobic microorganisms such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Prevotella intermedia*. Among the latter, many are Gram-negative oral bacteria whose proteolytic activity is associated with oral malodour and periodontal disease.\[^{3}\] Other bacteria associated with gingivitis and/or periodontitis (viz.- *Actinobacillus actinomycetemcomitans*, *Campylobacter rectus*, *Peptostreptococcus micros*, *Bacteroides forsythus*, *Eubacterium* species and *Spirochetes*) are known to produce large amounts of volatile sulfur compounds (VSC) which are malodorous.\[^{4}\]

Word halitosis is a Latin word which is derived from halitus means breathed air, and the osis means pathologic alteration and it is used to describe any unpleasant or disagreeable bad odor emanating from the mouth breath.\[^{5}\] Halitosis or bad breath is a disease caused by a lack of oral hygiene. Halitosis can cause harm not only to sufferers but also other people and can affect someone’s social life such as shame, avoidance of social interactions and decreased self-confidence.\[^{6}\]

Research by Ravindran et al. in India in 2015 showed that the number of patients with controlled diabetes mellitus had more halitosis (26.7%) than uncontrolled diabetes mellitus patients (20%). This occurs due to bacterial decomposition and the evaporation of Volatile Sulfur Compounds (VSC).\[^{7}\] Research Kumaresan et al. in India in 2017, 149 patients with type 2 diabetes mellitus showed that only 57 subjects knew and were aware of halitosis as a result of type 2 diabetes mellitus, while most subjects did not realize that halitosis was a manifestation of the oral cavity due to diabetes mellitus. This shows the lack of public knowledge about the impact of systemic diseases on oral health.\[^{8}\]

Research by Bisong et al. in Cameroon in 2015 shows that disease of the oral cavity is higher in people with diabetes mellitus compared with non diabetes mellitus, where hyperglycemia is suspected to be factors that play a role in the emergence of disorders in the patient’s oral cavity diabetes mellitus. This study revealed that plaque, calculus, xerostomia and halitosis was significantly higher in people with diabetes mellitus compared to non diabetes mellitus.\[^{9}\]

Everyone certainly wants healthy teeth and avoid bad breath. They think that by brushing their teeth alone, bad breath can be eliminated, even though they need continuous treatment to get maximum results. Apart from regular brushing, other efforts are also needed. Bad breath faced or experienced by a person has many causes, not only from infections in the oral cavity, it can be from other sources that can cause bad breath including throat, lung and stomach disorders, and cadaulin (Diabetes mellitus sufferers), and consumption of certain drugs.

Diabetes mellitus is a chronic disease with hyperglycemia and glucose intolerance that occurs because the pancreas cannot produce insulin adequately or because the body cannot use insulin. This disease affects many Indonesians. Oral complications that commonly occur in people with type 2 diabetes mellitus include xerostomia, periodontal disease, caries and halitosis. Halitosis describes breath that is smelly or unpleasant.\[^{10}\]

Oral and dental health is very important to maintain overall health. Good oral and dental health will improve our ability to speak, speak, smile better, taste food, chew, swallow, and can even improve our facial expressions when communicating.\[^{11}\] Teeth are a very important part of us, because teeth are directly related to health and
appearance. Oral health is often neglected by the public. They consider other health to be more important than oral health. In dental health problems, especially bad breath (halitosis). Many people experience a bad condition in their mouth (halitosis) but they lack awareness of the condition of their mouth. Everyone certainly wants healthy teeth and avoid bad breath. They think that by brushing their teeth alone, bad breath can be eliminated, even though they need continuous treatment to get maximum results. Apart from regular brushing, other efforts are also needed.

Word halitosis is a Latin word which is derived from halitus means breathed air, and the osis means pathologic alteration and it is used to describe any unpleasant or disagreeable bad odor emanating from the mouth breath. The bad breath of diabetes mellitus is very typical like acetone. The substance comes from ketone limbs that can be secreted from the breath. 

Aleman L F J et al conducted a study to determine the effectiveness and sustainability of three commercial mouthwashes against the halitosis and concluded that a decrease in VSC and organoleptic levels after use of mouthwashes for 1st and 3rd hours. Results obtained in that study indicate that mouth rinsing with essential oils, cetylpyridine chloride and triclosan represents a positive option for the treatment of halitosis.

Halitosis treatment aims to improve the quality of life of people with diabetes mellitus due to prolonged disturbances in the oral cavity in the form of halitosis and other oral diseases. Herbal medicine is one of the treatment efforts and/or other methods of treatment outside of medical science and treatment science, traditional medicine needs to be nurtured, developed and supervised so that its benefits and safety can be accounted for.

A preliminary study was conducted at the Tompobulu Public Health Center in October-November 2019 regarding the effect of video method counseling, treatment in improving the quality of life of people with Diabetes Mellitus due to halitosis. The results showed that there was a relationship between counseling, treatment and quality of life for people with diabetes. In that study, provided treatment with a combination of using 0.5% chlorhexidine mouthwash with herbal mouthwash, in this case lemongrass and ginger. Based on the results of the treatment given in the form of 0.5% chlorhexidine mouthwash and it was found that some still experienced bad breath with the criteria that there was moderate mouth rinse (50%) after giving mouthwash to (20%), bad breath was very strong (10%) becomes (0%). Patients using lemongrass and ginger leaves experienced changes in bad breath criteria, namely moderate bad breath (40%) to (15%), strong breath (15%) to (5%). The research objective was to formulate a herbal mouthwash from a combination extract of ginger and lemongrass as an antibacterial cause of halitosis in DM sufferers.

Material and Methods

Materials and tools

The main ingredients used are the formulations made in the Pharmaceutical Microbiology Laboratory of UMI. The bacterial cultures used in this study were Gram negative bacteria, namely P gingivalis and gram positive bacteria, namely Streptococcusmutans. The agar media used were Nutrient Agar (NA), Vogel Johnson Agar (VJA), and Eosin Methylene Blue Agar (EMBA). The chemicals used were 70% alcohol, spirits, pH 4 and pH 7 buffers. The equipment used was a Kotterman-Germany brand Clean Bench (aseptic room equipped with a UV lamp), incubator cupboard, autoclave, hotplate, petri dish, analytical balance, Erlenmeyer, test tube, dropper pipette, volumetric pipette, loop needle, measuring cup, measuring flask, Bunsen lamp, and other supporting tools.

Research Treatment

The treatment applied to this test bacteria is the concentration of formulation A added. The concentration of formulation A (100 gr ginger, 100 gr lemongrass, 100 clove seeds in 100 ml water) used was 100%, 75%, 50%, 25%. For formulation C, it is 10000 ppm, 8000ppm, 7500ppm, 6000ppm with a concentration. Testing the antibacterial activity of the sample using the
agar diffusion method. The experiment was carried out with 3 replications. Observation data are presented in tabular form and then analyzed descriptively.

**Preparation of Test Bacterial Culture**

The test bacterial culture to be used is prepared by taking one bacterial loop from an NA agar slant, then inoculating it into 10 ml of sterile NB. Furthermore, it was vortexed to even out the bacteria in NB, then incubated at 37°C for 24 hours. After 24 hours, an inoculum is obtained which can be directly used for testing antibacterial activity.

**Testing Antibacterial Activity with the Well Method**

The well method (agar diffusion) is based on the ability of the tested antibacterial compound to produce the radius of the inhibition zone around the test well against the bacteria used as the tester. Testing the antibacterial activity of formulation A and formulation C was started by preparing the bacterial growth medium. Making the media begins with weighing the powder media and adding distilled water as directed on the packaging. Then, stirring while heated using a hot magnetic stirrer until the media solution is homogeneous which is marked by a clear color of the solution, then Erlenmeyer is covered with cotton and sterilized at 1210°C for 15 minutes. After sterilization the media is cooled closed at room temperature until the temperature reaches 400°C then 0.1% test bacteria are inoculated into each growth medium (0.1 mL of culture in NB into 100 mL of growth media) then homogenized. The growth medium used for each of the test bacteria. The media that has been inoculated with the tested bacterial culture is poured into a dish and allowed to freeze. Then five holes (wells) were made aseptically with a diameter of 7 mm and put a sample solution of 60 µL containing the added formulation A. The concentration of formulation A used was 100%, 75%, 50%, 25%. For formulation C it is 10000 ppm, 8000ppm, 7500ppm, 6000ppm. The sample solution was prepared by dissolving the sample according to the concentration in a 1% acetic acid solution. Incubation was carried out statically at 37°C for 48 hours.

**Inhibition Zone Calculation**

The zone of inhibition of chitosan antibacterial compounds was measured based on the radius (rp, mm) of inhibition in the form of a clear area around the test well. Measuring radius (rp, mm) is done by measuring the distance from the edge of the test well to the boundary of the zone of the inhibition zone using a caliper (accuracy of 0.05 mm) on several sides of the test well, then averaged. The value of the diameter (d, mm) of the inhibition zone as a result of direct observation is obtained using the formula d = 2 x rp.

**Results**

**Table 1. The results of the antibacterial activity test of formulation A and formulation C with several concentrations**

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Antibacterial Activity Test</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. gingivalis</td>
<td>Streptococcus mutans</td>
</tr>
<tr>
<td></td>
<td>R1</td>
<td>R2</td>
</tr>
<tr>
<td>100%</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>75%</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>50%</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>25%</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 1. The results of the antibacterial activity test of formulation A and formulation C with several concentrations

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Formulation A</th>
<th>Formulation B</th>
<th>Formulation C</th>
</tr>
</thead>
<tbody>
<tr>
<td>10000 ppm</td>
<td>10</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>8000 ppm</td>
<td>9</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>7500 ppm</td>
<td>8</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>6000 ppm</td>
<td>7</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 1. Antibacterial activity test results p. gingivalis (PG) with concentration (w/v) and ppm

Figure 2. The results of the antibacterial activity test for Streptococcus mutans (SAMA) with a concentration of % (w/v)

Figure 3. The results of the antibacterial activity test for Streptococcus mutans (SAMA) with a concentration of ppm
Discussion

The extract was obtained through an extraction process using 95% ethanol as a solvent. Extraction using ethanol as a solvent was chosen in order to obtain a polar active ingredient. 95% ethanol was chosen as a solvent or extractor because through a series of initial trials it was found that ethanol showed the best effect on the withdrawal of the active ingredient contained in male mayana leaves. The extract obtained is then filtered with filter paper and then evaporated under vacuum pressure, the results obtained have the physical properties of the extract, namely in the form of a very thick precipitate, which indicates that the viscosity level is very high.

Ethanol was used as the solvent because it has two groups with different polarity which are hydroxyl group that is polar and alkyl group that is non-polar, so by the existence of these groups it is expected that the compounds with different polarity levels will be extracted into the ethanol.[15]

The results of the effect test on the growth activity of Gram negative, namely P. gingivalis and Gram positive bacteria, namely Streptococcus mutans. In observations after 24 hours of incubation with 3 repetitions. The test results of male mayana leaf polar extract against the growth activity of Streptococcus mutans and P. gingivalis has a positive value. This is indicated by the presence of a bright area around the culture hole in each of the tested bacteria. There was also inhibition against the comparative antibiotic.

In the table, it can be seen that the sample gives an inhibitory effect, on p. gingivalis but the pattern of values is uncertain. In a solution formulation A with a concentration of 75%, there was an inhibition with an inhibition diameter of 15.7%. This can occur because of the presence of acetic acid in the solution as a solvent. This inhibitory effect is not an activity of formulation A, because acetic acid also has the ability to inhibit bacteria. In the sample solution with a concentration of 100% chitosan (w/v), the highest inhibition occurred with an inhibition diameter of 18.7 mm / mg of the sample extract. This is presumably because the viscosity of formulation A is still low so that it can still diffuse into the medium so that the place where it grows p. gingivalis. In formulation A with a concentration of 25% (w/v), the lowest inhibition occurred.

The modified well method from the Kirby Bauer method was used because this method is more suitable and practical for drug testing than the diffusion method using a disc. Whereas with discs, the volume of the test solution is very limited. The volume of samples that is more than 20 µl per disc has resulted in interference of observation and measurement. The use of distilled water as a negative control is absolutely necessary to rule out the possibility of an antimicrobial effect. In the observations, it turns out that for all wells with distilled water, none of them provide an inhibition zone.

Observations on the Streptococcus mutans test bacteria (table 1), showed that distilled water as a negative control did not have an inhibitory power against the growth of Streptococcus mutans, which was indicated by the absence of an inhibition zone in the area around the well containing the distilled water. The 50 µg/50 µl ciprofloxacin comparison solution had a large zone of inhibition against the growth of Streptococcus mutans. In Table 1, the test solution of formulation A with a concentration of 25% has shown an inhibitory effect on the growth of Streptococcus mutans. This effect was stronger at concentrations of 100%, 75%, and 50% (Table 1). The antimicrobial effect actually increased with an increase in the concentration of the test solution in succession of 25%, 50%, 75%, 100%. This shows that there is a strong positive relationship between concentration and inhibition zone. This relationship can be seen in Figure 2. This means that the extract solution with the ethanol extractor has an antimicrobial effect against Streptococcus mutans.

The results of further observations using formulation C with a concentration of 10000ppm, 8000ppm, 7500ppm, 6000ppm showed that the antimicrobial effect was actually increasing with an increase in the concentration of the test solution in succession. This shows that there is a strong positive relationship between
concentration and inhibition zone. This relationship can be seen in Figure 2. This means that the extract solution of formulation C with the ethanol extractor has an antimicrobial effect on P. gingivarlis and Streptococcus mutans. From the data of all the tables above, it can be shown that the order of the strength of the antimicrobial activity of the laurant test concentrations of 10000ppm, 8000ppm, 7500ppm, 6000ppm.

Even though it has antibacterial ability, it does not mean that male mayana leaf extract is called an antibiotic substance because there is no resistance standard and an assessment of bacterial sensitivity. The ratio of the size of the light zone formed in the extract solution was smaller than that of ciprofloxacin as a positive control. The antibacterial abilities contained in formulations A and C are not only limited to the two tested bacteria used in this study but may still have antimicrobial abilities against other bacteria. The antioxidant activity of astaxanthin is stronger than other carotenoids in reducing free radical activity as a trigger the emergence of such degenerative disease cancer, heart disease, and diabetes mellitus.[16]

One of the herbal medicines that have antioxidants is ginger, lemongrass and cloves.

This research is in line with research conducted by Nivetha R at al. that the Chinese used cloves more than 2000 years ago, to get rid of bad breath. The synergistic effect of clove oil along with other coriander oils, anise, coriander, and cilantro eucalyptus shows a higher level of inhibition in Gram-negative bacteria, thus proving that synergism worsens the antimicrobial activity of clove oil. Clove

The oil can be a short term remedy for halitosis because it is antimicrobial, but cannot be used long term because it is lacking probiotic activity.[17] Halitosis is not a disease but an inconvenience, probiotics are marketed for the treatment of oral and intestinal halitosis. Few clinical studies have proven different strains or probiotic products potent.[18]

Studies have shown an increasing number of type 2 diabetes (T2D) patients with concomitant obesity and hyperlipidemia syndromes, caused by relevant metabolic disorders. However, there are several drugs and therapies that can solve this problem completely. Research conducted by Xiaotong Yu at all stated that herbal formula JTTZ menghasilkanpeningkatan yang aman dan signifikan pada glukosadarah, lipid darah, dan tingkatberatbadan; gejalalægæ; dannemeningkatkan ungssel β untukpasien T2D denganobesitas dan hiperlipidemia. Herbal fomula JTTZ telahmenunjuk kanbahaiberpotensidikembangkansiebagaipengobatanalternatifuntukpasien T2D, terutamamereka yang tidakdapatmentolerir metformin atauobathipoglikemiklianynya.[19]

**Conclusion**

Based on the results of the antibacterial activity test, it can be said that all tests for both formulation A and formulation C using several concentrations showed quite good results with the antibacterial activity being directly proportional to the concentration, the greater the concentration the greater the activity. It should be given the opportunity to continue this research in testing and exploring herbal plants using other bacteria.

**Financial Support and Sponsorship:** Costs from research institutions Universitas Muslim Indonesia, Makassar

**Ethical Considerations:** Ethical clearance was obtained from Universitas Muslim Indonesia; with number” 579/A/KEPK- UMI/V/2020. Just before the interview, written (or thumb impression) consent was obtained from each participant in Universitas Muslim Indonesia guidelines.

**Conflicts of Interest:** The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated.

**References**


The Concordance of Dysmorphic Erythrocyte and Cast Erythrocyte Examination using Flowcitometry, Low Condenser Light Microscope, and Phase Contrast Microscope in Children with Glomerular Hematuria

Maya E. Roring¹, Jusak Nugraha², Risky V. Prasetyo³, Ferdy R. Marpaung²

¹Resident, Clinical Pathology Specialist Programme, ²Lecturer, Departement of Clinical Pathology, ³Lecturer, Departement of Child Health, Faculty of Medicine, Airlangga University - Dr. Soetomo Academic Hospital, Surabaya, Indonesia

Abstract

This was an observational analytic research with cross sectional design carried out at the Clinical Pathology Laboratory and Department of Child health of the Dr. Soetomo Hospital Surabaya in April - November 2020. Urine sediment of glomerular hematuria pediatric patient examined using flowcytometry, low condenser light microscope (LLCM), phase contrast microscope (PCM). The concordance of the results of the examination of dysmorphic erythrocytes and erythrocyte cylinders using flowcytometry with PCM was determined by Cohen’s Kappa Coefficient (κ) and Bland Altman. There were 40 urine samples of children with glomerular hematuria with many diagnose was lupus nephritis of 60%. The analysis of dysmorphic erythrocyte examination from LLCM and PCM had a very strong kappa agreement κ 0.828 (p <0.05); The concordance analysis of LLCM and PCM cast erythrocyte had a strong agreement, κ 0.625 (p <0.05) and the concordance analysis between Flowcitometry and PCM dysmorphic erythrocyte flagging had a weak agreement, κ 0.302 (p> 0.05). LLCM can be considered to replace PCM to determine the origin of hematuria because it is very effective in detecting dysmorphic erythrocytes in patients with glomerular hematuria. The use of automatic tools is useful for pathological urine samples, but it is advisable to review them using a manual macroscope.

Keywords: Dysmorphic erythrocytes, erythrocyte cylinders, phase contrast microscopy, light microscopy, flowcytometry

Introduction

Glomerular hematuria is classified according to the number of erythrocytes found in urine into microhematuria and macrohematuria. The causes of glomerular hematuria in children are IgA nephropathy, post streptococcal acute glomerulonephritis (GNAPS), primary glomerulonephritis, systemic lupus erythematosus (SLE), henoch-schonlein purpura (HSP), membranoproliferative glomerulonephritis (MPGN), rapidly progressive glomerulonephritis (RPGN). The diagnosis of hematuria in children is more difficult than in adults because the symptoms are non specific and often without symptoms. Invasive methods such as kidney biopsy is the gold standard to establish the diagnosis. Birch and Fairy recommend examining the urine sediment using a phase contrast microscope to determine the origin of glomerular or non-glomerular hematuria by assessing the size and formation of erythrocytes in urine. The incidence of asymptomatic isolated hematuria in children ranges from 0.5% and 1%, mostly due to glomerular abnormalities and most often in boys than girls, regardless of age.

Urine sediment examination is a test that can be used to diagnose glomerular hematuria. Dysmorphic erythrocytes and erythrocyte cylinders found in urine are one of the markers of glomerular damage. Examination
of urine sediment with manual microscopy recommended by several international guidelines is a phase contrast microscope (PCM). PCM is considered to be better at identifying cells and formations found in urine compared to low light condenser microscopy. PCM as a reference method has several limitations, namely it is expensive so that it is not available in all laboratories, time consuming and requires reading expertise.

Study by Barthe et. al, 1986 used a non staining light microscope to evaluate dysmorphic erythrocytes, and the results were similar to studies using PCM. Different results were found in the study by Ince et. al. which states that light microscopy is inadequate for the detection of bacteria, erythrocytes and hyaline cylinders. Flowcitometry is a method used in examining urine sediment with an automatic analyzer. Several journals report the use of flowcitometry can overcome the limitations of PCM because the results are more standardized and the operation is fast, thus saving time and effort, however, a scientist stated that flowcitometry was inadequate in identifying particles such as cylinders and crystals in pathological urine samples so that a manual microscope review is still needed.

Examination with a low condenser light microscope and flowcitometry, if it gives the appropriate results as obtained at PCM, can be used as a substitute for examining dysmorphic erythrocytes and erythrocyte cylinders. This prompted researchers to examine the agreement of the results of the examination of dysmorphic erythrocytes and erythrocyte cylinders using flowcytometry, low light condenser microscopy (LLCM), and phase contrast microscopy in children with glomerular hematuria.

Materials and Methods

This study was an observational analytic with a cross sectional design which was carried out in the Laboratory of Clinical Pathology and the Department of Children Health of Dr. Soetomo Hospital Surabaya, Indonesia. Samples were pediatric patients who had been diagnosed with glomerular hematuria by the clinician of the Nephrology Division of Pediatrics, who met the inclusion and exclusion criteria. Samples were collected from April-November 2020.

The first urine sample in the morning from a patient with glomerular hematuria was examined for urine sediment. The volume of urine collected is 10-12 mL. The urine was initially examined with Flowcitometry method by Sysmex UF 5000 automatic tool. Then, the urine was centrifuged at 2000 rpm for 5 minutes, the supernatant was removed by decantation. The remaining sediment was then resuspended with 0.3-0.5 mL of the remaining supernatant. 1 drop of urine was placed between a slide and cover slip then examined under a phase contrast microscope and a low light condenser microscope. The data from the automatic tool was in the form of dysmorphic erythrocyte flagging and the number of cylinders/µL, while PCM and LLCM were the number of dysmorphic erythrocytes and erythrocyte cylinders in 10 fields of view.

The concordance for the examination of dysmorphic erythrocytes and erythrocyte cylinders using flowcytometry with phase contrast microscopy was determined using the Cohen’s Kappa Coefficient (κ) and The concordance of the examination of dysmorphic erythrocytes and erythrocyte cylinders using a low condenser light microscope with a phase contrast microscope was determined by Bland Altman.

Results and Discussion

There were a total of 40 study patients who met the inclusion and exclusion criteria consisting of 23 male patients (57.7%) and 17 female patients (42.5%). The mean age of patients in this study was 11.92 ± 3.323 and most diagnosis was lupus nephritis (Table 1).
Table 1. Characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>11.92 ± 3.323</td>
</tr>
<tr>
<td>Median (min-max)</td>
<td>12.5 (5 – 17)</td>
</tr>
<tr>
<td>Sex</td>
<td>n %</td>
</tr>
<tr>
<td>Boy</td>
<td>23 57.5</td>
</tr>
<tr>
<td>Girl</td>
<td>17 42.5</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Lupus Nephritis</td>
<td>24 60</td>
</tr>
<tr>
<td>GNAPS+RPGN</td>
<td>2 5.0</td>
</tr>
<tr>
<td>IgA Nephropathy</td>
<td>2 5.0</td>
</tr>
<tr>
<td>Nephrotic Syndrome</td>
<td>1 2.5</td>
</tr>
<tr>
<td>Nephrotic Nephritic Syndrome</td>
<td>6 15</td>
</tr>
<tr>
<td>RPGN+CKD</td>
<td>5 12.5</td>
</tr>
</tbody>
</table>

The results of dysmorphic erythrocytes examined using LLCM showed dysmorphic erythrocytes in 32 samples (80%) and using PCM 34 samples (85%) of the 40 samples in this study. For erythrocyte cylinders, 4 samples (10%) and 5 samples (12.5%) were obtained from the entire study sample using LLCM and PCM. Dysmorphic erythrocyte flagging was released by the Sysmex UF 5000 as many as 24 samples (60%). Evaluation by LLCM and PCM showed dysmorphic erythrocytes in 32 samples (94.1%). The Kappa result between LLCM and PCM dysmorphic erythrocytes obtained a Kappa coefficient of 0.828 with p <0.001 (Table 2).

Table 2. Agreement of LLCM and PCM dysmorphic erythrocytes.

<table>
<thead>
<tr>
<th>Dysmorphic Erythrocyte PCM</th>
<th>Dysmorphic Erythrocyte LLCM</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>32  (94.1%)</td>
<td>2  (5.9%)</td>
</tr>
<tr>
<td>No</td>
<td>0  (0%)</td>
<td>6  (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>32  (80%)</td>
<td>8   (20%)</td>
</tr>
</tbody>
</table>
The results of the Bland Altman test show that there is an agreement in the number and percentage of dysmorphic erythrocytes between LLCM and PCM (Figure 1). Evaluation by LLCM and PCM obtained erythrocyte cylinders in 3 samples (60%). The Kappa result between LLCM and PCM dysmorphic erythrocytes obtained a Kappa coefficient of 0.625 with p <0.001 (Table 3).

**Table 3. Agreement of LLCM Erythrocyte Cylinders with PCM.**

<table>
<thead>
<tr>
<th>Erythrocyte Cylinders PCM</th>
<th>Erythrocyte Cylinders LLCM</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Ada</td>
<td>1</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>36</td>
</tr>
</tbody>
</table>

Evaluation dysmorphic erythrocyte by PCM and Flagging on flowcitometry were obtained in 23 samples (60%). The Kappa result between PCM with flowcytometry flagging obtained a Kappa coefficient of 0.302 with p 0.019 (table 4).

**Table 4. Agreement of PCM Dysmorphic Erythrocytes with Flowcytometry Dysmorphic Erythrocyte Flagging.**

<table>
<thead>
<tr>
<th>Dysmorphic Erythrocyte PCM</th>
<th>Flagging Flowcytometry</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>23 (67.6%)</td>
<td>11(32.4%)</td>
</tr>
<tr>
<td>No</td>
<td>1 (16.7%)</td>
<td>5 (83.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (60%)</td>
<td>16 (60%)</td>
</tr>
</tbody>
</table>
The study subjects consisted of 40 pediatric patients who had been diagnosed with suspected glomerular hematuria. Most of the diagnosis were lupus nephritis. Study by Viteri et al. The most common cause of nephritis is GNAPS\(^1\). Study by Moustafa et al. in Egypt the most common cause of glomerular hematuria in children is Alport Syndrome\(^1\). Current data in Dr. Soetomo Hospital Surabaya, Indonesia in the period from January 1 2020 to December 31 2020, the most common diagnosis for the causes of glomerular hematuria in children were SLE and Nephrotic Syndrome. The difference in diagnosis that is often found as a cause of glomerular hematuria is probably due to differences in patient selection criteria for each study.

The age range of the entire study sample group was 5-17 years with a mean of 11.92 years, with the largest age group being 12-17 years. These results are the similar as studies by Pardede et al. 2005 that Acute glomerulonephritis in children is most often found is 6-11 years old and Huang et al. 2010, where in the population of children with SLE - LN, the mean age at diagnosis was 8.6-13.5 years\(^1\). Most of the subjects of this study were men (57.5%). These results are the similar as studies by Moustafa et al. that male subjects were 60%\(^1\) and Pardede et al. also found male subjects were 58%\(^1\). A literature states that glomerular hematuria is more common in men than in women, regardless of age\(^1\).

Urine sediment examination both microscopic and automatic analyzer can quantitatively describe the number of elements formed in urine\(^1\). Microscopic examination of urine sediment can improve diagnostic efficiency when performed early in the evaluation of a disease\(^1\). Dysmorphic erythrocytes are found in the urine if the glomerular filtration barrier (GFB) is impaired. These erythrocytes are smaller in size and have smaller cytoplasmic protrusions or cell fragments\(^1,15,16,17\). From LLCM examination result by 3 readers, dysmorphic erythrocytes were positive in 32 samples (80%) and negative in 8 samples (20%), while the dysmorphic erythrocytes from PCM was slightly more, positive in 34 samples (85%) and negative in 6 sample (15%). This result was in accordance with previous study by Da Silva et. al. where there was no significant difference in the number of dysmorphic erythrocytes found in low-light condenser microscopes and contrast-phase microscopes in glomerular and non-glomerular hematuria patients\(^9\).

Damage of the GFB can also cause protein and erythrocytes to appear in the urine. Erythrocytes that enter the tubule will be trapped in uromodulin (Tamm-Horsfall protein) to form a cast (erythrocyte cylinder)\(^1\). The results of this study showed that erythrocyte cylinders in LLCM were negative in 36 samples (90%) and positive in 4 samples (10%). The results were not much different from the review with negative erythrocyte cylinder PCM in 35 samples (87.5%), positive in 5 samples (12.5%). Previous study by Ringsrud et al. in patients with interstitial nephritis, cylindrical erythrocytes were only found in 4 patients\(^19\). This result is in accordance with previous study. One literature states that erythrocyte cylinders are rare but highly pathognomic and have a high specificity of 97% for detecting glomerular hematuria\(^20\).

Examination of urine sediment using a conventional microscope, although considered a reference method, has several limitations in its operation. To increase the accuracy and precision of urine sediment examination, several studies have been conducted to compare automatic instruments with manual examination using a microscope\(^21,22,23\). In addition, agreement analysis of dysmorphic erythrocyte count from LLCM and PCM was carried out on 40 child participants in this study. Dysmorphic erythrocytes were positive in 32 samples (94.1%) and 6 samples (100%) negative from both microscopes. Two samples (5.9%) were positive for dysmorphic erythrocytes in PCM but negative in LLCM (Table 1). The kappa test result was 0.828 (p<0.01), it suggest that there is a very strong agreement for the results of dysmorphic erythrocytes in LLCM and PCM. These results are consistent with studies by Barros Silva et. al. PCM has a higher sensitivity than LLCM, both methods have the same accuracy and can be used for examination and a study by Chu shu et al. that LLCM have the same accuracy to recognized isomorifk and
The Kappa coefficient of 0.625 was obtained in the test results between LLCM and PCM erythrocyte cylinders which showed a strong agreement (p <0.05). Furthermore, LLCM and PCM have the same ability to detect dysmorphic and isomorphic erythrocytes. LLCM ability on urine sediment examination was good enough, similar to PCM.

Conclusion

There is a strong agreement for examination of dysmorphic erythrocytes and erythrocyte cylinders using a low light condenser microscope and a contrast phase microscope in children with glomerular hematuria with kappa coefficients of 0.828 and 0.625. With the results, LLCM can be used to replace PCM. The weak agreement between flowcitometry and PCM shows that for pathological urine samples using an automatic flowcitometry method it is recommended to do a review using a conventional microscope.

Conflict of Interest: The author declare that they have no conflict of interest.

Source of Funding: None.

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Ethical Approval

This study was approved by Health Research Ethics Committee of Dr. Soetomo Surabaya, Indonesia (approval number: 1810/KEPK/II/2020).

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Knowledge and Practice Regarding Prevention of Renal Calculi among Non Teaching Staffs of a Selected College of Health Sciences at Mangaluru

Maya Shaju1, Amala Elsy Mathai1, Dina Joseph1, Albert Thampi1, Jyothi2

1IVth Year B.Sc Nursing Students, 2Lecturer, Department of Medical Nursing, Yenepoya Nursing College, Yenepoya Deemed to be University, Mangaluru, Karnataka

Abstract

Background and Aim: Mankind has been afflicted by urinary stones and is the most common disease of the urinary tract. It affects all ages, sexes, and races but occurs more frequently in men than in women within the age of 20–49 years. Generally, to prevent the first episodes of kidney stone formation or its secondary episodes, proper management of diet is required. The study aim to assess the correlation between knowledge and practice regarding prevention of renal calculi among Non teaching staffs.

Methods: A descriptive correlation approach was adopted for the study and by purposive sampling technique 95 non-teaching staffs were selected. The data was collected using demographic proforma, structured knowledge questionnaire and Rating scale. Collected data were analyzed by Descriptive and Inferential statistics using SPSS version16.0.

Results: Results showed that majority (75.8%) of the subject had average knowledge and 72.6% of the subjects had good practice towards the prevention of renal calculi. There was a significant correlation (p<0.05) between knowledge and practice among non-teaching staffs regarding the prevention of Renal calculi.

Conclusion: The study concluded Non teaching staffs had average knowledge and good practice towards prevention of renal calculi.

Key words: Knowledge, Practice, Non teaching staffs, Renal calculi

Introduction

Globally, kidney stone disease prevalence and recurrence rates are increasing, with limited options of effective drugs. Urolithiasis affects about 12% of the world population at some stage in their lifetime. It affects all ages, sexes, and races, but occurs more frequently in men than in women within the age of 20–49 years. In Indian population, about 12% of them are expected to have urinary stones and out of which 50% may end up with loss of kidney functions.1

Mankind has been afflicted by urinary stones and is the most common disease of the urinary tract. Kidney stone, also known as urolithiasis, is when a solid piece of material formed within the urinary tract. Kidney stones typically form in the kidney and leave the body through the urine stream.2

The stone formation include anything that either causes stasis or supersaturation of the urine. Immobility and a sedentary lifestyle, which increases stasis. Dehydration, which leads to supersaturation. Metabolic disturbances that results in an increases in calcium or other ions in the urine. Previous history of urinary
calculi. Living in stone-belt areas, high mineral content in drinking water, a diet high in purines, oxalates, calcium supplements, animal proteins, urinary tract infections, prolonged indwelling catheterization, neurogenic bladder, history of female genital mutilation also play a role in kidney stone formation.

Effective kidney stone prevention depends upon addressing the cause of stone formation. Generally, to prevent the first episodes of kidney stone formation or its secondary episodes, proper management of diet and the use of medications is required. Primary prevention of kidney stone disease via dietary intervention is low-cost public health initiative with massive societal implications. Thus nutritional management is the best preventive strategy against urolithiasis.

Good voiding habits, particularly frequent urination, and regular exercise are useful in preventing the stones from forming. Most importantly, a high fluid intake should be maintained at all times, especially during hot, dry weather when the risk of kidney stone formation is greatest. Therefore, of all the preventive recommendations is the most important guideline for people with any type of kidney stones. Hence the awareness regarding the prevention of renal stone is necessary to prevent the occurrence and also helps to modify the life style pattern.

Material and Methods

A Descriptive correlational research is being conducted in Yenepoya Nursing college, Karnataka, India after obtaining the ethical clearance(Protocol no 2019/024) from Institutional Ethics Committee, Yenepoya( Deemed to be University). Nonteaching staffs between the age group of 20-49 years were included in the study and who had a previous history of renal calculi were excluded. 95 Non teaching staffs were selected by Non-probability purposive sampling technique. Informed consent was obtained from the respondents after proper explanation about the purpose, the usefulness of the study and assurance was given about the confidentiality of their responses. Data was collected by using structured knowledge questionnaire and self reported rating scale.

The data were analyzed by descriptive and inferential statistics using SPSS version 16.0. Demographic variable, knowledge questionnaire, rating scale will be analyzed using the descriptive Statistics such as Frequency, Percentage, Mean and Standard Deviation. To correlate the knowledge and practice score of non teaching staffs, Karl Pearson co-relation co-efficient will be used. Chi-square test was used to find the association between the Knowledge and practice score with selected demographic variable

Results

Description of sample characteristics:

Frequency and percentage characteristics distribution was computed to describe the sample characteristics. The baseline sample characteristics of the participants showed that majority (31.57%) of the subject belongs to the age group of 20-30 and majority (82.1%) of the subjects was females. Majority (58.8%) of the subjects belongs to Hindu religion, and most (58.4%) of the subject had completed Degree. Majority of the subjects (56.8%) were resides in rural area and most (90.5%) of the subjects are non-vegetarian. Majority of the subjects (49.5%) had previous information through internet.
Table 1: Frequency and percentage distribution of non teaching staffs according to the grading of their knowledge score.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor Knowledge</td>
<td>5</td>
<td>5.3</td>
</tr>
<tr>
<td>Average Knowledge</td>
<td>72</td>
<td>75.8</td>
</tr>
<tr>
<td>Good Knowledge</td>
<td>18</td>
<td>18.9</td>
</tr>
</tbody>
</table>

Table 1 shows that Majority (75.8%) of the subjects had average knowledge, 18.9% of the subjects had good knowledge and only 5.3% of the subjects had poor knowledge.

Practice of Non teaching staffs regarding prevention of renal calculi.

Data presented in the Figure 1 shows that majority (72.6%) of the subjects had good practice and 27.4% of the subjects had average practice.

Correlation between knowledge and practice regarding prevention of Renal calculi.

Karl Pearson correlation coefficient was used to correlate the knowledge and practice score of non teaching staffs. There was a positive correlation between knowledge and practice score of non teaching staffs regarding prevention of renal calculi among non teaching staffs (p =0.027).
Association between knowledge score with selected demographic variables

Chi square association with knowledge score indicated that there is significant association between knowledge and demographic variable i.e, education ($\chi^2=0.017$), $P<0.05$. The findings also revealed that age, gender, religion, residence, dietary pattern, previous information from the internet, health professionals in the family was statistically non significant at 0.05 level.

Association between Practice score with selected demographic variables

Chi square association with practice score indicated that there is no significant association between practice and demographic variable.

Discussion

This study was designed to collect information regarding knowledge and practices of renal calculi among nonteaching staffs. Results indicate that the non teaching staffs had average knowledge regarding renal calculi. This findings are supported by a study conducted by Soni GP (2016) on Prevention of Renal Calculi in Terms of Knowledge and Dietary Pattern among Primary School Teachers of Moodabidri, Dakshina Kannada District. Findings of the study showed that less number of primary school teachers had adequate knowledge regarding renal calculi and its prevention.3

Practice of non teaching staffs regarding renal calculi was better than their knowledge. Majority (72.6%) had good practice towards the prevention of renal calculi. This findings are supported by a descriptive cross sectional study (2017) was conducted in the University of Peradeniya, Sri Lanka to determine knowledge, attitude and practices regarding urinary tract stones among final year medical students ,which showed that majority( 55.55%) of them had a good practice towards the prevention of renal calculi.4

In the present study positive correlation was observed between knowledge and practice. Supportive to this findings, Pethiyagoda et al conducted (2015) a study revealed that There was a correlation between knowledge level and Practice.5

This study finding revealed that there was no significant association between knowledge score and practice score with demographic variables. Supportive to this study ,Bakunts V conducted a study (2011) on Knowledge, Attitude and Practice of Kidney Stone Formers in Armenia regarding Prevention of Kidney Stone Disease which revealed that there was no significant association between knowledge and practice score with selected demographic variable.6

Conclusion

Renal calculi is a global health problem that seriously affects human health. Prevention plays a major role in controlling the incidence of renal calculi. In conclusion, findings indicated that Non teaching staffs had average knowledge and good practice towards prevention of renal calculi.

Acknowledgement: We thank Yenepoya nursing college, (Yenepoya Deemed to be University) for providing the opportunity to conduct the study and for their constant support for the competition of the work.

Conflict of Interest: All the authors declare that they have no conflict of interest.

Informed Consent: Informed consent was obtained from all the study participants

Ethical Approval: Obtained the ethical clearance from Institutional Ethics Committee, Yenepoya( Deemed to be University) (Protocol no 2019/024).

Funding Sources: Self funded

References

3. Soni GP. A Study to Evaluate the Effectiveness of


Financial Obstacles and Disadvantages of E-Learning From The Viewpoint of Dammam Teachers

Mayada Abu Alhomos¹, Tala H. Sasa², Nawal.H.Bahtiti², Tasneem Alayed², Omniya Miri³

¹Assistant Professor, ²Lecture in Professor in Applied science Private University, P.O.Box 166Amman 11931, ³Department of Basic Sciences, Deanship of Preparatory Year and Supporting Studies, Imam Abdurrahman Bin Faisal University, P.O. Box 1982, Dammam 34212, Saudi Arabia

Abstract

The efforts of containing the novel virus (COVID-19) have begun to show signs of psychological impact on teachers and students across the globe. As a result of the COVID-19 pandemic, many school districts have closed for the remainder of the academic year. These closures are unfortunate because, for many students, schools are their only source of trauma-informed care and supports. When schools reopen, they must develop a comprehensive plan to meet their students’ the potential mental health needs as social distancing and awareness campaigns can be a double-edged sword, if handled inadequately. This paper evaluated the challenges of teachers in Dammam in e-learning during the period of the new coronavirus (COVID-19) global pandemic.

Key word: E-Learning, COVID-19, Teachers, Ancient learning, Students

Introduction

E-learning: It is defined as providing electronic educational content through computer-based media and networks to the learner in such a way that they can interact actively with this content [1]. With the teacher and their peers, whether simultaneously or concurrently. Also the possibility of completing this education in time and space and at a speed that suits its circumstances and abilities, as well as the possibility of managing this learning through those media [2]. Many sources define e-learning as “an interactive system of distance learning, delivered to learners on-demand and based on an integrated electronic environment designed to build and connect courses online, mentoring, testing, resource management, and processes [3,4]. Stein, S. J., Shepherd, K., & Harris, I. (2011) was a lot of inclusive, summary totally different views on e-learning, the argument that e-learning encompassed a spread of patterns [5]. We’ve little doubt that the management of the academic method depends directly on the human workers of academics and directors WHO area unit able to carry on with the technological developments required by this method, in reviewing the capabilities of employees during this field, we have a tendency to should note, in line with out there data, that a minimum of half the employees within the public education sector area unit academics, academics and supporting bodyworkers WHO area unit nearly old, wherever there are a unit a minimum of 2 totally different generation employees WHO adopt ancient education which Education has principally supported the teacher within the schoolroom, and depends on the book and also the direct paper communicating, that is that the direct tool for the worth of scholars, and within the case of the ministry’s reliance on education (simultaneous), this class won’t be able to influence the talents and technological needs of this method, and also the ministry has not provided the mandatory coaching for this class of teachers, that indicates a scarcity of coming up with for this method to launch this model while not taking into consideration the skills of teachers to keep with the programs and tools needed for the success of this year. [6] The second generation is most capable of handling technological reality and group action data technology.
into the education method - UN agency had effective initiatives within the early stone amount - with their own capabilities and straightforward provision tools from a smartphone and a portable computer, and these initiatives have had an energetic impact in overcoming the primary crisis, and this generation has had innovative initiatives within the entire instructional method from innovative initiatives in tributary to the education method. Benefits: flexibility, convenience, straightforward, and quick access anytime, anyplace [7]. The chance of choosing elements from online courses is consistently increasing in quantities, instant feedback once victimization online assignments, exams and exercises straightforward and quick review, updating, piece of writing and distributing instructional elements. Asynchronous permits the scholar to review consistent with his ability (quickly or slowly), offers numerous instructional facilities and ways that stop ennui, it’s straightforward to follow the scholars notwithstanding there several quick access for thousands of same-time sources aside from paper sources. Time: Save time, organize time in order that he schedules his lessons consistent with his work and his family. Quicker as a result of it permits the scholar to leap faraway from subjects and activities, he is aware of. Money: wherever it cuts the value of travel, quality, and living. Also, the value of production and distribution of instructional materials. Cost of offices and lecturers, the value of wasting workers’ time. Communication and interaction: The possibility of communication and interaction between students and lecturers through online lessons [8]. The chance to review anyplace with a pc and web. The interaction between the lecturer and also the student is best within the case of overcrowded categories. Disadvantages: eLearning suffers from some determinants, that area unit incentives for study and analysis to resolve them: [9]. You need a technological infrastructure that will not be out there in some places. Limited pandowdies might hinder the method of education, particularly in loading and handling multimedia system. [10]. The start value is high. Some students might feel lost or confused regarding instructional activities Some students might feel isolated from their peers and their teacher. This study un concealed academics’ opinion regarding e-learning; Some courses are troublesome to conducted online since it would like a lot of personal communication and require the student to learn laptop skills. The bulk of Arab countries have resorted to e-learning to finish the syllabus. During this paper, the analysis house on the obstacles and challenges of e-learning, wherever there are obstacles for the college, that could be a lack of expertise and mastery of laptop skill [11], and there are issues facing students, the most important of them is the material potential of some and also the temperament to hitch the electronic categories of learning. In Addition that the majority of scholars have an absence of technical expertise [12]. This paper additionally addressed the way to overcome such obstacles by seeking out solutions to every downside so as to finish the course of education, particularly for college students in their final stages of education as they’re near to graduate, by motivating students to be told e-learning, and coaching school members through awareness courses and workshops on e-learning [13].

Method

A. Participants

This survey was conducted from the 10th of September 2020 to 25th of October 2020. In eastern Dammam office /Saudi Arabia. A total of 130 were recruited to the study, the majority of study (91.5%) respondents were in the middle age between 29 to 48 years old. Males were accounted approximately one fourth (26.2%) of sample. The majority of respondents (73.1%) were bachelor degree holders. About one third of the sample had experience between 6 to 11 years. About 60.0% of respondents were from international and private schools.

B. Constructs of the study

We have three constructs Availability of E-learning requirements related to technical, administrative and financial aspects, assessment environment Learning, and E-learning challenges and obstacles. Each category includes several variable: age, gender, educational level and experience.
Statistical Analysis

Data were analyzed by the Statistical Package for Social Sciences (SPSS) software version 25.0 (SPSS®: Inc., Chicago, IL, USA). Means, standard deviations, frequencies and percentages were produced. Independent t-test, one-way ANOVA test with Scheffe post hoc were used to compare between subgroups as appropriate. Furthermore, Pearson correlation coefficient used to test the correlation between overall mean of the outcomes. The level of significance was set at (P \leq 0.05).

Results

A. Sample description

A total of 130 were recruited to the study, the majority of study (91.5%) respondents were in the middle age between 29 to 48 years old. Males were accounted approximately one fourth (26.2%) of sample. The majority of respondents (73.1%) were bachelor degree holders. About one third of the sample had experience between 6 to 11 years. about 60.0% of respondents were from international and private schools.

B. Reliability coefficient

The reliability coefficient (Cronbach’s Alpha) of the study is 0.829, which is a good value reflecting a reliable measure of the study tool.

D. Study of items’ means

The following part shows responses to the levels of the variables. Here, mean and standard deviation are calculated for each item. Higher mean value indicates more agreement on that item.

i. Availability of E-learning requirements related to technical, administrative and financial aspects

Table 1 shows that all items have “agree” attitude. Item 3-The school administration encourages teachers to use the e-learning and computerized curriculum in teaching has the highest mean value of 4.12(SD=0.813) with ‘agree’ attitude. Item 4-E-learning goals branch into more commercial goals than educational and Item 5-The speed of the internet is always suitable to take advantage of the website services at all times “ has the lowest mean with 3.22(SD=1.09, 1.22) respectively with ‘agree’ attitude. In general respondents’ attitude was “agree” regarding the availability of E-learning requirements related to technical, administrative and financial aspects with mean 3.59(SD=0.5).

<table>
<thead>
<tr>
<th>Item</th>
<th>Mean</th>
<th>SD</th>
<th>Attitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-The prevailing educational system continuously supports e-learning</td>
<td>3.75</td>
<td>0.827</td>
<td>agree</td>
</tr>
<tr>
<td>2-School administration allocates part of the school’s budget to support e-learning at the beginning of the school year</td>
<td>3.45</td>
<td>1.027</td>
<td>agree</td>
</tr>
<tr>
<td>3-The school administration encourages teachers to use the e-learning and computerized curriculum in teaching</td>
<td>4.12</td>
<td>0.813</td>
<td>agree</td>
</tr>
<tr>
<td>4-E-learning goals branch into more commercial goals than educational</td>
<td>3.22</td>
<td>1.019</td>
<td>agree</td>
</tr>
<tr>
<td>5-The speed of the internet is always suitable to take advantage of the website services at all times</td>
<td>3.22</td>
<td>1.226</td>
<td>agree</td>
</tr>
<tr>
<td>6-The computer lab official helps teachers and students constantly</td>
<td>3.38</td>
<td>1.045</td>
<td>agree</td>
</tr>
<tr>
<td>7-The platform used for e-learning is easy to handle</td>
<td>3.75</td>
<td>0.798</td>
<td>agree</td>
</tr>
</tbody>
</table>
Table 2 shows that Item 11-Teachers encourage students to enter the platform and follow up on assignments and tests has the highest mean of 4.34 (SD=0.551) with attitude of ‘strongly agree’. In the second rank Item 12-Availability of educational material on the platform all the time increases the ability to understand the educational material with mean 4.02 (SD=0.821). The minimum mean is for Item 6- It reduces students’ skills in cooperative learning and learning by playing with only 2.00 (SD=0.956). In general, ii. assessment environment Learning is of ‘neutral’ attitude with mean 3.15 (SD=0.405).

Table 2. B. assessment environment Learning

<table>
<thead>
<tr>
<th>Item</th>
<th>Mean</th>
<th>SD</th>
<th>Attitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-E-learning lacks effective communication between students and the teacher</td>
<td>2.67</td>
<td>1.278</td>
<td>neutral</td>
</tr>
<tr>
<td>2-E-learning is a heavy burden on the head of the family when pursuing his children</td>
<td>2.05</td>
<td>1.063</td>
<td>disagree</td>
</tr>
<tr>
<td>3-The student is convinced of the necessity of e-learning as an alternative to traditional learning</td>
<td>3.50</td>
<td>1.021</td>
<td>agree</td>
</tr>
<tr>
<td>4-E-learning lacks confidentiality and honesty when answering tests</td>
<td>2.15</td>
<td>1.229</td>
<td>disagree</td>
</tr>
<tr>
<td>5-E-learning weakens students’ attitudes and educational values, which are stipulated in the philosophy of education</td>
<td>2.25</td>
<td>1.023</td>
<td>disagree</td>
</tr>
<tr>
<td>6- It reduces students’ skills in cooperative learning and learning by playing</td>
<td>2.00</td>
<td>0.956</td>
<td>disagree</td>
</tr>
<tr>
<td>7-The teacher feels that e-learning reduces his control over the course of the educational process</td>
<td>2.62</td>
<td>1.190</td>
<td>neutral</td>
</tr>
<tr>
<td>8-The teacher has technological skills that enable him to deal with the system</td>
<td>3.72</td>
<td>0.881</td>
<td>agree</td>
</tr>
<tr>
<td>9-The lack experience for students and their parents in using computer software</td>
<td>2.32</td>
<td>1.025</td>
<td>neutral</td>
</tr>
<tr>
<td>10-The teachers communicate with the students and answer their questions</td>
<td>4.00</td>
<td>0.844</td>
<td>agree</td>
</tr>
<tr>
<td>11-Teachers encourage students to enter the platform and follow up on assignments and tests</td>
<td>4.34</td>
<td>0.551</td>
<td>Strongly agree</td>
</tr>
</tbody>
</table>
12- Availability of educational material on the platform all the time increases the ability to understand the educational material 4.02 0.821 agree

13- The student will benefit from the educational platform services and continuously follow them 3.76 0.888 agree

14- Lessons are recorded for students using multimedia so the student can follow them 3.78 0.767 agree

15- Students are encouraged with rewards and certificates of appreciation for their continuous follow up 3.33 1.052 neutral

16- There is a phone number or email to communicate with the school administration and teachers to answer inquiries of students and their parents 4.02 0.802 agree

Overall 3.15 0.405 neutral

E-learning challenges and obstacles

Table 3 illustrates that in the first rank is Item 4-cheat prevention is difficult to control during electronic tests with mean 4.29(SD=0.731) with attitude of ‘strongly agree’, while Item 5-Students can see their evaluations’ results and scores is in the second rank with mean of 4.12(SD=0.722) with ‘agree’ attitude. Item 10- Do encourage continuous use of E-learning has the lowest mean of 3.14(SD=1.193) with ‘neutral’ attitude. In general, E-learning challenges and obstacles have a mean of 3.73(SD=0.401) with ‘agree’ attitude.

Table 3: C. E-learning challenges and obstacles

<table>
<thead>
<tr>
<th>Item</th>
<th>Mean</th>
<th>SD</th>
<th>Attitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Some e-learning programs are expensive</td>
<td>3.65</td>
<td>0.904</td>
<td>agree</td>
</tr>
<tr>
<td>2 - E-learning is difficult to apply in materials that require practical application and laboratories</td>
<td>4.10</td>
<td>0.905</td>
<td>agree</td>
</tr>
<tr>
<td>3 - Students respond positively to the offered subject</td>
<td>3.39</td>
<td>1.075</td>
<td>neutral</td>
</tr>
<tr>
<td>4 - Cheat prevention is difficult to control during electronic tests</td>
<td>4.29</td>
<td>0.731</td>
<td>Strongly agree</td>
</tr>
<tr>
<td>5 - Students can see their evaluations’ results and scores</td>
<td>4.12</td>
<td>0.722</td>
<td>agree</td>
</tr>
<tr>
<td>6 - The connection to the system is frequently interrupted due to internet pressure</td>
<td>3.95</td>
<td>0.951</td>
<td>agree</td>
</tr>
<tr>
<td>7 - There is coordination between the Ministry of Communications and the Ministry of Education in supporting distance learning</td>
<td>3.58</td>
<td>0.930</td>
<td>agree</td>
</tr>
<tr>
<td>8 - There are deficiencies in the services provided by the system and the mechanism for answering questions</td>
<td>3.32</td>
<td>0.890</td>
<td>neutral</td>
</tr>
<tr>
<td>9 - It is difficult for all parents to subscribe to the Internet and provision of computers</td>
<td>3.77</td>
<td>1.019</td>
<td>agree</td>
</tr>
<tr>
<td>10 - Do encourage continuous use of E-learning</td>
<td>3.14</td>
<td>1.193</td>
<td>neutral</td>
</tr>
</tbody>
</table>

Overall 3.73 0.401 agree
D. Correlation analysis

From table 4, the relation between construct A “Availability of E-learning requirements related to technical, administrative and financial aspects” and construct B “assessment environment Learning” is \((r=0.390)\) which is a moderate value. There is no relation between construct A “Availability of E-learning requirements related to technical, administrative and financial aspects” and construct C “E-learning challenges and obstacles” \((r=0.02)\) which is non-significant and the relation of construct C “E-learning challenges and obstacles” with construct B “assessment environment Learning” which is negative \((r=-0.232)\).

Table 4. Correlation analysis (N=130).

<table>
<thead>
<tr>
<th>Construct</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>0.390*</td>
<td>0.020*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P&lt;0.000</td>
<td>P&lt;0.823</td>
</tr>
<tr>
<td>B</td>
<td>0.390*</td>
<td>1</td>
<td>-0.232*</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.055</td>
<td></td>
<td>P&lt;0.008</td>
</tr>
<tr>
<td>C</td>
<td>0.020*</td>
<td>-.232*</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.823</td>
<td>P&lt;0.008</td>
<td></td>
</tr>
</tbody>
</table>

G. T tests and Analysis of variance

Are there significant differences in the levels of the study constructs that can be attributed to age, gender, Educational level, Experience and Type of school? Independent samples t-test will be used to test for gender while, analysis of variance (ANOVA) will be used to test for other personal variables. Age, gender, Educational level, Experience and Type of school.

Table 5: Mean, standard deviation, P-value and F –value according to part A and part B.

<table>
<thead>
<tr>
<th>Part</th>
<th>Variable</th>
<th>category</th>
<th>Mean (SD)</th>
<th>P- value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-</td>
<td>Availability of E-learning requirements related to technical, administrative and financial aspects</td>
<td>Educational level</td>
<td>Diploma Bachelor Master Ph.D.</td>
<td>4.1(0.168) 3.56(0.508) 3.54(0.505) 3.66(0.136)</td>
</tr>
<tr>
<td></td>
<td>Type of school</td>
<td>Government International Private</td>
<td>3.69(0.493) 3.63(0.509) 3.24(0.315)</td>
<td>F=-6.901 P=.001</td>
</tr>
<tr>
<td>B-</td>
<td>E-learning disadvantages</td>
<td>Type of school</td>
<td>Government International Private</td>
<td>3.30(0.518) 3.05(0.364) 3.1(0.664)</td>
</tr>
</tbody>
</table>
From table 5 part A, there is a significant difference in the mean of Availability of E-learning requirements related to technical, administrative and financial aspects for educational level diploma (mean = 4.1) p=0.016 <0.05 compared with another education levels, also there is a significant difference in the mean of Availability of E-learning requirements related to technical, administrative and financial aspects for type of schools where government schools have (mean 3.69) p=0.001<0.05 while private schools have mean 3.24.

From table 5 part B, there is a significant difference in the mean of E-learning disadvantages for type of schools where government schools have (mean 3.30) p=0.02<0.05 while international schools have mean 3.05. there is no significant difference in the mean of E-learning obstacles that can be attributed to Age, gender, Educational level, Experience and Type of school.

**Discussion**

From the teachers’ point view E-learning is not a heavy burden on the head of the family when pursuing his children, E-learning doesn’t lack confidentiality and honesty when answering tests, E-learning doesn’t weaken students’ attitudes and educational values, which are stipulated in the philosophy of education and It doesn’t reduce students’ skills in cooperative learning and learning by playing. The teachers saw that cheat prevention is difficult to control during electronic tests [2].

E-learning objectives correspond to traditional learning objectives, as well as to life-long learning objectives related to cognitive, affective and psychomotor domains. The continuous and rapid change of contemporary society and existing technologies leads to life-long education. E-learning is a part of life-long learning that may become more popular [7]. The lack experience for students and their parents in using computer software is not necessary a problem in E-learning since all middle and high school students have access to mobile devices [3]. We agree with others all over the world that teachers are needed more training courses to develop their experiences of software applications [5].

Online teaching and learning were an unprecedented experience for most teachers and students; although nowadays platforms, training course, and software applications are available [5]. Although teachers at Dammam schools feels that e-learning can’t reduce the control over the course of the educational process. Jordanians teachers found that “the continuing with the online learning model is not acceptable because it is socially and psychologically unhealthy Measures of lockdown, closures, and quarantine, brought by COVID-19 caused stress, frustration, and depression. [11]. Also we can summarize the effect of E-learning on university students and their instructor, the opinions of students that studying using e-learning is not equal as direct education [12].

**Conclusion**

The COVID-19 pandemic has modified schooling forever; The COVID-19 has ended in colleges close all throughout the world. As a result, schooling has modified dramatically, with the one-of-a-kind upward push of e-getting to know, wherein coaching is undertaken remotely and on virtual platforms. Research shows that online getting to know has been proven to boom retention of information, and take much less time, that means the adjustments coronavirus have brought on is probably right here to stay.

**Recommendations**

Educators should be motivated to participate in the training of obtaining

Informed knowledge about new technological changes so as to form a positive attitude towards e-learning for both teachers and students. Whereas, training will not only benefit teachers, but students at the same time.

The smooth implementation of the e-learning system can be ensured by providing the internet for each
as a tool, which will motivate students to improve their knowledge, technologies and skills.

**Acknowledgement:** All thanks and appreciation to the administrative and academic staffs of Applied Science Private University and everyone who contributed to the completion of this scientific study.

**Declaration of Competing Interest**

Authors declare that they have no conflicts of interest to disclose.

**Source of Funding:** there is no financial support.

**Ethical Clearance:** Taken from Applied Science private University

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Stature Prediction and Formulation of Regression Equation from Hand and Foot Anthropometry

Megha Rapotra¹, Jaswinder Kaur²
¹Ph.D Scholar, ²Professor, Department of Anatomy, MMU, Mullana, Ambala, Haryana

Abstract

Background: Estimation of stature is a key feature of personal identification. Evaluation of stature from incomplete skeletal and decomposing human remains is particularly important in personal identification for forensic experts and anthropologists.

Methods: The data was collected from 1000 asymptomatic, healthy adults (500 males and 500 females) belonging to border areas of Punjab region of age group ranged between 18-50 years.

Results: The comparison of each parameter of the study shows that all parameters have higher values in males than in females. It is also observed that in males highest correlation is exhibited by Right foot length (r = .418) and in females it is exhibited by Left Foot Length (r = .354). The calculated linear regression formulae show good reliability and applicability for stature estimation.

Conclusion: In all parameters the foot length and hand length are better predictors of stature in both sexes. Using regression formulae the height of subject can be easily determined and this will be used as research tool in anthropological and medicolegal issues.

Keywords: Stature, Anthropometry, Hand length, Foot length, Forensic investigations

Introduction

Stature is considered as one of the important and significant parameters for establishment of personal identification. Anthropometry is being widely used in medical sciences especially in Forensic Sciences for helps in narrowing down the investigation process. These techniques are commonly used by anthropologists especially adopted by forensic anthropologists to estimate body size for the purpose of identification. Estimation of stature from decomposing and incomplete skeletal is important in personal identification.

Anthropometry as adopted by medical scientist is described as a technique of expressing the form of human body quantitatively as it is the systematic collection and correlation of measurement of the human body. Origin of anthropometry dates back to as early as the ancient civilizations of Rome, Egypt and Greece. They use anthropometric measurements for cultural purposes to represents beauty, power and other desirable attributes of the human Form. so it has been the focus of scientists, anatomists and anthropologists for many years.

There is an established relationship between stature and various body parts like head, trunk, upper and lower extremities. It is common to find the peripheral parts of the body such as hand and imprints of the hand, foot or footprints or from shoe left at scene of a crime or in explosions, aircraft and railway accidents. The relationship between specific body dimensions / proportions can be used to help solve crimes in the
absence of complete evidence. Therefore, many different parts of the body have been used to estimate height by different workers globally.9

Many studies have been conducted for assessing stature by anthropometric measurements of different parts of the body. 2,8-15

India is a vast country with varied geographical conditions so these parameters vary from population to population due to the differences in genetic makeup of person, nutrition, environment and levels of physical activity.16

Therefore the purpose of this study is to find the correlation between body height, foot length, foot breadth, hand length and hand breadth measurements in both the sexes and also to derive regression equations for the calculation of stature. The present study is unique in its sample selection as there is no existing anthropological data on the estimation of stature from hands and feet measurements in this group of population of border areas of Punjab, India.

Materials and Methods:

The present study was conducted out in the region of Punjab, mainly border areas like Amritsar, Pathankot, Gurdaspur, Dinanagar Disticts and their surrounding villages. The material comprised of 1000 young healthy adults (500 males and 500 females) of the group 18-50 years. The subjects were from general public. The data was conducted by doing Random sampling.

Steps of collection of data: Inclusion and Exclusion criteria: Study included Apparently healthy, asymptomatic adults, age ranges between 18 to 50 years. Males and females with any physical deformities of stature, hand and foot were excluded from the study.

In order to avoid inter-observer error in methodology, the measurements were taken by one observer. Diurnal variations have been reported in the stature of an individual thus, all measurements were taken during afternoon hours in a well lighted room and were recorded in measurement performa.

The objectives and steps of the study were explained to the study population Prior to the investigation, written informed consent was obtained by taking their signature and thumb impressions. Foot and hand measurements were taken independently on the left and right side of each individual, apart from this stature were also recorded.

Instruments used: Stadiometer was used to measure verticle height for stature estimation. The measurements were recorded in centimeters. Sliding caliper was used for feet measurements.

The data were subjected to statistical analysis using statistical package for social sciences (SPSS21).

Land marks and technique involved in anthropometric measurements:

1. Stature (S): It is the vertical distance between the highest point on vertex and platform of measuring bar. The subject should stand erect, barefoot on level platform, with his back hips and feet touching the bar, arms hanging by the side and head should rest in Frankfurt plane.

2. Hand length (HL): It is the distance between the mid-point of a line connecting the styloid processes of radius and ulna to the most anterior of the middle finger. The subjects will asked to place their hands supine on a flat hard horizontal surface with fingers extended and adducted, following which the hand length were measure by sliding caliper.

3. Hand breadth (HB): It is the distance between the most prominent point of the Lateral aspect of the head of the second metacarpal to most prominent point of the medial aspect of head of the fifth metacarpal.

4. Foot length (FL): It is the distance between the Most prominent posterior point on the heel to the tip of first toe foot lengths were taken independently on left and right side of each individual using a spreading caliper.
5. **Foot breadth (FB):** It is the distance between the most prominent point of the medial aspect of the head of the first metatarsal to most prominent point of the lateral aspect of head of the fifth metatarsal.

**Results:** The following observations were tabulated after statistical evaluation of the observations recorded in the study. The age of study population ranged between 18 and 50 years for both males and females. The mean age of females was 32 years and that of males was 33 years.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Stature</th>
<th>Foot Length</th>
<th>Foot Breadth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td>Male N=500</td>
<td>Female N=500</td>
<td>Rt side</td>
</tr>
<tr>
<td>Mean</td>
<td>171.13</td>
<td>159.91</td>
<td>25.05</td>
</tr>
<tr>
<td>Std.D</td>
<td>9.47</td>
<td>7.24</td>
<td>1.69</td>
</tr>
<tr>
<td>Minimum</td>
<td>149.35</td>
<td>149.35</td>
<td>17.6</td>
</tr>
<tr>
<td>Maximum</td>
<td>198.13</td>
<td>192.03</td>
<td>29.5</td>
</tr>
</tbody>
</table>

[Table-1] shows comparison of descriptive statistics for the stature, foot and hand parameters in both sexes. The average stature of males was 171.13±9.7 cm and for females it was 159.91±7.2 cm. The foot lengths measured 25.05 cm (approx) and hand breadth measured approximately 9.57 cm. Similarly in case of female foot lengths and foot breadths approximately measured 23.08 and 8.83 cm respectively.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Hand Length</th>
<th>Hand Breadth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td>Rt side</td>
<td>Lt side</td>
</tr>
<tr>
<td>Mean</td>
<td>18.50</td>
<td>18.48</td>
</tr>
<tr>
<td>Std.D</td>
<td>1.12</td>
<td>1.15</td>
</tr>
<tr>
<td>Minimum</td>
<td>12</td>
<td>12.3</td>
</tr>
<tr>
<td>Maximum</td>
<td>21.4</td>
<td>21</td>
</tr>
</tbody>
</table>

[Table-2] shows comparison of hand dimensions among male and females in study population. The average of the hand lengths measured 18.50 cm (approx) and hand breadth measured approximately 8.24 cm in males. Similarly in female hand length and hand breadth approximately measured 17.17 cm and 7.46 cm respectively.

The comparison of respective readings of various parameters studied shows that all parameters have higher values.
in males than in females. [Table-3] shows correlation between the stature of individual and various parameters studied. All the parameters exhibit statistically highly significant (p <0.001) positive correlation with the stature except female foot breadth.

Correlation co-efficient of the length measurements is higher than that of breadth measurements. It is also observed that in males the highest correlation is exhibited by right foot length (r=0.418) and the lowest by Left Foot Breadth (r=0.152). In females highest correlation co-efficient is exhibited by left foot length (r=0.354).

Table 3: Showing Karl Pearson’s Correlation Coefficients between the stature and various parameters studied in sample.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Study Parameters</th>
<th>Karl’s Pearson Correlation</th>
<th>Sig (2-tailed) (p&lt;0.05)</th>
<th>Karl’s Pearson Correlation</th>
<th>Sig (2-tailed) (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>MALE SAMPLE</strong></td>
<td></td>
<td><strong>FEMALE SAMPLE</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Right Hand Length</td>
<td>.403</td>
<td>.000*</td>
<td>.348</td>
<td>.000*</td>
</tr>
<tr>
<td>2</td>
<td>Left Hand Length</td>
<td>.374</td>
<td>.000*</td>
<td>.346</td>
<td>.000*</td>
</tr>
<tr>
<td>3</td>
<td>Right Hand Breadth</td>
<td>.249</td>
<td>.000*</td>
<td>.174</td>
<td>.000*</td>
</tr>
<tr>
<td>4</td>
<td>Left Hand Breadth</td>
<td>.212</td>
<td>.000*</td>
<td>.191</td>
<td>.000*</td>
</tr>
<tr>
<td>5</td>
<td>Right Foot Length</td>
<td>.418</td>
<td>.000*</td>
<td>.351</td>
<td>.000*</td>
</tr>
<tr>
<td>6</td>
<td>Left Foot Length</td>
<td>.415</td>
<td>.000*</td>
<td>.354</td>
<td>.000*</td>
</tr>
<tr>
<td>7</td>
<td>Right Foot Breadth</td>
<td>.154</td>
<td>.001*</td>
<td>.117</td>
<td>.009</td>
</tr>
<tr>
<td>8</td>
<td>Left Foot Breadth</td>
<td>.152</td>
<td>.001*</td>
<td>.118</td>
<td>.008</td>
</tr>
</tbody>
</table>

* statistically significant value

Linear regression analysis of the observations was performed separately for each sex and also for each parameter studied. (Table 4) The equations also exhibit standard error of estimation (SEE). The SEE predicts the deviations of the estimated stature from the actual stature.
Table 4: Linear Regression equations to determine stature from different parameters in sample.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Parameters</th>
<th>Linear Regression Equations</th>
<th>SEE**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FEMALE SAMPLE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Foot Length</td>
<td>( S = 116.69 + 1.87 \times FL )</td>
<td>5.1</td>
</tr>
<tr>
<td>2</td>
<td>Foot Breadth</td>
<td>( S = 159.38 + 0.05 \times FB )</td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>Hand Length</td>
<td>( S = 110.47 + 2.88 \times HL )</td>
<td>5.9</td>
</tr>
<tr>
<td>4</td>
<td>Hand Breadth</td>
<td>( S = 138.04 + 2.84 \times HB )</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>MALE SAMPLE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Foot Length</td>
<td>( S = 110.87 + 2.40 \times FL )</td>
<td>5.8</td>
</tr>
<tr>
<td>6</td>
<td>Foot Breadth</td>
<td>( S = 158.13 + 1.35 \times FB )</td>
<td>3.7</td>
</tr>
<tr>
<td>7</td>
<td>Hand Length</td>
<td>( S = 106.32 + 3.50 \times HL )</td>
<td>6.6</td>
</tr>
<tr>
<td>8</td>
<td>Hand Breadth</td>
<td>( S = 143.37 + 3.37 \times HB )</td>
<td>4.8</td>
</tr>
</tbody>
</table>

The accuracy of the regression equations was verified by comparing the estimated stature with actual stature. The estimated stature values are found very closer to actual stature value in both male and females. (Table 5)

Table 5: Difference of Actual Stature and Estimated Stature in Males and Females.

<table>
<thead>
<tr>
<th>Estimated Stature using Regression Equations for</th>
<th>Mean Estimated Stature (in Cm)</th>
<th>Difference Between Means= Mean Actual Stature – Mean Estimated Stature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male Mean Actual Stature (171.39)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot Length</td>
<td>171.11</td>
<td>0.02</td>
</tr>
<tr>
<td>Foot Breadth</td>
<td>171.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Hand Length</td>
<td>171.11</td>
<td>0.02</td>
</tr>
<tr>
<td>Hand Breadth</td>
<td>171.11</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Female Mean Actual Stature (159.91)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot Length</td>
<td>159.84</td>
<td>0.07</td>
</tr>
<tr>
<td>Foot Breadth</td>
<td>159.90</td>
<td>0.01</td>
</tr>
<tr>
<td>Hand Length</td>
<td>159.83</td>
<td>0.08</td>
</tr>
<tr>
<td>Hand Breadth</td>
<td>159.86</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Discussion

The mean stature estimated in the present study in males is 171.13 cm (Range: 149.35-198.13) whereas in females mean stature is cm 159.91 (Range: 149.35-192.03). The stature obtained by different researchers such as Ilayperuma ,Sunil, Manpreet Kaur et al, Rati Tandon, Sunil, was similar to the present study.17-20 In this study, the mean body height of males is higher than that of mean body height of females. This is in agreement with the result of Ebite et al., and Ilayperuma et al.3,17 There are also other studies by Qamara SR et al, Ozaslan A et al and Duyar I et al, Nath S et al, indicating varied mean height due to variations in the morphology of different population group.2,6,20,21

It is also observed that in males the highest correlation is exhibited by right foot length (r=0.418) and the lowest by Left Foot Breadth (r=0.152). In females highest correlation co-efficient is exhibited by left foot length (r=0.354). All the parameters exhibit statistically highly significant (p <0.001) positive correlation with the stature except female foot breadth. There are some studies by Saxena, Bhatnagar et al, Abdel-Malek AK et al, Krishan, in which an attempt has been made to establish correlation between stature and hand dimensions.8,10,11,15 Deopa Deep and Charnalia also observed a significant and positive correlation between foot length and height in individuals of Uttarakhand region.22,23 GN Geetha et al, Manpreet Kaur et al, Rati Tandon, and O.P. Jasuja et al showed the significant correlation between height and hand parameters.16,18,19,24

The presence of positive correlations between stature and other study parameters of hand and foot, facilitates formulation of regression equations which can be successfully utilized for stature estimation in the population. There are various methods to estimate stature but the easiest and reliable method is by regression analysis. In the present study, we had formulated different regression equations separately for both the sexes and on both sides. Research indicated that regression formulae can be derived for stature estimation using foot and hand measurements with a great accuracy and a small SEE, i.e. about 2–6 cm.

Conclusion

The study indicates that stature can be predicted accurately by linear regression analysis even when only some remains of the body are found as in bomb explosions, terror events, mass disaster, accidents, wars etc. If either of the measurement is known the other can be calculated and it will help in establishing identity in certain medicolegal cases and for forensic experts. For calculating height, we used higher number of population and formed different regression equations using hand breadth, with different values, resulting in more correct regression equations. These regression equations are specific for this region only because of geographical variations in the morphology of different population group.

Ethical Clearance: Institutional Committee (IEC), MMIMSR, Mullana (Ambala).

Source of funding: Self

Conflict of Interest: Nil

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Assessment of Knowledge Regarding First Aid among the Undergraduate Students: A Cross-Sectional Study

Melvin Jacob¹, Hezil Reema Barboza²

¹MSc Nursing, ²Lecturer, Dept of Medical Surgical Nursing, Yenepoya Nursing College, Yenepoya (Deemed to be University), Deralakatte, Mangaluru, Karnataka, India

Abstract

Background: First aid knowledge is very valuable for every individual in the community. It enables the individual to assist the injured person in an emergency situation until medical help arrives at the site.¹ Students are highly exposed to different kinds of physical injuries. Lack of knowledge regarding managing these medical injuries was noted among the students.² Careful management recommended for reducing the complication. This study was conducted to determine the knowledge of first aid and its associated factors among undergraduate students.³⁻⁵

Materials and Methods: A descriptive cross-sectional study was conducted among 100 undergraduate students. Subjects were selected using purposive sampling techniques. The information was collected using a demographic proforma and a self-administered questionnaire. The overall knowledge on first aid was graded as adequate, good and inadequate.

Results: The overall knowledge score was 13.81 ± 4.41. The mean percentage of the overall level of knowledge was 57.54% which indicate the majority had good knowledge on aspects of first aid. In this study there was no significant association between knowledge score and selected demographic variables.

Conclusion: The study showed the majority had good knowledge of first aid. But also identified some key areas in which first aid knowledge was deficient in the management of shock, burns and fracture. Thus there is a need for first aid training to be introduced in the undergraduate curriculum of different disciplines. The study recommends the use of modern techniques such as simulation with computerized mannequins as a teaching-learning method in all the educational institutions.

Key words: First aid, Knowledge, Students, Shock, Undergraduate.

Introduction

First aid is the immediate support given to the person with minor or major injuries. Knowledge regarding basic first aid measures is curtailed to save the life of an injured. It facilitates the rescuer to assist the injured person until the emergency helps arrives. Various types of accidents and injuries are part of our day to day life. Lack of skillful personals in and around the accident sites usually delays the basic management, later it will become a serious life-threatening condition. Prompt action taken during the initial hours will helpful for the person to come back to a normal productive life.¹

Accidents are not completely preventable or unavoidable. Managing the victims in a professional manner from the accident area will be help to reduce the deformity.² The number of persons killed in the road traffic accidents is drastically increased, 2.7 per one lakh population in the year 1970 is upturn to 11.8 per one lakh population in 2015.³ Studies conducted in various parts of the country also focusing the need for basic first aid training. One of the South Indian study results revealed that 12 % of the students are only undergone specific first aid training sessions.⁴ Developing countries like India it is needed to incorporate basic first aid in the graduation level curriculum will help to reduce mortality.
and morbidity related to the mishandling of affected persons.\textsuperscript{5}

**Materials and Method**

The research design adopted for the present study was a descriptive research design. The study was carried out in a professional college, Karnataka. Samples consist of 100 undergraduate students who were selected by purposive sampling technique, based on inclusion criteria. Undergraduate students studying in the first year were selected as study participants. Students who underwent training in first aid were excluded from the study. In the present study first aid refers to immediate care given to the person suffering from sudden illness or injury related to conditions such as cardiac resuscitation, chest pain, ventilation, stroke, shock, fracture, poisoning, burns and snakebite.

**Tools and techniques**

Blueprint of the questionnaire was prepared on the basis of specific areas such as first aid knowledge on cardiac problems 33.32%, ventilation 4.16%, stroke 16.66%, shock 12.5%, fracture 8.35%, poisoning 8.35%, burns 12.5%, snake bite 4.16%. In order to obtain validation of the data collection tool, the draft of the problem statement, objective, demographic proforma, and knowledge questionnaire was submitted to 5 experts. To ensure reliability, the tool was administered to 10 undergraduate students after obtaining permission from the authority. Reliability of the tool was analyzed by split-half method followed by Spearman-Brown prophecy formula. The reliability coefficient of the tool was found to be 0.8. This indicated that the tool was reliable.

The baseline Proforma was used to assess the baseline characteristics of undergraduate students. It had 4 items which include age, gender, type of family, and area of residence. The knowledge questionnaire had 24 items. A score of ‘1’ was given for the correct answer and ‘0’ for the wrong answer. The maximum score was 24. Knowledge score 75% and above was graded as adequate knowledge, 51-74% was good and less than 50% was graded as inadequate knowledge.

Data collection was done after obtaining ethical clearance from the institutional ethics committee (Protocol No: 2017/068). Prior permission from the concerned authority was obtained to conduct the study. Subjects were asked to participate in the study after self-introduction by the investigators. The subjects were informed about the purpose of the study and their consent was attained. The participants were assured about the confidentiality of their information. The data were analyzed in terms of the objectives of the study using both descriptive and inferential statistics. The data obtained were plotted in the master sheet.

**Data Analysis:** The data entry and analysis was performed using Statistical Package for Social Sciences software package version 23. Chi-square test was used to find out the association of socio demographic variables with knowledge score regarding first aid and $P < 0.05$ was taken as statistically significant association

**Results**

The baseline characteristics of the study population showed a majority of them (66%) were 18 years and 24% were 19 years old. The maximum numbers of the subjects (90%) were females. Most of the subjects (88%) belong to the nuclear family and 55% were living in an urban area.

Figure 1 shows almost half of the proportion (49.0%) was with good knowledge and only 36% were with inadequate knowledge regarding first aid. The overall knowledge score was $13.81 \pm 4.41$. The mean percentage of the overall level of knowledge was 57.54% which indicate the majority had good knowledge of the management aspects of first aid which was showed in table 1. Table 2 depicts the analysis of knowledge on first aid management in the specific area found that participants had had poor knowledge regarding first aid management for shock, burns and fracture. Most of the participants had very good knowledge to identify and manage cardiac problems. Data presented in the table 3 revealed that there was no significant association was
found between knowledge of undergraduate students regarding first aid and selected demographic variables such as age, gender, area of resident and type of family.

**Figure 1: Grading of knowledge score**

**Table 1: Mean, median, mean% and standard deviation of overall knowledge score**

<table>
<thead>
<tr>
<th>Description</th>
<th>Mean</th>
<th>Mean%</th>
<th>SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall knowledge score</td>
<td>13.81</td>
<td>57.54</td>
<td>4.41</td>
<td>14.5</td>
</tr>
</tbody>
</table>

**Table 2: Mean scores for the areas of knowledge questionnaire**

<table>
<thead>
<tr>
<th>Area</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac problems</td>
<td>4.76</td>
<td>1.87</td>
</tr>
<tr>
<td>Stroke</td>
<td>2.22</td>
<td>2.10</td>
</tr>
<tr>
<td>Shock</td>
<td>1.72</td>
<td>0.92</td>
</tr>
<tr>
<td>Burns</td>
<td>1.70</td>
<td>1.14</td>
</tr>
<tr>
<td>Poisoning, snake bite</td>
<td>2.28</td>
<td>1.08</td>
</tr>
<tr>
<td>Ventilation</td>
<td>2.28</td>
<td>1.08</td>
</tr>
<tr>
<td>Fracture</td>
<td>1.14</td>
<td>0.77</td>
</tr>
</tbody>
</table>

SD: Standard deviation
Table 3: Association between knowledge of undergraduate students regarding first aid and selected demographic Proforma

\[ N = 100 \]

<table>
<thead>
<tr>
<th>SL No</th>
<th>Demographic variable</th>
<th>Median (≤14.5)</th>
<th>Median (≥14.5)</th>
<th>X2</th>
<th>df</th>
<th>P-value</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18 years</td>
<td>34</td>
<td>32</td>
<td>.58</td>
<td>2</td>
<td>0.74</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>19 years</td>
<td>11</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 years</td>
<td>4</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>7</td>
<td>3</td>
<td>1.96</td>
<td>1</td>
<td>0.16</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>42</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Type of family</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Joint family</td>
<td>5</td>
<td>7</td>
<td>.29</td>
<td>1</td>
<td>0.58</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Nuclear family</td>
<td>44</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Area of residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urban</td>
<td>31</td>
<td>24</td>
<td>2.65</td>
<td>1</td>
<td>0.10</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Discussion**

Awareness and knowledge regarding first aid play a crucial role in undergraduate students, especially those who are pursuing a medical career, as they are tomorrow’s lifesavers. In the present study it is observed that the majority of the subjects (49%) were having good knowledge regarding first aid, followed by (36%) were having inadequate knowledge and (15%) were having adequate knowledge. A study done by Swetha et al reveals that 96% of the nursing students had basic knowledge of first aid. A study was conducted by Alsayali et al shows that 56% of participants had good knowledge regarding first aid and basic life support.

The majority of study participants were aware of dealing with cardiac emergencies and the study recommends the training of the general population in basic cardiac emergencies. A review article by Toresdahl et al, suggests training for medical providers at sporting events. An efficient response to cardiac emergencies requires a planned emergency action plan, training of potential first responders in cardiopulmonary resuscitation and use of an automated external defibrillator, communication, transportation and coordinated work systems. Prompt recognition and early defibrillation are crucial in the management of athletes suffering sudden cardiac arrest during athletic events. This article reviews cardiac care in athletics, with special considerations to the school and outdoor school events.

Several studies showed poor knowledge in the specific areas of first aid management such as shock,
burns, fracture, seizures and choking. The results of a prospective study of school injuries showed an incidence rate of 5.4 injury events/100 children annually, which appears to be an underestimate of the actual rate. Among all the injury events 28.7% resulted in serious injuries. Most of the children during the study period with either serious or minor injuries were sent to the school office or returned to the classroom, which indicates that the present level of first-aid training among school personnel is inadequate. In this view every teaching and nonteaching staff needs in-service education of management of musculoskeletal injuries.

All students and staff need to be competent in first aid skills and apply in various real-life situations. All felt that these skills need to be taught from the school level onwards and all of them were willing to participate in the first aid training sessions at the college. Courses on First aid and Basic Life Support (BLS) should be made mandatory in universities and this should be done through lectures with hands-on skills to make it more effective. Countries like England have made BLS courses mandatory in the school curriculum and research showed that 86% of school children effectively demonstrated performing CPR correctly. Simulation-based education provides an opportunity for the students to improve education, competency and safety of the patients. Students reported a high level of satisfaction with the hands-on experience as a member of the health care team in a simulated cardiac arrest. With the limited sample size, the researchers acknowledge the limitation of the study regarding the generalization of results of this project to the entire population of students and in the present study samples were selected from a single college.

**Conclusion**

Present study findings showed that the majority undergraduate students had good knowledge about first aid. The incorporation of first aid education and training will be able to improve the knowledge of remaining students with inadequate knowledge. Proper knowledge and efficient skills in different techniques, materials used in first aid and Basic Life Support (BLS) play a crucial role in the effective management of victims of accidents and injury. Institutions should give importance to first aid and BLS skills of their students by conducting refresher courses on a regular basis. Simulation-based education should be incorporated into the medical education curriculum to train the graduates.

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**Conflicts of Interest:** There are no conflicts of interest

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Evaluation of Dental Implant Stability Regarding All-on-4 Concept Utilizing Two Different Angles

Mohammad A. Hameed1, Thair A. Hasan2

1Ph.D. Student, Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Baghdad, Baghdad-Iraq, 2Professor, Chairman of Scientific Iraqi Council of Maxillofacial Surgery, Baghdad-Iraq

Abstract

Background: The “All-on-4” technique using 4 implants that has gained popularity in full arch edentulism cases in recent years was presented as a modern technique in implant-denture rehabilitation by Malo in Lisbon, Portugal, for the first time in 2003 and the all-on-four concept that began to be used in atrophic full arch mandibular and in the maxilla in 2005 has emerged.

Aim: evaluation of dental implant stability in relation to All-on-4 concept.

Materials and Methods: This was a clinical prospective comparative study organized from February 2019 to March 2020 in the College of Dentistry Teaching Hospital, Department of Oral & Maxillofacial Surgery/Dental Implant Unit/University of Baghdad. A total of 15 Iraqi patients aged 41-73 years, 9 males & 6 females were enrolled in this study receiving 80 Dental implants (All-on-4 concept). Patients classified into two groups, group A (All-on-4 with 2 anterior axial and 2 posterior with angulation of 17°) and group B (All-on-4 with 2 anterior axial and 2 posterior with angulation of 30°). For both, the surgical site of dental implant was examined for the hard and soft tissues clinically and radiographically utilizing OPG and CBCT.

Results: The mean of ISQ values obtained in this study at each time point in the total dental implant, axially placed and angled implant stability demonstrated significant increase (P = 0.000, 0.031 and 0.004) respectively at different times. There was a significant increase in the mean stability of axial and angled implants concerning males (P = 0.001 and P =0.002) respectively than females. Significant correlation between implant stability and the regions at the three times periods. There was a significant difference in angled implants stability between different intervals (P = 0.001) in patients >50y. Success of dental implants 95.24%.

Conclusion: All-on-4–style full-arch dental implant procedures have one of the highest success rates of dental treatment; the dental implant stability had significant effect in mandible in comparison to maxilla. There was irrelevant clinical analysis result in comparison to the statical analysis in relation to stability with different variables.

Keywords: All-on-4, Implant stability and Dental implant.

Introduction

All on 4 Concept Principle

In 1993, Dr Paulo Malo performed the pilot study to establish All-on-4 concept (1). The design of the “All-on-4” immediate-function concept was developed in 2003 by Malo and colleagues (2), the approach to rehabilitate the fully edentulous mandibular jaw by placing only 4 implants in the following combination: 2 anterior implants placed axially and 2 posterior implants placed distally tilted within the mandibular parasymphysel region. These implants were immediately loaded with a full fixed acrylic prosthesis within 2 hours of surgery.

Corresponding author:
Mohammad A. Hameed
email: mohamed.alrubae84@gmail.com
Building on the mandibular “All-on-4” success, Malo and colleagues replicated the same design for the maxilla in 2005 (4).

**Biomechanical advantages of “All-on-4” design** (3).

1. Implants follow a dense bone structure.
2. Longer implants can be placed by tilting them posteriorly.
3. Tilting improves A-P spread of implants.
4. A-P spread enhances load distribution for prosthesis.
5. Shorten cantilever (maximum of 7 mm for maxilla and 1.5–2.0 _ A-P spread for mandible) reduces prosthetic fracture/instability and marginal bone height stability.
6. Marginal bone height of implants is maintained with rigid prosthesis.
7. Tilted implants have similar success rate as traditional implants when splinted together.

According to Sennerby and Meredith, 2008 and Sachdeva et al., 2016 dental implant stability can be divided into:

- Primary stability refers to the mechanical bracing of the implant in bone and absence of any micromovement.
- Secondary stability refers to successful osseointegration of the implant with the surrounding bone. The majority of implant losses may be explained as biomechanically induced failures, since low primary implant stability, low bone density, short implants and overload have been identified as risk factors.

Resonance frequency analysis (RFA): Meredith, Alleyne (5) suggested a non-invasive method of analyzing peri-implant bone by connecting an L shaped transducer to an implant in an animal study. The transducer provides a high frequency mechanical vibration and record the frequency and amplitude of the signal received. The resonance frequency was thus defined as the peak of frequency-amplitude plot and converted to a value representing stiffness of bone implant interface (6).

A new generation of RFA technology has been developed by members of the original team behind the commercialisation of RFA (Integration Diagnostics Sweden AB, Gothenburg, Sweden). A small pen-like battery-driven instrument (PenguinRFA) is used together with reusable transducers (MulTiPeg™) as (Fig. 1.5B). These are made from biocompatible titanium and can consequently be autoclaved and used numerous times. The instrument can be packed in a sterile pouch and kept on the surgical tray and used without the assistance from a second person (7). The instrument measures the frequency of the vibration and translates it to an ISQ scale value between 1 and 99. The higher the ISQ value, the better the stability. RFA measures implant stability as a function of interface stiffness, which correlates with implant displacement, i.e. micro-mobility. More than 700 references, ISQ> 70 represents “high stability”, ISQ between 60 and 69, “medium stability”, and ISQ <60 is considered “low stability” (8). Values above ISQ 70 indicate a very stable implant with low micro-mobility. This value is typically recommended for one-stage and immediate loading. A second measurement is recommended before the final restoration to verify osseointegration (9).

**Materials and Methods**

A total of 15 Iraqi patients aged 41-73 years, 9 males & 6 females were enrolled in this study receiving 80 DI (All-on-4 concept).

Patients classified into two groups, group A (All-on-4 with 2 anterior axial and 2 posterior with angulation of 17°) and group B (All-on-4 with 2 anterior axial and 2 posterior with angulation of 30°). For both, the surgical site of DI was examined for the hard and soft tissues clinically and radiographically utilizing OPG and CBCT.
The total performed implants was 80 DI was for both groups, 40 DI for each group.

**Inclusion Criteria:**

1. Patient’s ≤18 years with complete jaw edentulism.

2. Patient was with good general condition or with other diseases that not influences BHP (like hypertension … etc.).

3. Completely edentulous maxilla and mandible or presence of teeth with an unfavorable long-term prognosis to be extracted.

4. Adequate available alveolar bone height and width in between premolars regions.

5. Patients who refused any kind of sinus augmentation procedure on undergoing any kind of Inferior Alveolar Nerve (IAN) transposition procedure (lateralization) when indicated.

**Exclusion Criteria:**

1. Presence of local acute infection at the implant site or any evidence of local pathological conditions in implant zone.

2. Poor oral hygiene and poor motivation to initiate/maintain good oral hygiene.

3. Any systemic diseases or condition that influence bone healing.

4. Anatomical limitation that interfere with the performance of this procedure.

5. Clinical evidence of parafunctional habits.

**Clinical examination:**

A detailed medical, family and dental history were taken from the patients regarding any systemic diseases, smoking and other conditions that could influence bone healing. A clinical examination of the patient to evaluate regional lymph nodes, facial profile and symmetry, and temporomandibular joint function. The intraoral examination assessed mouth opening, oral hygiene, any clinical evidence of parafunctional habits.

**Radiological examination**

A preliminary preoperative OPG was taken as a standard radiograph for documentation and assessment of the available alveolar bone height taking in consideration the amount of magnification and important anatomical structures such as (the anterior wall of maxillary sinus, floor of nasal cavity, mandibular canal and mental foramen and its loop). Also, it was important to identify the presence of any pathology in the implant zone and the relation of the proposed DI to the anatomical limitations. CBCT for indicated cases (candidates) was taken to evaluate the available bone of the maxilla and mandible in a 3-dimensions view.

**Surgical Procedure**

Prophylactic oral rinse with 0.2% chlorhexidine was performed about 1 minute before surgery. Topical spray anesthesia of Lidocaine 10% was applied to the buccal/palatal and lingual mucosa to decrease pain of local anesthesia. Surgical procedures were performed under local anesthesia infiltration with Lidocaine 2% (Septodent). A crestal incision was made from first molar to first molar region with distally bilateral buccal vertical oblique incision, full-thickness mucoperiosteal flap reflection using Molt # 9 and/or Haworth periosteal elevator was performed to locate a bluish hue and bulge of maxillary sinus to determine anterior boundary of maxillary sinus and attention to locate and avoid damaging the mental nerve and locating neurovascular bundle clinically with direct vision, alveolar osteotomy was made with surgical bur. The ridge crest was trimmed to remove any sharp edges and irregularities using surgical bur and surgical hand piece with copious irrigation of normal saline solution, as seen in Figure (1A).

After drilling with a ø 2.4 mm drill to 10-mm depth using a dental engine handpiece set at 800 rpm and torque equal to 35 N/cm, an All-on-4 Guide (Trigonometer, Nucleoss Turkey) was placed in the midline. The
contour of the guide should be adjusted so as it follows the opposing arch; this allows implants to be directed against the opposing arch for proper inclination, as presented in Figure (1B).

Starting with the anterior implant sites, drilling with a pilot drill (starter drill) at least 10 mm from the previously midline guide (lateral incisor position) according to jaw size and anatomical variation, sequential drilling until reaching the final size with 0°. The two axially anterior implants were installed in the anterior region parallel to the midline following the jaw axially to avoid buccal bone plate penetration, Implant placed with 35 rpm speed using motorized way and then finally seated with manual ratchet Figure (1C&D).

Posterior implant position at second premolar position with the same technique and insertion torque. The exact location of the anterior wall of maxillary sinus was important to be located and with attention to locate and avoid damaging the mental nerve and neurovascular bundle clinically with direct vision because it allows the posterior implants to be placed angulated distally with either 17° or 30° with respect to the guide and with anterior wall of maxillary sinus according to group selection as illustrated in Figures (E & F).

After the instillation of dental implants in their position using same speed and torque, a multipeg on each fixture was applied and measure the primary implant stability at time of surgery mesio-distally and buccolingually using penguin® device, as demonstrated in Figure (1G).

This step was followed by seating the multiunit abutment on implant fixture. Wound closure was performed with interrupted (3/0) black silk suture.

Within 72 hours, the provisional denture was inserted with the use of temporary multiunit collar as demonstrated in Figure (2A&B).

The stability was measured after 12 weeks and 24 weeks. The metal framework and final restoration was performed after 12 weeks. A passive fit was essential to ensure accuracy and not to translate undue strain onto the implants. The occlusal contacts were adjusted. Regarding the screw-retained restoration, the abutments and restorations were gently tightened to the implant bodies using a manual screwdriver to facilitate it is opening as seen in Figure (2C&D).

**Results**

Distribution of patients data concerning the age and gender

Fifteen patients contributed to this study aged from 41-73 years with an average of 59.8 years. The highest percentage (73.3%) was reported >50y this study included 9 males and 6 females (60% vs 40%) respectively with a male to female ratio of 1.5:1.

**Distribution of dental implants (DI) per patients according the gender in each jaw**

Eighty-four DI were installed in twenty-one jaws according to All-on-4 concept, one case dropped out after four weeks. Nine male patients (twelve jaws) were contributed in this study with six females (eight jaws).

The effect of time on the dental implants stability.

Table (1) explain the statistical correlation between dental implants stability in ISQ that were installed in axial (anterior), angled (posterior) direction in relation to time interval T1 (baseline), T2 (12 weeks) and T3 (24 weeks). In the total DI, axially placed and angled implant stability demonstrated significant increase (P = 0.000, 0.031 and 0.004) respectively at different times.

Relation of gender and times differences regarding the stability of dental implants

There was a significant increase in the mean stability of axial and angled implants concerning males (P = 0.001 and P =0.002) respectively, on the other hand, the axial inserted implants stability presented a significant correlation between gender at T2 & T3 (P =0.010 & 0.023) as explained in a table (2).
Age and time differences regarding the stability of dental implants

There was significant difference in angled implants ISQ between different intervals (P = 0.001) in patient >50y.

**Jaws and time difference regarding the stability of dental implants**

Regarding to mandible there was a significant difference in the mean of ISQ values at different periods (p = 0.024) as explained in a table (3).

Angle and time differences in the posterior region regarding the stability of dental implants

The posterior implants were inserted at two different angles (17° and 30°), there was no significant difference in ISQ value concerning DI angle at each time data. Furthermore, there was a significant difference in the mean of implant stability for DI inserted at (30°) (P=0.040) as demonstrated in the table (4).

**Survival rate**

The total number of DI installed in the study was 84. Four dropped out of the total number due to failure during healing period. All implant undergoes early loading and success rate was 95.24%.

<table>
<thead>
<tr>
<th>Table 1: Correlation of the effect of time on the stability of dental implants.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variable</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Anterior (Axial)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Posterior (angled)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Highly significant = **, significant = *, Min. =Minimum, Max. = maximum, No. = Number, S.D. = standard deviation.
Table 2: Descriptive statistics and gender difference regarding the stability of dental implants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>Descriptive Statistics</th>
<th>Gender difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Males (No.=12)</td>
<td>Females (No.=8)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
</tr>
<tr>
<td>Total</td>
<td>T1</td>
<td>74.38</td>
<td>3.07</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>75.71</td>
<td>2.77</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>75.93</td>
<td>3.25</td>
</tr>
<tr>
<td></td>
<td>F-test</td>
<td>11.34</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.000**</td>
<td>0.215</td>
</tr>
<tr>
<td>Ant.</td>
<td>T1</td>
<td>74.35</td>
<td>3.38</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>75.65</td>
<td>3.03</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>75.69</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td>F-test</td>
<td>11.10</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.001**</td>
<td>0.412</td>
</tr>
<tr>
<td>Post.</td>
<td>T1</td>
<td>74.40</td>
<td>3.46</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>75.77</td>
<td>3.07</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>76.17</td>
<td>3.76</td>
</tr>
<tr>
<td></td>
<td>F-test</td>
<td>8.68</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.002**</td>
<td>0.274</td>
</tr>
</tbody>
</table>

Table 3: Descriptive statistics and jaw difference regarding the stability of dental implants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>Descriptive Statistics</th>
<th>Jaw difference</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Maxilla (No.=9)</td>
<td>Mandible (No.=11)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
</tr>
<tr>
<td>Total</td>
<td>T1</td>
<td>70.34</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>71.21</td>
<td>3.69</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>71.76</td>
<td>3.12</td>
</tr>
<tr>
<td></td>
<td>F-test</td>
<td>2.48</td>
<td>6.69</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.127</td>
<td>0.024*</td>
</tr>
</tbody>
</table>
Cont... Table 3: Descriptive statistics and jaw difference regarding the stability of dental implants.

<table>
<thead>
<tr>
<th>Time</th>
<th>Descriptive Statistics</th>
<th>Angle difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17º (No.=10)</td>
<td>30º (No.=10)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>T1</td>
<td>74.78</td>
<td>4.86</td>
</tr>
<tr>
<td>T2</td>
<td>76.05</td>
<td>3.03</td>
</tr>
<tr>
<td>T3</td>
<td>77.18</td>
<td>2.56</td>
</tr>
</tbody>
</table>

Table 4: angle and time differences in the posterior region regarding the stability of dental implants.

<table>
<thead>
<tr>
<th></th>
<th>F-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>3.89</td>
<td>0.055</td>
</tr>
<tr>
<td>T2</td>
<td>4.98</td>
<td>0.026*</td>
</tr>
<tr>
<td>T3</td>
<td>5.03</td>
<td>0.040*</td>
</tr>
</tbody>
</table>
Figure 1 (A) bluish hue and bulge of maxillary sinus and osteotomy of alveolar ridge. (B) Trigonometer placement in the midline following the opposing arch. (C) Drilling with final drill (D) Installation of anterior implant at 0°. (E) Drilling of posterior implant at 30°. (F) Installation of posterior implant at 30°. (G) Measurement of implant stability by penguin and multipeg intraoperatively.
Discussion

Dental implant stability in all-on-4 design in the current study, the mean of the 2nd measurement of total DI stability (12 weeks following DI placement) was significantly increased when compared with the primary stability (baseline) with values of 74.42 vs 73.34 respectively. Furthermore, the 3rd reading (24 weeks following DI placement) was significantly increased also compared to the primary stability and the 2nd measurement after the healing period (74.76 vs 73.34 and 74.42) respectively. However, there was significant increase in both axial and angled insertion dental implants separately (P= 0.031 and 0.004) respectively.

One important point to mention is that the increase in stability may be attributed to that all of DI were placed in the anterior region of maxilla and mandible. the bone density than the posterior mandible, followed by anterior maxilla, this opinion is supported by Farré (10) who stated that there was a significant statistical relationship obtained between the implant location and Hu and was in turn between the location and primary stability in terms of ISQ measurements.

All the initial measurements of DI stability registered higher than 60 ISQ so there was a contrast with Suzuki (11) who found that The ISQ values increased when the initial ISQ is lower than 60 ISQ, whereas ISQ values mostly stay unchanged or decreased when the initial ISQ is higher than 60 ISQ.

The present study is supported by Olsson (12) who examined a total of 61 oxidized implants (6 or 8 implants per maxilla). The mean ISQ, which was 60.1 at placement and increased to 62.8 after 4 months.

Hassan and Emarah (13) is contradict in their
study with the present research, they reported two groups, Group 1 included 5 patients received all on four maxillary prosthesis, and group 2 included 5 patients received all on six maxillary prosthesis. The Implant stability significantly increase from base line (60) to (62) after 6 months then insignificantly increased at 12 months (63). The reduced number of the implants in group 1 may subject the implants in this group to higher biomechanical load, which may decreased percentage of bone to implant contact and decrease implant stability. In contrast, wide implant distribution in the group 2 may cause physiologic loading of the bone, thus increasing the implant stability.

In the present study, there was a significant change in stability for males 9 (60%) than females (40%) (P=0.000 and 0.215) respectively this may be related to the small sample size.

On the other hand there was a significant change in implant stability in both axial and angled dental implants separately (P= 0.031 &0.004). This is in close relation to the study performed by Wentaschek \(^{(14)}\) who stated that the mean ISQ value for osseointegrated of 57 all on six implants after 3 months was significantly higher (p < 0.001) than their means at baseline. The DI were separated into axial (No. = 39) and tilted (No. = 18) implants, the differences were also significant (p < 0.005).

The patients that were treated according to all-on-4 concepts presented with significant change in total DI stability in relation to mandible than maxilla (P=0.024) this may be contributed to the bone type in the mandible which is highly compact than cancellous bone in the maxilla and this is supported by Sthita \(^{(15)}\).

In the present study, on the other hand, a significant increase in stability of angled DI was noted in the maxilla this may related to the high bone implant contact because the adequate available bone quantity in the maxilla than mandible which may be returned to the fact that of the resorption in mandible more than maxilla, in addition to that there was insufficient articles written about stability of both jaws.

In this research, there was a significant increase in the 2nd & 3rd values of total dental implants ISQ in patients aged > 50 y; this may be related to that, the majority of patients was enrolled in this age group.

In addition to the stability was increased in patient > 50 in angled dental implants.

**Clinical Analysis**

Considering the clinical analysis one important point to be mentioned is that according to the ISQ device manufacturer that is based on more than 700 references, the ISQ values are divided into three levels Andreotti et al., 2017.

1. ISQ < 60 = low stability.
2. ISQ 60-69 = medium stability.
3. ISQ ≥ 70 = high stability.

Accordingly, in the present research, a clinical analysis of the data is contemplated and performed by the researcher based on calculating the number of DI in which their ISQ values remained or changed to a different level when compared to the baseline data. The results were considered clinically significant or not, dependent on the number of DI that resided in the same level of ISQ or changed to another level regarding the 2nd and 3rd measurements compared with primary stability. This clinical analysis of data illustrated and confirmed that not all the statistically significant results essentially being clinically relevant as follows:

The statistical analysis reported a significant increase in the 2nd measurement in the age group >50 y and an increase in the 3rd one in the same age group of males. While, the clinical analysis demonstrated no significant change in age group > 50 y (15 out of 16 DI resided in the same level).

In spite of that, the statistical analysis reported a significant increase in stability of dental implants in relation to males in regards to the total, axial and angled DI implants (P= 0.000, 0.001&0.002) respectively, and the mandible showed non-significant change in relation
to any site and angle.

The clinical analysis using McNemar test demonstrated non-significant change in both genders in relation to different site and angles (P=1).

The patients >50y showed statically a significant change in total DI stability (P= 0.000) while clinically there was non-significant change (P= 1).

Regarding the angles, the statistical analysis was reported a significant change in axial and angled DI stability, however it was clinically irrelevant.

The stability of total DI of the mandible, tilted implant in maxilla and angled 30° inserted dental implants presented with statistically significant changes (P= 0.024,040, 0.026 & 0.040) respectively, in spite of that there was no clinical relevant in relation to that (P=1,0.25&1).

**Conclusion**

All-on-4–style full-arch dental implant procedures have one of the highest success rates of any treatment in dental treatments; the technique is also among the most difficult and can be fraught with obstacles. With careful planning and knowledge of potential pitfalls associated with performing, the procedure clinicians can incorporate All-on-4–style dental implant treatment into their practices with a high degree of confidence and less stress.

Survival rate if DI was 95.24% and patients benefited from the use of the All-on-4 treatment concept. The present study showed good clinical outcomes when using two tilted and two axial implants and a fixed prosthesis for rehabilitation of the edentulous ridges. The DI stability had significant effect in mandible in comparison to maxilla.

**Declarations**

Conflict of Interest the authors declare that there are no potential conflicts of interest related to the study.

**Source of Funding:** Nil

**Ethical Clearance:** This research has exemption as it a routine treatment (no new materials were used).

**References**

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15. Sthita Gurrala VD, Francis Akkara. All-on- 4 dental implant concept in immediate rehabilitation of failing dentition—a prospective study to evaluate the efficacy and cost effectiveness in Indian population. 2020.
The Effect of Oxidative Stress on Vitiligo Patients

Mohammed Hasnawi Ali¹, Muthanna M. Awad²

¹Master of Biology Student. ²Prof., University of Anbar, College of Education for Pure Sciences, Department Biology

Abstract

The purpose of the study to shed light on one of the hypotheses keys in causing disease vitiligo is the stress hypothesis of oxidative, stable of vitiligo have been identified active stages or on the basis of the development or the emergence of new spots in the past three months and the absence of new or patches development in the past six months, the in a row, this study included three groups, all of them were male. The first group included the active group, patients with vitiligo of the active type 30, the second person, the dormant group, includes static type vitiligo patients 25, one person, the third, the control group (apparently healthy) included 20 People. We have examined the serum levels of Superoxide Dismutase enzyme, catalase enzyme, and Malondi- aldehyde, our results revealed that the level of higher than Malondi- aldehyde at the level of probability (P <0.05) In the serum of the disease group compared to the control group, a significant increase in the activity of superoxide dismutase was observed in the serum of the disease group at the level of probability (P <0.05) Compared to the control group, the catalase activity decreased at the probability level (P <0.05) In the pathological group compared with the control group, our study shows that stress Oxidative has a role in the physiology pathogenesis of vitiligo active and static. An imbalance of antioxidants was observed in the blood of active Vitiligo patients.

Key Words: Vitiligo, Oxidative Stress, SOD, MDA, CAT

Introduction

Vitiligo Of skin diseases resulting from the absence of melanin pigment in some areas of the body (1). The skin is the outer covering of the body and works to protect it from external influences and the melanocytes are counted Melanocyte One of the components of the skin and these cells are responsible for the formation of the pigment melanin Pigment Which give your skin color, people who suffer from vitiligo disappear to have this dye due to the absence of cells melanocytes of the skin complexion (2,3), Vitiligo appears in both sexes and in all age groups, as it spreads among 2 - 1(%) Of the world’s population and the true causes behind the occurrence of Vitiligo are not clearly known and may be the result of the contribution of several pathological factors (4), Vitiligo disease appears in the form of milky spots is crusted with distinct margins (5). There are theories that explain the breakdown of melanocytes and the pigmentation process in the skin that ends with the emergence of Vitiligo. One of these theories is the theory of oxidative stress. This theory refers to the case of disturbance balance between the oxidizing substances of the types of oxygen and effective types of nitrogen effective and between the systems defense antioxidant during metabolism (6). Oxidative factors contribute to the pathophysiology of vitiligo patients through a significant increase in antioxidants in the serum of vitiligo patients. The antioxidant is Superoxide Dismutase enzyme, catalase enzyme, and Malondi- aldehyde.

Literature Review

Superoxide dismutase enzyme(SOD): a commonly discovered enzyme 1969 by Worlds Mikord
and Friedovich\(^{(7)}\). Works of this enzyme to convert the root of the super Oxide to hydrogen peroxide H2O2 And oxygen O 2 As in the equation:

\[
O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2
\]

Catalase enzyme\((\text{CAT})\): It is an enzyme found in all living things, which is considered one of the main antioxidants \(^{(8)}\), the dismantling of hydrogen peroxide The H \(_2\) - O \(_2\) to oxygen and water \(^{(9)}\), as in the equation:

\[
2H_2O_2 \xrightarrow{\text{Catalase}} H_2O_2 + O_2
\]

Malondialdehyde\((\text{MDA})\): It is a compound that results from the process of fat oxidation and represents the final product of the oxidation of membrane lipids in melanocytes \(^{(10)}\), a compound aldehyde tri - high interaction produces peroxide polyunsaturated fatty acids \(^{(11)}\).

Materials and Methods of Work

Study type was determined and is a pathological study of vitiligo patients compared to control. Samples were collected from a consultant dermatologist from Ramadi General Hospital and with the help of Dr. Abdullah Saleh Al-Hassan, a dermatologist in Anbar Governorate, during the period of time. 2020/7/1 Up to 2021/1/1. As it included the patient group 55 Sick and this group is divided into 25 Patient with static state, 30 Patients with active status in addition to the control group and their number 20, a person who was shown to be free of any pathological symptoms in this group. With the exclusion of smokers, patients taking Vitiligo treatment, and those suffering from other diseases, it was withdrawn 5MI of venous blood by medical syringe for the subjects under study. Blood samples were collected in tubes EDTA Anticoagulant and the serum was separated using a centrifuge. 3000 A diuretic course 5 For minutes, the serum was collected in clean and sterile Abendorf plastic tubes and kept in the freezer at some degree-20M until use. And the use of a serum to check for levels of antioxidants SOD, CAT, MDA In the study groups. The method for determining the efficacy of the enzyme superoxide dismutase in the blood serum depends on the susceptibility of the enzyme SOD On activating the oxidation of epinephrine into adenine and chromium, as the latter is a chemical substance that is produced naturally when the oxidation of epinephrine and the reaction takes place at a temperature 37°C \(^{(12)}\), Take 1 ML of serum and diluted in 1.8 ML of solution Carbohydrate buffer Concentrated 50m It is an acidic function 10.2 Then add 0.1ML of epinephrine and 1ML of solution EDTA (Ethylene diamine Tetracetic acid ) Function acid10.2 I read the absorbance with a spectrophotometer at a wavelength 480 Nanomer, as the first reading took the case of adding epinephrine A Control The second reading was taken after 5Minutes from the first reading A Sample The effectiveness of the catalase enzyme in the blood serum was estimated, and this method depends on the ability of the catalase enzyme to break down the hydrogen peroxide compound into water and oxygen gas, thus reducing the absorption during the reaction period \(^{(13)}\), 50 MI of serum has been added to 5MI of Phosphate buffer solution At PH = 7. The reaction begins immediately after adding hydrogen peroxide to the solutions, and after mixing them the first absorbance is read. \((t1)\) After passing \((15)\) Sec and then I read the second absorption \((t2)\) at a wavelength 240 Nm using a Spectrovoltmeter. Then estimating the concentration of peroxidation of lipids in the blood serum by measuring the amount of Malondi- aldehyde and the method depends on the interaction between the lipid peroxides. MDA And between thiobarbituric acid and this reaction takes place in an acidic medium and is a colored product, the intensity of absorption for it is measured when 532 Nanometer using a spectrophotometer \(^{(14)}\), Use 200 ml of serum 4 ml of solution TCA Trichloroaetic acid plus 1 ml of thiobarbiturl acid (TBA The solutions were mixed well and then placed in a water bath 100C For a period 15th A minute was then left to cool, the solution was placed in a filtrate separator device, and the spectrometer was read at a wavelength of 532 nm.

Results

The results are indicated in the figure (1) High level of superoxide dismutase at the level of probability \((P <0.05)\) in serum in the disease group compared to the
control group. The results indicated in the figure (2) Low levels of catalase at the level of probability (P <0.05) in the disease group compared to the control group in serum. The results are shown in Figure (3) High Malondi- aldehyde at the level of probability (P <0.05) in the disease group compared to the control group in serum.

Figure number (1): To estimate the efficacy of an enzyme SOD In the study groups

Figure number (2): To estimate the efficacy of an enzyme CAT In the study groups
Discussion

Vitiligo is one of the diseases resulting from the disappearance of melanin pigment from the skin due to the absence of melanocytes, and among the theories that lead to the destruction of these cells is the theory of oxidative stress that leads to the production of toxic compounds, these compounds have a great effect on melanocytes and their destruction. A significant increase in the enzyme superoxide dismutase was observed at the level of probability (P <0.05) In the disease group compared to the control group, as a result of the continued production of these toxic compounds in the blood serum, due to the oxidative stress on melanocytes (15). The This enzyme disposal of toxic super oxide, which is considered toxic compounds in the Melanotene cell formed as a result of oxidative stress, which leads to damage to DNA as well as destroying the enzymes that make proteins and other molecules in the cell as a result of oxidative stress (16), and Superoxide Dismutase enzyme works on converting the super Oxide root of hydrogen peroxide H2O2 and oxygen. Low levels of catalase were observed at the probability level (P <0.05) In the disease group compared to the control group. Schalreuter and his group (1999) Confirmed the reason for this enzyme decrease is because it helps the cell get rid of the toxicity of the toxic compound (H2O2) In the cell, which consists of this compound as a result of oxidative stress in the cell melanocytes and thus the catalase enzyme to neutralize the toxic action of this compound was observed high levels of Malondi- aldehyde (MDA) at the level of likelihood (P <0.05) In the disease group compared to the control group. Malondi- aldehyde (MDA) it is a final product of lipid oxidation in the melanocyte as a result of the oxidative stress process in the cell (18). The significant increase in Malondi- aldehyde in vitiligo patients illustrates a state of oxidative stress in the cell (18, 19, 20). The lipid peroxidation leads to the destruction of the cell membrane of the melanocytes, which leads to the removal of the melanin pigment in the skin of vitiligo patients (21).

Conclusion

Oxidative stress is one of the hypotheses that have a role in the destruction of melanocytes in vitiligo patients and the disappearance of melanin pigment from the skin due to the resulting disturbances between antioxidants and oxidants in the cells.

Conflict of Interest – Nil

Source of Funding: Self
Ethical Clearance – Not required

Reference


Effect of Exotoxin a isolated from *Pseudomonas Aeruginosa* against Human Cancer Cell Lines

Muthanna Hamid Hassan

Assist. Prof., Department of Biology, College of Science, University of Anbar, Iraq

Abstract

In this study, the presence of *Pseudomonas aeruginosa* was investigated from many clinical samples, and then about 54 isolates were obtained, diagnosed with all different diagnostic methods, and then the bacterial isolates produced for Exotoxin A were detected by the ELISA method and then the most productive isolate was chosen for purification by means of precipitation with ammonium sulphate and ion-exchange column and detection of molecular weight was (M.W 65.03 KD). The competent isolation tested its toxicity against two cancer cell lines HeLa and PC3 which were an inhibition ratio 69.1% and 61.6% respectively in high concentration.

*Key words*: p.aeruginosa, exotoxin A, chromatography, cancer cell line

Introduction

*Pseudomonas aeruginosa* is considered an important opportunistic pathogen\(^{(1,2)}\), causing dangerous infections, especially in immunocompromised patients \(^{(3)}\). This infection is difficult to treat due to the great resistance it possesses \(^{(4)}\). In addition; it has a great ability to resist a wide range of antimicrobial agents. Exotoxin A (ETA) is regarded as the important virulence factor secreted by *P. aeruginosa*. Liu was 1st who disgonized and purified it \(^{(5,6)}\). That ETA is considering an ADP-ribosylating toxin which works in the inhibition of protein synthesis and finally cell death \(^{(7)}\). ETA is a single chain polypeptide of a MW 66-kDa. It is binding to receptors as specific via endocytosis receptor-mediated \(^{(8)}\).

Material and Methods

Collection of Samples

This study have collection about 73 samples from pathogenic cases as Burns, Wounds, UTI infection, Otitis media, in AL-Ramadi Teaching Hospital and AL-Fallujah Teaching Hospital, throughout the period from 1/7/2020 to 20/9/2020. Samples were obtained by utilizing sterile cotton swaps whereas sterile UTI infection was taken by sterile container. Samples were streaked directly on agar of MacConkey and incubation was done for 24 at 37 °C.

*P. aeruginosa* Isolation and Identification

For the purpose of isolating and diagnosing bacteria, several tests were performed:

**Morphological examination**: Morphological examination was performed with Gram stain and spore-forming stain by light microscopy

**Culture examination**: Culture examination was performed by cultured bacterial colonies on the selective media MacConkey agar, cetrimide agar and incubation was done for 24 at 37 °C.

**Biochemical exams**: many biochemical exams were conducted for diagnosis bacteria including: IMVC, urease, triple sugar iron, oxidase and catalase tests for
diagnosing confirmed of the isolates were performed vitek-2 system.

**Production of pyocyanine:** The bacteria were cultured on agar of cetrimide and incubation was done for 24 at 37 °C, as a result, the green color presence indicates +ve results.

**Growth ability at 42 °C:** The bacteria were cultured on agar plates and incubation was done for 24 at 42 °C, bacterial growth at such temperature mentions +ve results.

**Protease test:** For the detection the ability of bacteria to produce protease enzyme that were streaked on skim milk agar.

**Detection of ETA:** For the detection the ability of bacteria to produce Exotoxin A were tested using ELISA kit.

**Partial Purification of ETA**

**Precipitation with (NH₄)₂SO₄:** the procedure adopted in this study was performed according to (9). Toxin was precipitated by addition of (NH₄)₂SO₄ at (20, 30, 40, 50, 60, 70, 80) % saturation. The product of 80% saturation that precipitated was utilized for obtaining complete toxin precipitation, and centrifugation for 30 min at 10,000 rpm was done for separating the precipitant.

**Ion exchange chromatography Purification:** Such was performed based on (10) through using column of DEAE-cellulose 2.5 x 15 cm that several times was washed with buffer of equilibration 0.01 M Tris-OH of pH 8.

**Toxin MW determination:** gel filtration chromatography was used for toxin MW purified partial determination, toxin A was kept to flow via a glass column (1.5 x 60) cm which packed with Sepharose 6B, and then in a flow rate eluted ( 3 ml /fraction). Trypsin 23KDa, ovalbumin 43KDa and bovine serum albumin (BSA) 67 KDa were protein standards.

**Protein concentration determination:** concentration of protein was performed based on (11) as following: A standard curve of BSA was carried out by utilizing various concentrations from stock solution of BSA based on volumes.

**Cell lines and growth conditions:** Cell lines of cervical cancer (HeLa), prostate cancer (PC-3), and normal ones were utilized for determining the ETA effect. Such cells were on media of MEM and RPMI were cultured that were enriched with mixtures of FBS (10%) and penicillin streptomycin (1%). Incubation was done at 5% CO2 and 37 °C (12).

**MTT cytotoxicity assay:** Based on (13) Exotoxin A cytotoxicity was performed.

**Statistical Analysis**

The data that obtained were displayed as mean ± SD and statistical significances were measured utilizing test of ANOVA(14).

**Results and Discussion**

The Results of this study refer to clinical samples as total of 73 were taken from various cases as showed in (Table1). About only 54 isolates (71.05 %) gave morphological features and biochemical tests in relation to *P. aeruginosa*, while the remaining isolates of 19 might related to other bacteria being pathogenic from various genera as illustrated in (Figures 1,2). Diagnosis of the bacterium was done by means of Gram stain, and looks as non-sporulating, slightly or a straight curved, motile G- rod that aerobically growing, this is agreement with (15). Generally, once clinical samples are cultured on agar of MaConkey, it showed all the essential traits of growing on that medium. The isolates cultured on agar of blood and appear as β hemolytic. Morphological suspected isolates features were perforemed based on the shape of colonies which if formed as colorless if cultured on agar of MaConkey because of no fermentation of lactose. Also, sub-culturing on agar of Cetrimide was added for bacterial inhibition other than *P. aeruginosa*(16). According to the results of biochemical tests that revealed that only 54 isolates were belong to
*P. aeruginosa*. These isolates gave +ve results for tests of catalase, oxidase, citrate utilization, while -ve results were obtained in production of indol and test of methyl red- vogesproskour.

Colonies cultured on nutrient agar for 24 h at 42°C can grow normally; where growth appearance mentions +ve results. also All the bacterial isolates are represented *P. aeruginosa*, and were positive for protease this is in agreement with (17) who revealed that isolates of *P. aeruginosa* as over than 95% were +ve for Exotoxin A and protease. For the purpose of pigment production from such bacteria, it cultured on agar of cetrimide for production of pyocyanin (H$_2$O soluble, a blue green, non-fluorescent, phenazine pigment) that is stimulated by the inclusion of MgCl$_2$, and K$_2$SO$_4$ in the broth. Cetrimide was added to inhibit other bacteria. Its function as cationic detergent being quaternary ammonium leads to release of N and P from cells of bacteria other than *P. aeruginosa*(16).

**Exotoxin A Detection**

For exotoxin A detection produced by *P. aeruginosa* isolates, kit of ELISA was utilized. About of 54 isolates, only 21 were detected of exotoxin A production. For concentrations measurement, the same kit was utilized also as the displayed in (Table 2). Isolates screening mentions that isolates mostly show +ve results. Nevertheless, only one isolate was elected based on their productivity being the highest besides their distribution in various sites when infections, highest isolates called *P. aeruginosa* 15, which produce (27.56) ng/ml and distributed in burn cases.

**Purification of Exotoxin A: such steps are includes:**

**Proteins precipitation by (NH$_4$)$_2$SO$_4$:** for toxin crude extract concentration and H$_2$O removing as abundant as possible, (NH$_4$)$_2$SO$_4$ was utilized at (20, 30, 40, 50, 60, 70, and 80) % saturation. For exotoxin A precipitation, the ratio of saturation as 80% was elected. Such step permits the molecules salting out of from H$_2$O. Since (NH$_4$)$_2$SO$_4$able to neutralize charges at the protein surface and disrupting layer of H$_2$O contiguous the protein, it will cause eventually a decline in the protein solubility that in turn cause protein precipitation via the salt effect (18,19).

**Exotoxin A partial purification via ion-exchange chromatography:** Exotoxin A partial purification via ion-exchange chromatography was done by DEAE-cellulose utilizing. Figure 3 illustrated the DEAE-cellulose column wash and elution to select isolate. In the wash steps, exotoxin A was detected, whereas the fractions as eluted were revealed. The obtained results revealed the existence of two peaks. Nevertheless, just one peak for each select isolates elution exposes activity as found via kit of ELISA. The purified partial proteins amounts mention as much as protein of 0.053 mg/ml produced via isolates PA15, the number fraction was between 35-37. Results revealed that there are one protein peak will seemed following elution by gradient NaCl concentration, and then the absorbance was measured at 280 nm.

**Toxin M.W detection**

The M.W was assigned to ETA produced by *P. aeruginosa* was measured via gel filtration utilizing Sepharose 6B in the existence of three proteins as standards (BSA, ovalbumin, trypsin). Every standard of protein and Exotoxin A were column-applied and individually eluted and then Ve of every standard protein were measured then recorded Ve/Vo for every one. The Results indicate that ETA has M.W (65.03) as illustrated in Figure 4.

**Assay of Cell viability**

Effect of cytotoxicity was done by using technique of MTT. Figure 4 showed that declining in cells viability of PC3 and HeLa by concentrations and incubation period increasing of the extracts was noticed. Significant inhibition against HeLa (P<0.05) in most concentrations and incubation periods was observed. ETA extract of (400 µg/mL) as the highest concentration caused effect in maximum time as highest inhibition against PC3. High effect as cytotoxic against Hela and PC3.
was noticed if with ETA extract were treated which were 61.6% and 69.1%, respectively. However, they were of week activity versus cell line being normal. Normal cells were growing well in around 91%. These results might be explained due to cancer cells death that occurred by apoptosis that is recognized as a controlled event. The cytokines production are known to be as anti-inflammatory molecules besides phagocytosis can cause this type of cell damage\(^{(20)}\). Consequently, it was believed that the ETA was of biological activity that might inhibit proliferation of cancer cells\(^{(21)}\).

Figure (1): percentage of isolated *P. aeruginosa*

![Pie chart showing percentage of isolates: P. aeruginosa 71.05%, other bacteria 29.95%]  

Figure (2): Number of the isolates *P. aeruginosa*

![Bar chart showing Series 1, P. aeruginosa 54, Series 1, other bacteria 19]
Table (1) Source and number of *P. aeruginosa*

<table>
<thead>
<tr>
<th>Sources</th>
<th>No. of samples</th>
<th>No .positive isolates</th>
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<tbody>
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<tr>
<td>Wound</td>
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<td>11</td>
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<td>Otitis media</td>
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<td>8</td>
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<tr>
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<tr>
<td>Total</td>
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Table (2) The concentration of ExotoxinA isolated from *P. aeruginosa* isolates

<table>
<thead>
<tr>
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<th>NO. isolates of produced ETA</th>
<th>Specimen</th>
<th>Concentration toxin ng/ml</th>
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<tr>
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</tr>
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<td>21</td>
<td>52</td>
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Conclusion

This study was conducted on extracted and purification of exotoxin A produced by local strains of *P. aeruginosa* in Iraq then detection the molecular weight of toxin and have cytotoxicity against cancer cell lines. approved by the Institutional ethical Committee.

Conflict of Interest : No conflict of interest

Funding: Self ,

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

References

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A Comparison between Retro-Clavicular and Proximal Infraclavicular Approaches for Brachial Plexus Block for Anesthesia of the Upper Limb

Mohammed Sami Hassan 1, 2, Mohammad Asi Jabbar 2, Mortada Abd Al-Hussian Jubara 2, 3

1 Lecturer, Consultant, F.I.C.M.S., Hamorabi Medical College, University of Babylon, Babylon, Iraq, 2 Specialist of Anesthesia, C.A.B.A., Department of Anesthesia, Al-Shaheed Ghazi Al-Harriri Hospital, Baghdad Medical City, Ministry of Health and Environment, Baghdad, Iraq, 3 Specialist of Anesthesia, F.I.C.M.S., Department of Anesthesia, House Nursing Private Hospital, Baghdad Medical City, Ministry of Health and Environment, Baghdad, Iraq

Abstract

Background: This comparative study hypothesize there are differences between ultrasound guided retro-clavicular and proximal infraclavicular approaches for brachial plexus block for surgeries involving lower part of arm, elbow, forearm and hand with the use of tourniquet.

Method: About 36 patients randomly allocated into 2 groups, first group block done through retro-clavicular approach (RCB) and the other group done by using proximal infraclavicular block (PIB). In RCB group needle inserted posterior to the clavicle and directed posterior to the first part of axillary artery. In PIB needle inserted from lateral to medial after abduction of the arm more than 90° where the cords are clustered together.

Results: Block performance time was (6.11±2.58) and (6.05±2.61) for RCB and PIB, respectively without significant difference (p= 0.94). The onset of sensory was (12.8±1.4) and (11±2.3) for RCB and PIB, respectively. For motor onset (17.4 ±2.3) and (15.3±3.3) and there is significant difference between both groups (p=0.009 and 0.04), respectively. There was no significant difference in block-related pain between the two groups (p=0.809) VRS for RCB was (1.41±0.712) and for PIB was (1.32±0.67). Tourniquet pain reported by the patient for RCB was 1.24±0.56 and for PIB was 1.11±0.315, there is no significant difference between both groups (p=0.392)

Conclusion: Ultrasound guided BPB by retro-clavicular and proximal approaches are with a comparable quality of surgical anesthesia for operations of the lower arm, elbow, forearm and hand with the use of tourniquet, the onset of surgical anesthesia is earlier for PIB, the RCB is preferred for patients who cannot abduct the arm.

Keywords: Retro-clavicular; Proximal infraclavicular; Brachial plexus block; Upper limb anesthesia

Introduction

Infraclavicular block is easy to learn effective and useful to provide analgesia and anesthesia below the shoulder. Although Ultrasound guidance regional anesthesia have increased the interest in ICB it is still underused regional anesthesia technique of the upper limb (1, 2).

The common approaches are: Medial approach around the middle of clavicle; Lateral approach around the coracoid process; Retro-clavicular; Parasagittal (3). Coracoid approach is most popular (3, it has gained

Corresponding author:
M AsiJabbar
Baghdad, Iraq
7733962400
Medicalresearch68@yahoo.com
popularity because of the presence of a consistent bony landmark, less chances of vascular puncture or pneumothorax and adequate neural blockade\(^{(4)}\).

The ultrasound-guided parasagittalmidclavicular method has several disadvantages: Needle visualization can be difficult because of its steep insertion angle; The lateral cord is vulnerable to injury; The acromial branch of the thoraco-acromial artery is prone to puncture\(^{(5,6)}\).

Retro-clavicular approach (RCB) was first described in 2007 by Hebbard and Royse\(^{(7)}\), the needle is inserted posterior to the clavicle and passes underneath it in a cephalad-to-caudal direction. This allows excellent needle visualization as the ultrasound beam is perpendicular to the shaft of the needle. The needle trajectory is posterior to the lateral cord and acromial artery and therefore might reduce the chance of direct needle trauma to these structures during the procedure\(^{(7)}\).

The technique is quick and reliable, Reduced performance time, and fewer paresthesias than with the conventional approach. However, there is one significant disadvantage to the retro-clavicular approach: the bony clavicle causes acoustic impedance, creating a “blind spot” that the needle must traverse before appearing on the ultrasound image. Structures behind the clavicle cannot be visualized and therefore are at risk of damage. Preexisting anatomical descriptions of the area indicate that the subclavius muscle, supraspinatus muscle, and subscapularis muscle may be encountered during needle insertion, and the supra-scapular nerve and its attendant artery and vein may be vulnerable to injury\(^{(6-8)}\).

Proximalinfraclavicular approach (PIB) done by use Ultrasound-guided infraclavicular approach at a more proximal position in the costo-clavicular space, where the cords are grouped together cephalad to the artery this allows access to all three cords along one needle path. Using this technique, the onset time is faster, the axillary nerve is more reliably anesthetized, and the intercostal brachial nerve is often blocked as well when compared to a more lateral approach\(^{(8)}\). The drawback is that the cords lie close to the first and second ribs so that pleural puncture is more likely if the user does not have a thorough understanding of the anatomy and good technical skills. The proximal approach is performed with the blocked arm abducted same as axillary block moving the brachial plexus away from the thorax and closer to the dermal surface because the pectoralis major muscle, which is located superficial to the brachial plexus and the axillary artery, is stretched by the arm abduction this position elevates the clavicle slightly in the cephalad direction\(^{(9)}\).

The aim of our study was to compare two approaches of ICB (ultrasound-guided retro-clavicular and proximal infraclavicular) for surgery of lower arm, elbow, forearm, wrist or hand with tourniquet use. The outcomes to be studied include time for performing the block, pain during performance of the block, complications and pain related to tourniquet.

**Methods**

This study was conducted in AlKafeel Super-specialty Hospital from June 2018 to January 2019. Patients belonging to ASA status I to III admitted for elective or urgent lower arm, elbow, forearm, wrist, or hand surgery were divided into two groups. Patients were randomly allocated into two groups using randomization.com where random sequence generated by the website. Informed written consent was taken, the procedure explained to the patient and the 5-point verbal rating scale (VRS) score explained to the patient. Age, sex, BMI, type of surgery was recorded. Patient excluded from the study: refusal of regional anesthesia, coagulopathy, infection at the site of needle entry and inability to abduct the arm for PIB group.

Block done in the surgery operating room, standard monitoring was applied and a 18-gauge intravenous cannula was inserted in the contralateral arm. Intravenous midazolam 1 to 2 mg was given based on patient preference.

Patient positioned supine in a comfortable position to the patient make placement of the ultrasound transducer and needle insertion easier, small folded towel inserted behind shoulder of the side to be blocked to make the...
needle advancement easier with the head elevated 30 degree and turned away from the side to be blocked. The arm is by patient side and not abducted for RCB. The high-frequency linear transducer 12M Hz GE Healthcare used to scan from medial side of the clavicle in parasagittal planeto the Deltopectoral groove, Sonographically visualize the first partaxillary artery lies lateral to the first rib and proximal to the pectoralis minor crossing over the 2nd rib, thencourses away fromthe thoracic cage. The injection target which lies just posterior to the axillary artery.

We used Vishal Uppal technique where the needle length to the target place was marked and held by the index and thumb fingers for more safety and to avoid needle probe mal-alignment or acoustic shadow of the clavicle. After sterile preparation, Needle insertion point was 1-2 cm posterior to the clavicle after palpating supraclavicular fossa medial to the trapezius muscle insertion on the clavicle, the needle directed more towards the anterior axillary line. Under sterile condition a skin wheal raised using a 30-gauge needle, 100-mm 18-gauge needle inserted into the skin wheal and advanced strictly in-plane with the US beam, the needle should be appeared at the predetermined length behind the posterior wall of the clavicle and must not be angled posteriorly, to avoid any risk of pneumothorax.

The PIB group arm is abducted and externally rotated, the linear transducer is placed adjacent to the inferior border of the clavicle, parallel to the clavicle, and lateral to the midclavicular line, the axillary artery was seen in short axis. Needle was inserted from lateral to medial in plane to the transducer, the needle directed to the clustered cords and local anesthesia injected in single shot. All blocks were performed with 25-32 mL of bupivacaine 0.5% with adrenaline 1:200,000.

The collected data were:
1. Performance time: expressed in minutes. The duration of each block procedure was measured from first needle insertion for skin infiltration to the termination of the injection of local anesthetic and removal of the block needle. The time recorded by the anesthesia assistant.
2. Block onset assessed for sensory and motor: Onset time was defined as the time from block completion to the onset of analgesia and motor block checked every 1 minute after 5 minutes from the end of the block to decrease discomfort to the patient from repeating assessment by pinprick the area supplied by five nerves and movement as follow:
   - Radial (lateral aspect of the dorsum of the hand and wrist extension)
   - Median (volar aspect of the index and thumb-fifth finger opposition)
   - Ulnar (volar aspect of the fifth finger and fifth finger abduction)
   - Musculocutaneous (lateral aspect of the forearm elbow flexion)
   - Medial cutaneous nerve of the arm (medial aspect of the arm)
3. Pain during the procedure: after finishing the needling 5-point verbal rating scale (VRS) score after the removal of the needle. The VRS was scored from 0 (no pain) to 4 (the worst pain imaginable).
4. Tourniquet pain assessed 30, 45 and 60 minutes after inflation by using the 5-point verbal rating scale.
5. Complications: Any complication related to the procedure was searched and recorded intraoperatively and post operatively including: pneumothorax, vascular injury, paresthesiadiuring the procedure, nerve injury, phrenic nerve block, Horner’s syndrome and hematoma formation.

Exclusion criteria: Patient with infection at site of injection, bleeding tendency, inability to abduct the arm, patient refusal.

Statistical Analysis
Statistical analysis was performed using the SPSS
version 23 statistical software (SPSS Inc., Chicago, IL, USA). All numerical data were tested for a normal distribution. The continuous variables are presented as the mean±standard deviation (SD). Categorical variables are presented as the number (n) and the percentage (%) of patients. Differences between mean values for normally distributed variables were compared using Student’s t test. Fisher’s Exact test was used for categorical data where appropriate. A value of (p<0.05) was considered to indicate statistical significance.

**Results**

About 36 patients enrolled in this study seventeen patients given RCB, nineteen patients given PIB. In group RCB the mean age was (42.2±20.4) years while for the PIB was (34.8±15.2) years. In RCB group nine male patients and eight female patients, while in group PIB thirteen male and six female (Table 1). BMI was (28.3±4.8) m²/Kg for RCB and (27.6±5.2) m²/Kg for PIB.

Block performance time was (6.11±2.58) and (6.05±2.61) for RCB and PIB respectively without significant difference (p=0.94). The onset of sensory block was (12.8±1.4) and (11±2.3) for RCB and PIB, respectively (p=0.009). For motor block onset (17.4±2.3) and (15.3±3.3)(p=0.04), there was a significant difference between both groups, (Table 2).

There was no significant difference in block-related pain between the two groups (p=0.809) VRS for RCB was (1.41±0.712) and for PIB was (1.32±0.67), (Table 3). Tourniquet pain reported by the patient for RCB was (1.24±0.56) and for PIB was (1.11±0.315), there is no significant difference between both groups (p=0.392), (Table 4).

Dose of local anesthesia used for both groups was 28.5±1.5 and 28.6±1.4 and there is no significant difference (p >0.933). Procedures done under both approaches are shown in (Table 5).

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<th>RCB</th>
<th>PIB</th>
<th>p-value</th>
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<td>Age (years+SD)</td>
<td>42.2±20.4</td>
<td>34.8±15.2</td>
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<tr>
<td>Dose of LA used(ml)</td>
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<td>28.6±1.4</td>
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<td>Sex(M/F)</td>
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<tr>
<td>ASA(I/II/III)</td>
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<td>(14,3,2)</td>
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<td>BMI (m²/Kg)</td>
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Table 3. Pain during needling

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Table 4. Prevalence of tourniquet pain in both groups

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<th>mild pain</th>
<th>Moderate pain</th>
<th>Severe</th>
<th>Very severe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCB</td>
<td>14</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>PIB</td>
<td>17</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>36</td>
</tr>
</tbody>
</table>

Table 5. Type of surgery for both groups

<table>
<thead>
<tr>
<th></th>
<th>RCB</th>
<th>PIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Forearm</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Elbow</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Lower arm</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Discussion

Although the two approaches differ in direction of needle when injection of local anesthetiabut both approaches are considered proximal approaches to the ICBPB where the needle directed toward first part of axillary artery, the main concern with needletip visualization was the clavicle shadow before visualize needle at its trajectory to the target site in RCB while in PIB approach the needle is well visualized throughout its whole length and this will avoid the penetration of the superficial pleura at this site, in our study no vascular or pleural injury noticed\(^7, ^15\).

One case was planned to be given RCB but we faced technical difficulties in insertion of the needle and directing it to the target of posterior cord due to morbid obesity, short neck and acutely angulated clavicle and we decide to use the other approach and it was successful this is also what was seen by Zhi Yuen Beh\(^16\).

One case planned to be given PIB according to the randomization and the patient can’t abduct the arm and he preferred regional anesthesia so given RCB which...
was more comfortable to the patient this is what was seen by ChandniSihawho prefer the RCB approach for acute trauma patient who can’t abduct his arm \(^{(17)}\).

Performance time: the two approaches studied not differ in duration of block performance and Block performance time was 6.11±2.58 and 6.05±2.61 for RCB and PIB respectively without significant difference \((p 0.94)\) other studies show performance time shorter (median 3.77 (2.90–6.53)) in RCB \(^{(18)}\).

While Bigeleisen findings for PIBwas median time nine(7–12) min and the difference from our study related to including the scanning time and identification of the nerves before needling\(^{(19)}\).Sensory onset: there is insignificant difference between both groups in sensory andmotor onset they are earlier in PIB and this also found by P. Bigeleisen where motor block was complete in all nerves at 20 minutes after the block also this finding noticed by Manoj Kumar \(^{(15, 18)}\).

Pain during procedure performance no significant difference in pain experienced by the patients during the block and \((p=0.567)\) and most of the patients report (70% of RCB and 78% of PIB group) it is mild pain, Charbonneau et al \(^{(20)}\) reported that in the retro-clavicular approach, the needle path avoids the puncture of the pectoralis major and minor, which results in less pain during the procedure. And the results of this study are also found by Nilgun\(^{(21)}\).

Both groups show well tourniquet pain toleration and no pain reported by most of the patients and for both groups (82.4 and 89.5% for RCB and PIB respectively). These findings goes with many studies comparing ICB with other brachial plexus blocks they found tourniquet tolerated better and it is explained by local anesthetic spread to the intercostobrachial nerve\(^{(22)}\).

Maximum Bupivacaine dose given in our study was 160 mg while in the published literature of 175-225 mg with epinephrine still regarded safe dose although site of injection not included in these literatures and we didn’t notice any complication related to this dose further study to be done for plasma concentration in these blocks approaches \(^{(23, 24)}\).

This high dose not gone with findings of Jia Wei Li et al, where relatively small volume and dose used for proximal or costo-clavicular approach with 100% success rate and explained by the grouping of the cords at the costo-clavicular space together lateral to the axillary artery\(^{(24)}\).

Complications no complications from both groups noticed for the cases included in the study and this may be related to small number included\(^{(18)}\).

Conclusion
Ultrasound guided BPBby retro-clavicular and proximal approaches are with a comparable quality of surgical anesthesia for operations of the lower arm, elbow, forearm and hand with the use of tourniquet, the onset of surgical anesthesia is earlier for PIB, the RCB is preferred for patients who cannot abduct the arm.

Declaration of Competing Interest- Nil

Source of Funding- Self.

Ethical Clearance- Taken from AlKafeel Super-specialty Hospital committee.

References
2. Chin KJ, Alakkad H, Adhikary SD, Singh M. Infraclavicular brachial plexus block for regional anaesthesia of the lower arm. Cochrane Database of Systematic Reviews 2013;8


A Pathologist’s Perspective on Histopathology Examination of Autopsies and Various Incidental Findings

Mohanvir Kaur1, Lachhima Bhandari2, Vijay Kumar Bodal3, Sarbhjit Kaur4
1Associate Professor, 2Junior Resident, 3Professor, Department of Pathology, 4Associate Professor, Department of Gynaecology & Obstetric, Government Medical College, Patiala

Abstract

Background-The current study aims to highlight the importance of autopsies in not just finding the cause of death; but also, incidental discovery of various rare lesions, that may or may not be the cause of death. The study also aims to highlight the common cause of death, spectrum of common incidental findings and reporting of various rare, interesting cases. Method- This is a retrospective study conducted between year 2013 to 2020 on 800 autopsies and their histopathological examination in Pathology Department, GMC, Patiala. Conclusion- Finding of this study concludes the importance of histopathological autopsy in not just highlighting the cause of death but also finding many rare diseases, thus enriching the field of medicine and pathology by observing the histological findings and also sometimes the course of rare diseases.

Keywords- Autopsy, Histopathology, medico-legal autopsy.

Introduction

An autopsy and histo-pathological examination are an important tool to establish the cause of death, manner of death, time of death and solving the medico-legal cases.[1] It can be carried for clinical purpose or medico-legal purpose. Clinical autopsy or pathological autopsy is done not just to establish the cause of death but also to study the course of a disease and highlight rare disorders with help of histopathology and adding to the knowledge pool.[2]

A medico-legal autopsy is on the other hand carried with a sole purpose to establish the cause of death and confirm the physical findings of autopsy with histological findings. [2,3]

Many times, findings which are unrelated to the cause of death are noticed in routine histo-pathological examination of medico-legal autopsies. These findings provide an opportunity to study various infrequent lesions which go unnoticed when a person is alive. It has been documented that autopsies have led to discovery and clarify the pathology of many medical disorders (87 over a span of 46 years).[4] They help in studying not only medically diagnosed and treated neoplasm’s, but also natural evolution of many untreated diseases.[5] Many incidental findings have been highlighted on histo-pathological examinations which have proven to be a great learning tool for the pathologists as well as the forensic experts. It is also important for assessing statistics of mortality which are essential for public health and health service planning.[6]

The current study was carried out with the purpose to establish the importance of autopsy, histological examination of viscera to find the cause of death, common cause of death and its use as a tool to study various rare lesions. Also, to report interesting cases which otherwise would have gone unnoticed.
Material and Methods

The present study is a prospective study conducted in Pathology Department, GMC, Patiala between year 2013 to 2020 on 800 autopsy viscera received from the forensic department of our institution and adjacent civil hospitals of many districts. The viscera was received in 10% formalin solution along with post-mortem papers containing the personal details of the deceased, post-mortem examination report and suspected cause of death. The tissue was processed after fixation, stained with haematoxylin-eosin stain and viewed under light microscope to outline cause of death. Special stains like PAS, Zeil Neilson were done as and where required for confirmation of the histopathological findings.

Results- Of the 800 studied cases, 30 were autolysed. Males were more common than females with a ratio of 5:1. Age distribution showed 21-40 years as the most common age group (45%). The youngest of the received viscera was from infants (3 cases) and the oldest was 100 years old. (Table 1)

System wise division showed, of the 800 cases studied, cardiovascular system (27%) was the most common system followed by renal (24%), hepatic system (11.5%), respiratory system (9.5%), spleen (8%), pancreas (6%), CNS (5%), uterus (4%) and products of conception (3%). (Table 2)

The most common (m.c) diagnosis was acute tubular necrosis (ATN) (44%) followed by fatty liver (17.6%), congestive liver (8%) and atherosclerosis (7.7%).

System wise comparison of different cases was done and common as well as incidental findings were noted.

Cardiovascular system: This is the most common system which was affected. Various spectrum of lesions observed were- atherosclerosis (7.7%), MI (1.8%) and cardiomegaly (1.4%). MI acts as a direct cause of death. Out of 25 cases of MI, 2 were acute infarcts leading to death and rest were old healed infarcts.

Renal system: This is the second most common system to be affected. The spectrum of lesions affecting kidneys were- ATN (44%), chronic pyelonephritis (0.5%) (Figure 1) and acute pyelonephritis (0.1%). Diagnosis of ATN is a histological diagnosis as grossly no abnormality is noted and it was also the m.c histological diagnosis and m.c incidental finding.

Liver: The various lesions noted in liver were- fatty liver (17.6%), congested liver (8%) and liver cirrhosis (3.3%). Fatty liver was the second m.c histological diagnosis made on visceral examination. Equal incidence was noted in both men and women.

Respiratory system: Spectrum of lesions noted in lung were- congestive lung (5.9%), pneumonia (1.8%), alveolar edema (1.4%), pulmonary TB (1.4%) and lung abscess (0.4%). Various other interesting as well as incidental findings noted were- miliary TB (0.2%), one case of metastatic deposits of sarcoma in lung, one case of extra-medullary deposits of blast phase of CML, one case of fungal infection of lung and one case of small cell carcinoma lung.

Central Nervous system: Brain was not a common specimen to be autopsied. The specimens that were received showed largely normal histology with occasional age related changes like gliosis seen in 0.2% cases. However, there were three cases of meningitis and one case of diffuse astrocytoma.

Spleen and Pancreas: One case of spread of miliary TB to spleen was noted. Pancreatitis was seen in two cases.

Miscellaneous: Products of conception were reported in 0.4% cases. These were received from females in the age group of 21-40 years. Autolysed viscera were received in 0.2% cases.

Cases with multiple diagnosis- Out of these, any one or their combined outcome could have been the cause of death (Table 3)

Rare and interesting Case diagnosis-

1) In one case, incidental finding of acute pyelonephritis was made. Gross showed areas of
suppuration involving the cortex along with areas of patchy necrosis. Microscopy showed collections of neutrophils in tubules with relative sparing of glomerulus. (Figure 2)

2) In a case of miliary TB, bilateral lung involvement and tubercular deposits were also noted in spleen and liver. This was an incidental finding as cause of death was unnatural. (Figure 3)

3) In another rare case, metastatic deposits of sarcoma was noted in lung. On gross lung presented with firm, grey-white coloured, irregular mass. On microscopy, highly pleomorphic spindle cells with bizarre tumor cells were seen. Diagnosis of sarcoma was made, which was an incidental finding. (Figure 4)

4) Fungal hyphae were detected in a case of lung, an incidental finding. (Figure 5)

4) In one case, small cell carcinoma of lung was diagnosed which presented as tan white coloured, firm small nodules in the hilar region. This was an additional finding to the unnatural cause of death in autopsy findings.

5) A case of extramedullary infiltration of CML, blast phase in lung was noted in another autopsy. On microscopic examination, there were seen collections of myeloid blasts and predominance of myeloid series of cells.

| Table 1: Age wise distribution of various cases |
|-----------------|--------|--------|
| Age group       | No of cases | % age  |
| <20 years       | 40     | 5%     |
| 21-40 years     | 360    | 45%    |
| 41-60 years     | 240    | 30%    |
| 61-80 years     | 112    | 14%    |
| >80 years       | 48     | 6%     |

| Table 2: Various histo-pathological diagnosis in order of frequency |
|-----------------|--------|--------|
| Diagnosis       | No of cases | % age  |
| Acute tubular necrosis | 603     | 44%    |
| Fatty liver     | 240    | 17.6%  |
| Liver venous congestion | 110    | 8%     |
| Atherosclerosis | 105    | 7.7%   |
| Venous congestion of lung | 81     | 5.9%   |
| Liver cirrhosis | 45     | 3.3%   |
| Pneumonia       | 25     | 1.8%   |
| Myocardial infarction (MI) | 25     | 1.8%   |
| Alveolar edema  | 20     | 1.4%   |
### Table 2: Various histo-pathological diagnosis in order of frequency

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary tuberculosis</td>
<td>20</td>
<td>1.4%</td>
</tr>
<tr>
<td>cardiomegaly</td>
<td>20</td>
<td>1.4%</td>
</tr>
<tr>
<td>Chronic pyelonephritis</td>
<td>08</td>
<td>0.5%</td>
</tr>
<tr>
<td>Lung abscess</td>
<td>06</td>
<td>0.4%</td>
</tr>
<tr>
<td>Products of conception</td>
<td>06</td>
<td>0.4%</td>
</tr>
<tr>
<td>Military tuberculosis</td>
<td>03</td>
<td>0.2%</td>
</tr>
<tr>
<td>Meningitis</td>
<td>03</td>
<td>0.2%</td>
</tr>
<tr>
<td>Gliosis</td>
<td>03</td>
<td>0.2%</td>
</tr>
<tr>
<td>Acute pyelonephritis</td>
<td>02</td>
<td>0.1%</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>02</td>
<td>0.1%</td>
</tr>
<tr>
<td>Diffuse astrocytoma</td>
<td>01</td>
<td>0.07%</td>
</tr>
<tr>
<td>Small cell carcinoma lung</td>
<td>01</td>
<td>0.07%</td>
</tr>
<tr>
<td>Metastatic deposits of Sarcoma in lung</td>
<td>01</td>
<td>0.07%</td>
</tr>
<tr>
<td>CML, blast phase, lung</td>
<td>01</td>
<td>0.07%</td>
</tr>
<tr>
<td>Fungal infection lung</td>
<td>01</td>
<td>0.07%</td>
</tr>
<tr>
<td>Autolysed</td>
<td>30</td>
<td>0.2%</td>
</tr>
<tr>
<td>Total</td>
<td>1362</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Table 3: List of cases with multiple diagnosis

<table>
<thead>
<tr>
<th>Serial no.</th>
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<th>Diagnosis 1</th>
<th>Diagnosis 2</th>
<th>Diagnosis 3</th>
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<tbody>
<tr>
<td>1</td>
<td>70/M</td>
<td>MI</td>
<td>Atherosclerosis</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>45/M</td>
<td>Cirrhosis</td>
<td>Chronic pyelonephritis</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60/F</td>
<td>MI</td>
<td>Cirrhosis</td>
<td>pneumonia</td>
</tr>
<tr>
<td>4</td>
<td>55/M</td>
<td>Cirrhosis</td>
<td>Meningitis</td>
<td>pneumonia</td>
</tr>
<tr>
<td>5</td>
<td>50/F</td>
<td>Pneumonia</td>
<td>Fungal hyphae in lung</td>
<td>Chronic pyelonephritis</td>
</tr>
<tr>
<td>6</td>
<td>68/M</td>
<td>Cardiomegaly</td>
<td>Atherosclerosis</td>
<td>Alveolar edema</td>
</tr>
<tr>
<td>7</td>
<td>30/F</td>
<td>Acute pyelonephritis</td>
<td>Fatty liver</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>46/M</td>
<td>Acute MI</td>
<td>Atherosclerosis</td>
<td>Fatty liver</td>
</tr>
<tr>
<td>9</td>
<td>50/F</td>
<td>Miliary tb</td>
<td>ATN</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>57/M</td>
<td>ATN</td>
<td>Alveolar edema</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>65/M</td>
<td>ATN</td>
<td>Cirrhosis</td>
<td>Congested lung</td>
</tr>
<tr>
<td>12</td>
<td>70/M</td>
<td>ATN</td>
<td>Pneumonia</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: Chronic pyelonephritis showing thyroidisation of tubules. (H&E, 40x)

Figure 2: Acute pyelonephritis showing neutrophilic abscess in interstitium with sparing of glomeruli. (H&E, 40x)
Figure 3: Case of miliary Tb with epithelioid granuloma in liver (H&E, 400x)

Figure 4: low power and high power view of sarcoma lung (H&E, 40x, 400x)
Discussion

Current study was conducted between the year 2013-2020 and 800 cases were studied in the Department of Pathology, GMC, Patiala for histo-pathological examination. The male: female ratio was 5:1. Similar observations were made in different studies. [2,6,7,8] Most common age group according to present study was 21-40 years. The results were similar to the studies published. [2,7,8]

The present study highlights the importance of microscopic examination in addition to autopsy not just for exact cause of death but also to study rare diseases. Similar findings were suggested by Selvam et al, Costache et al and Vougiouklakis et al. [7,9,10]

The most common overall incidental finding was ATN seen in 44% cases. The results were similar to studies. [6,11] And the second mc incidental finding was fatty liver. These results were similar to the studies published. [8,12]

Maximum cases were seen involving the cardiovascular system (27%) followed by renal system (24%). This was similar to studies published by Pathak et al, Jhajj et al, Vij et al and Reddy et al.[2,6,13,14] Atherosclerosis was m.c finding in cardiac autopsies. Other lesions detected were MI (1.8%) displaying fibrosis in old infarcts. The findings were in accordance with published studies. [15, 16]

Renal system was the second m.c system involved and ATN as the leading diagnosis, as already discussed above. Also it is the m.c cause of acute renal failure. [11,17] ATN can be either due to direct effect of toxin/chemical substance or due to ischemia. In current study, these were mostly seen in cases of poisoning and trauma leading to myoglobinuria and hemoglobinuria, which is directly nephrotoxic.

In the hepatic system, the m.c diagnosis was fatty liver followed by venous congestion and cirrhosis. These findings were similar to studies published by Algarsamy et al, Singal et al and Simon et al. [18,19, 20]
The m.c finding in lung was CVS lung, followed by pneumonia, pulmonary edema, pulmonary TB and lung abscess. This is in agreement to study published by Kaur et al. but in contrast to studies published by Arunlatha et al and Patel et al, showing pulmonary edema as the m.c finding followed by CVS lung. A case of fungal hyphae in lung was reported using PAS stain. In our study pulmonary TB was noted in 1.4% cases and miliary TB was noted in 0.2% cases. The results were similar to study published by Patel et al with low incidence seen in 3.4% cases, but in contrast to study published by Garg et al where incidence of pulmonary TB was high. Many cases of tuberculosis remain undiagnosed and are diagnosed only at autopsy, thus highlighting the importance of autopsy. In a study of 73 cases of sudden deaths, by Rastogi P, 61.64% were due to tuberculosis.

One case of extramedullary CML (blast phase) was seen in the lung. CML has been reported in extramedullary organs in only 10% of cases. In most reported cases extramedullary involvement was indicative of impending progression of CML into blast crisis. In patients with blast phase, extramedullary blast crisis has been reported in about 7-17% cases.

One rare case of lung sarcoma was seen (0.07%). Primary lung sarcomas are rare, less than 0.5% of lung malignancies. Cameron and Miller and Allen reported lung sarcomas in 0.15% and 0.3% of lung neoplasms in lung malignancies, respectively.

In CNS, 03 cases of meningitis, 01 case of diffuse astrocytoma and 03 cases of gliosis were reported. This is significant because normally we don’t receive biopsy for cases like meningitis, hence autopsy plays a role here.

**Conclusion**

The study stresses on the need of histopathological autopsy in not just highlighting the cause of death but also studying many rare disorders. It thus enriches the field of medicine and pathology by not just studying the histological findings but also sometimes the course of rare diseases.

**References**


Incidental Neoplastic Tumours on Histo Pathology of Medico Legal Post Mortem Cases: A Ten Year Study

Mohanvir Kaur¹, Kanwardeep Kaur Tiwana², Lachhima Bhandara³, Shaina Goyal³, Shiv Kumar³, Monika Kalyan³

¹Associate Professor, ²Assistant Professor, ³Junior Resident Department of Pathology, GMC Patiala

Abstract

Background: Autopsy has been an important tool for detection of many rare lesions as well as studying the course of such lesions, highlighting the cause of death and also helps in indirect collection of data for mortality and forming health policies. Aim of present study was to detect the rate and type various neoplasms which were either clinically undiagnosed or not the direct cause of death and their system wise analysis.

Material and Methods: This is a retrospective study conducted on viscera Received between year 2011-20 on 810 medico legal autopsy cases coming to Department of Pathology for histopathological examination.

Result: Out of 810 cases, 09 tumours were diagnosed (1.1%). In 6 cases, it was completely incidental finding while in 03 cases history of malignancy was present. Tumours were mostly seen in older age group (>50 years).

Conclusion: The study concludes the importance of histopathologic study in autopsies in detection of clinically undiagnosed neoplasms and evaluating the cause of death. Thus, autopsies also indirectly aid to the true cancer incidence statistics.

Keyword: Autopsy, Neoplasms, Histopathology, Statistics.

Introduction

An autopsy is a highly specialised procedure that consists of a thorough examination of a dead-body to determine the cause, time and manner of death. It also helps to evaluate any disease or injury that may be present as an additional finding.[¹] It aids to the knowledge of pathologists, clinicians and forensic experts by incidental discovery of various lesions that otherwise would have gone un-noticed. The medico legal autopsy gives an opportunity to study medically diagnosed and treated neoplasms as well as natural evolution of untreated diseases.[²] It also aids in diagnosing various undiagnosed or misdiagnosed malignant tumours irrespective of the underlying cause of death, which may or may not be related to malignancy.[³] Thus, autopsy also helps in maintaining the mortality registers as well as collect data for cancer registry programmes.

The current study was conducted with an aim to diagnose various neoplasms, their type, rate and system wise distribution in the autopsies sent for other purposes. These neoplasms were either an incidental finding or already diagnosed and treated for the same with recurrence. This study also aims to establish the importance of histo-pathological autopsy.

Material and Method

This was a retrospective study conducted on 810
autopsy cases received in Department of Pathology between year 2011-20. The viscera were received in 10% formalin along with post-mortem report, personal details of deceased and suspected cause of death. multiple viscera were received in majority of cases. Grossing and processing of organs was done as per standard protocols and H&E staining was done with special stains wherever required.

**Results**

Out of 810 autopsy cases, 09 cases (1.1%) of tumour were diagnosed histologically. 08 cases were malignant and 01 case of benign neoplasm was diagnosed. Mostly the tumours were seen in older age group i.e > 50 years and the youngest age with tumour was 30 years. Males were more commonly affected than females with M:F ratio of 2:3. (Table 1)

Most commonly metastatic deposits were diagnosed rather than primary tumour of affected organs (4 cases-metastatic deposits of adenocarcinoma in lung, deposits of sarcoma in lung, infiltration by blast phase of CML in lung and deposits of adenocarcinoma prostate in liver). In one case, recurrence of tumour was seen in already diagnosed and treated case of Anaplastic Astrocytoma brain. (Table 2)

Most commonly affected organ was lungs; where total of 4 cases were diagnosed (0.49%). Second most common (m.c) affected organ was brain; 3 cases were detected (0.37%) followed by liver and kidney with one case each (0.12%).

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Age</th>
<th>Sex</th>
<th>Cause of death</th>
<th>Tumour detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 yr</td>
<td>M</td>
<td>Accident</td>
<td>Diffuse astrocytoma brain</td>
</tr>
<tr>
<td>2</td>
<td>35 yr</td>
<td>M</td>
<td>Asphyxia</td>
<td>Anaplastic Astrocytoma brain (recurrence)</td>
</tr>
<tr>
<td>3</td>
<td>36 yr</td>
<td>F</td>
<td>Road side accident</td>
<td>Meningioma</td>
</tr>
<tr>
<td>4</td>
<td>58 yr</td>
<td>F</td>
<td>Asphyxia</td>
<td>Metastatic deposits of sarcoma in lung</td>
</tr>
<tr>
<td>5</td>
<td>59 yr</td>
<td>F</td>
<td>Sudden cardiac arrest</td>
<td>Renal cell carcinoma</td>
</tr>
<tr>
<td>6</td>
<td>64 yr</td>
<td>M</td>
<td>Sudden cardiac arrest</td>
<td>Small cell carcinoma lung</td>
</tr>
<tr>
<td>7</td>
<td>66 yr</td>
<td>M</td>
<td>Asphyxia</td>
<td>Metastatic deposits of adeno-carcinoma in lung</td>
</tr>
<tr>
<td>8</td>
<td>70 yr</td>
<td>M</td>
<td>Myocardial infarction</td>
<td>Metastatic deposits of adeno-carcinoma prostate in liver</td>
</tr>
<tr>
<td>9</td>
<td>70 yr</td>
<td>M</td>
<td>Myocardial infarction</td>
<td>CML, blast phase, lung</td>
</tr>
</tbody>
</table>
Lung: It was the m.c system involved by malignancy. Total 4 cases of malignancy were noted in lung. Primary malignancy was small cell carcinoma seen in 64 yr male who was chronic smoker. Gross showed small tan white coloured firm, nodules in hilar region of lung. Two cases of metastatic deposits were noted-one squamous cell carcinoma (Figure 1) in 66 yr male and in second case deposits of Malignant Mesenchymal Tumour (Figure 2) were seen in 58 year female. In both cases, primary malignancy was unknown and deposits in lung were incidental finding on autopsy, with unknown primary. In another case, in 70 yr male, who was already a known case of CML. Extra-medullary infiltration of lung with blast phase of CML was an incidental finding. Microscopic examination revealed sheets of myeloid blasts.

Figure 1 Sections from lung shows Squamous cell carcinoma lung (H&E x400)

Figure 2 Sections from lung shows Malignant Mesenchymal Tumour (H&E X100)
**Brain:** It was next m.c system involved after lung. 3 cases of primary malignancy were detected in brain. Out of which 2 were malignant and 1 was benign. These were mostly seen in middle aged population. In one case, patient was already a known and treated case of Anaplastic Astrocytoma (Figure 3); had presented with recurrence which was an incidental finding as the cause of death was asphyxia. Diffuse Astrocytoma was detected incidentally in 30 yr male who died in road traffic accident. Gross examination showed a small, ill defined mass in frontal lobe of brain. One case of benign meningioma i.e angiomatous meningioma (Figure 4) was seen in 36 yr female who died by suicide. Gross showed a well defined, round mass attached to duramater. This mass was readily separable from durameter and not invading it.

![Figure 3 Sections from brain shows Anaplastic Astrocytoma (H& E X 100)](image)

![Figure 4 Sections show Angiomatous Meningioma (H& E x400)](image)
Liver: Only one case of metastatic deposits of adenocarcinoma in liver with known primary malignancy in prostate was seen in a 70 yr male. The metastatic deposits were an incidental finding during histo-pathological autopsy. Gross showed multiple grey-white coloured nodules of variable sizes on liver parenchyma.

Kidney: Single case of RCC was seen in 59 yr female. It was an incidental finding on grossing as a small, circumscribed growth less than 1 cm was seen on upper pole of kidney and microscopy showed clear cell features. Hence, a diagnosis of clear cell renal cell carcinoma was made (Figure 5)

![Figure 5 Sections examined from kidney exhibiting Clear cell carcinoma](image)

Table 2- Shows system wise distribution of various tumours.

<table>
<thead>
<tr>
<th>System</th>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
</table>
| Lung   |        | Primary- Small cell carcinoma lung (1)  
         |        | CML, blast phase (1)  
         |        | Metastatic deposits- Squamous cell carcinoma (1)  
         |        | Malignant Mesenchymal Tumour (1)  |
| Brain  | Angiomatous Meningioma (1) | Anaplastic Astrocytoma (1)  
         |        | Diffuse Astrocytoma (1)  |
| Liver  |        | Metastatic deposits of adenocarcinoma (1)  |
| Kidney |        | Clear cell carcinoma  |
Discussion

Current study was conducted on 810 autopsy cases over a period of 10 years to know the cause of death in a person and establish relation between autopsy findings and actual cause of death.

The total incidence of neoplasia was 1.1% (9/810 cases). The findings are close to studies by Patel et al and Sinhasan et al showing 2.47% and 4% incidence, respectively.[4,5] But the incidence was quiet high in studies published by Burton et al and Karwinski et al with 9% and 11%, respectively. [6,7]

Lung was the m.c system with malignancy in present study (0.49%). This is close to study by Sinhasan et al with a detection rate of 0.5%, but in contrast to Manser et al where detection rate is as high as 28%. [5,8] This discrepancy could be due to large number of cases included in the study.

One case of extra-medullary CML (blast phase) was seen in lung, which is again a rare presentation. The patient was already a known case of CML and had progressed into blast phase. CML has been reported in extra-medullary organs in only 10% of cases by Inverardi et al and between 4 to 16% by Gao et al. [9,10]

A case of metastatic deposits of sarcoma in lung was seen (0.12%). This was again an incidental finding on autopsy. Primary lung sarcomas are rare, less than 0.5% of lung malignancies. [11] While isolate pulmonary metastases occurs in 20% and 40% cases diagnosed with soft tissue sarcoma and primary bone sarcoma, respectively.[12]

In CNS, three tumours were detected which is 0.37% of the total cases. Out of these anaplastic astrocytoma is grade 3 tumour and fatal if not treated earlier. According to study published by Bogdanovic et al, undiagnosed brain tumours caused sudden death in 3.9% cases. The autopsy showed both benign and malignant tumours including meningioma, anaplastic astrocytoma which are included in present study. [13]

RCC was detected in 1 case (0.12%) incidentally as it was a small mass. However, it could have been detected with radiological tools in life of patient, later on. The findings are similar to Shah et al and Patel et al with detection rate of less than 1% and 1.98%, respectively. [14,15] However, there are studies where detection rate is as high as 26%. [16]

In liver, metastatic deposits from prostatic cancer were detected. According to study published by Wang et al, showed metastasis rate to liver from prostate was 4.29% and the mean survival was 38 months. [17] This shows, it is an uncommon finding with poor survival. Hence, autopsy has a role in detection of rare lesions.

Conclusion

The study stresses on the importance of autopsy in detection of many rare and important findings that would have a direct impact on life of the patient if alive. It also highlights the importance of histopathological study in autopsies in detection of clinically undiagnosed neoplasms and evaluating the cause of death. Thus, autopsies also indirectly aid to the true cancer incidence statistics

Ethical Clearance: Taken from committee.

Source of Funding: Self

Conflict of Interest: Nil

References

4. Patel S, Rajalakshmi BR, Manjunath GV. Histopathologic findings in autopsies with


Stature Estimation and Formulation of Regression Equation from Ear Anthropometry in Haryanvi Population

Monika Rathee¹, Jaswinder Kaur², Suresh Kanta Rathee³
¹Demonstrator, Department of Anatomy, Pt. B. D. Sharma PGIMS, Rohtak, ²Professor, Department of Anatomy, MMIMSR, Mullana, Ambala, ³Sr. Prof. & Head, Department of Anatomy, Pt. B. D. Sharma PGIMS, Rohtak

Abstract

Background: Human beings are unique in number of physical characteristics like shape and form of an individual. These characteristics are influenced by a wide range of racial, ecological, psychological, geographical, gender and biological variations. Human external ear is often overlooked during anthropometric measurements. The structure of human ear is so much variable that even two ears are different in same individual and it also varies according to age, sex, race and ethnic group. Authentic anthropometric data of ear for a particular population is necessary for designing of ear products for occupational environments, to find out the age, sex and stature of an individual in that population. This data on ear measurements can also be helpful in forensic and medico-legal cases for personal identification of an individual. The main objective of the study is to find any relationship between ear parameters and stature and also to formulate regression equation for estimation of stature in Haryanvi adult Jaats.

Methods: The present study was conducted on 300 healthy Haryanvi Jaats (150 females and 150 males) aged 20 to 60 years. Measurements of ear length, ear breadth and stature were taken on each subject.

Conclusion: Ear length and ear breadth are not significantly correlated with stature (p>0.05) in both the sexes except ear breadth which is significantly correlated with stature in males (p<0.05).

The present study concluded that stature cannot be estimated from ear length and ear breadth in Haryanvi population.

Key words: Anthropometry, Measurements, Parameters, Stature.

Introduction

Human beings are unique in number of physical characteristics like shape and form of an individual. These characteristics are influenced by a wide range of racial, ecological, psychological, geographical, gender and biological variations. Human external ear is often overlooked during anthropometric measurements.

corresponding author:
Ms. Monika Rathee,
Demonstrator, Department of Anatomy, Pt. B. D. Sharma PGIMS, Rohtak,
Email: monikarathee786@gmail.com

Anthropometric landmarks are described to measure the dimensions of external ear¹. People often feel embarrassed and depressed who have abnormal structure of ears that may be due to accidental or natural. To rectify the auricular defects like missing parts or inappropriate size, surgery is needed. For that, the data regarding bilateral position on face and normal auricular dimension in different ethnic groups is required. The structure of human ear is so much variable that even two ears are different in same individual and it also varies according to age, sex, race and ethnic group². Authentic anthropometric data of ear for a particular population is necessary for designing of ear products for occupational environments, to find out the age, sex...
and stature of an individual in that population. This data on ear measurements can also be helpful in forensic and medico-legal cases for personal identification of an individual. Stature prediction is required in people whose height cannot be measured due to neuromuscular disorders, lower limb or spinal deformity. For them it can be measured through anthropometry. For estimation of stature various body dimensions like arm span, hand and foot length and different cranio-facial parameters are used because they exist a definite biological relationship with stature. Agnihotri et al did not find any significant relationship between stature and ear parameters but Laxman K and Abdelaleem & Abdelbaky etc reported a significant relationship. Very few studies are there to estimate the stature from ear parameters. so, the present study attempted to find any relationship between ear parameters and to formulate regression equation for estimation of stature.

Material and Method

The present study was a community based anthropometric study conducted on 300 healthy Haryanvi Jaats (150 females and 150 males) of age group 20 to 60 years. Purposive sampling method was used to collect the samples. The purpose of the study was explained and consent from all the subjects were taken.

Along with stature, ear length and ear breadth were measured from healthy Haryanvi jaats without any craniofacial deformity by anthropometric rod and sliding caliper. The measurements were taken according to the landmarks and procedure recommended by Nath S and Krishan & Kumar. The taken anthropometric measurements are described as:

1. **Stature (S):** It is the vertical distance between the plane (where the subject stands barefooted) and the highest point on the vertex on the head. For measurement of stature, the subject was asked to stand straight upright on horizontal surface with arms hanging at sides and shoulders relaxed. The subject’s head was kept in Frankfurt Horizontal Plane and shoulder blades, buttocks and heels touching the measurement surface.

2. **Ear length (EL):** The straight distance between the superaurale (sa) and subaurale (sba).

3. **Ear breadth (EB):** The straight distance between the two most lateral points of the ear. One lies at the base of the ear and other lies at the helix.

Statistical analysis like mean, standard deviation using Z-test, Karl Pearson’s correlation coefficient and derivation of regression equation using Statistical Package for Social Sciences (SPSS) was done.

**Results and Discussion**

Table 1 shows the descriptive statistics i.e mean, standard deviations, minimum and maximum values for ear length, ear breadth and stature measurements in adult Haryanvi males and females respectively. All the parameters are higher in males than females.

<p>| Table 1: Descriptive Statistics for Ear Length, Ear Breadth and Stature in Adult Haryanvi Jaats (N = 300) |
|--------------------------------------------------|--------|--------|--------|--------|</p>
<table>
<thead>
<tr>
<th>Measurements in (cm)</th>
<th>Gender</th>
<th>Mean</th>
<th>S.D</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear Length</td>
<td>Male</td>
<td>6.54</td>
<td>0.484</td>
<td>5.63</td>
<td>7.96</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>6.26</td>
<td>0.425</td>
<td>5.36</td>
<td>7.56</td>
</tr>
<tr>
<td>Ear Breadth</td>
<td>Male</td>
<td>3.41</td>
<td>0.248</td>
<td>2.76</td>
<td>4.16</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>3.26</td>
<td>0.316</td>
<td>2.66</td>
<td>5.26</td>
</tr>
<tr>
<td>Stature</td>
<td>Male</td>
<td>172.14</td>
<td>6.547</td>
<td>151.40</td>
<td>192.00</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>156.07</td>
<td>6.061</td>
<td>141.46</td>
<td>177.00</td>
</tr>
</tbody>
</table>
Table 2 shows that ear length and ear breadth are not significantly correlated with stature (p>0.05) in both the sexes except ear breadth which is significantly correlated with stature in males (p<0.05).

**Table 2: Pearson Correlation Coefficients between stature and ear measurements**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Gender</th>
<th>Pearson Correlation Coefficient (r)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear Length</td>
<td>Male</td>
<td>-0.001</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.221</td>
<td>0.44</td>
</tr>
<tr>
<td>Ear Breadth</td>
<td>Male</td>
<td>0.194</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.063</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Significant (p<0.05)*

Table 3 shows the regression equations to calculate stature from cephalic measurements i.e. from ear length (EL), ear breadth (EB) by substituting the values of cephalic measurements.

**Table 3: Regression equations for estimation of stature from ear measurements**

<table>
<thead>
<tr>
<th>Regression Equation</th>
<th>Standard Error of Estimate (SEE)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Height = 172.23+(-0.14) EL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Height = 136.31+ 3.154 (EL)</td>
<td>6.56</td>
</tr>
<tr>
<td>Height = 154.68+ 5.114(EB)</td>
<td>Height = 152.10+1.214 (EB)</td>
<td>6.44</td>
</tr>
</tbody>
</table>

Table 4 shows the comparison of results of present study with similar available studies on different populations which states that mean values of ear length and ear breadth are higher in males as compared to females. The value of ear length and ear breadth is different in all studies that reveals that these parameters vary in different populations.

**Table 4. Studies showing comparison of Mean Ear Length and Mean Ear Breadth of present study with the mean of previous studies.**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Authors</th>
<th>Study Population</th>
<th>Mean Ear Length</th>
<th>Mean Ear Breadth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Present study</td>
<td>Haryanvi Jaat Population</td>
<td>6.51</td>
<td>3.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.26</td>
<td>3.26</td>
</tr>
<tr>
<td>2.</td>
<td>Japatti SR et al13</td>
<td>Maharashtrian Population</td>
<td>6.54</td>
<td>3.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.26</td>
<td>3.26</td>
</tr>
<tr>
<td>3.</td>
<td>Ekemini &amp; Ekanem11</td>
<td>Nigerian Population</td>
<td>5.89</td>
<td>3.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.65</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.72</td>
<td>3.03</td>
</tr>
<tr>
<td>5.</td>
<td>Laxmi et al15</td>
<td>Northern Indian Population</td>
<td>6.20</td>
<td>3.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.73</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.58</td>
<td>2.86</td>
</tr>
</tbody>
</table>
In the present study, ear length and ear breadth are not significantly correlated with stature (p>0.05) in both the sexes except ear breadth which is significantly correlated with stature in males (p<0.05). Agnihotri et al\(^6\) also finds that ear breadth is not significantly correlated with stature in Indo-Mauritius population but ear length is found to be significant. The other studies done by Laxman K\(^7\), Abdelaleem & Abdelbaky\(^8\), Ekemini & Ekanem\(^11\) and Taura MG et al\(^12\) shows that there is significant correlation between the stature and ear parameters of different population (p<0.05).

**Conclusion**

The present study concluded that stature cannot be estimated from ear length and ear breadth in Haryanvi population as these parameters are not significantly correlated with stature (p>0.05). Further it is believed that large sample size should be examined to validate the findings of this study and to make a definite conclusion.

**Ethical Clearance** - Taken from Institutional Ethical Committee of Maharishi Markandeshwar Deemed University, Mullana, Ambala, Haryana

**Source of Funding** - Self

**Conflict of Interest** - Nil

**References**


9. Nath S. Anthropometry— the measurement of body size, Shape and form. 1993;32.


Facial Anthropometry: A Reliable Tool for Stature Estimation in Haryanvi Population

Monika Rathee¹, Jaswinder Kaur², Suresh Kanta Rathee³

¹Demonstrator, Department of Anatomy, Pt. B. D. Sharma PGIMS, Rohtak, ²Professor, Department of Anatomy, MMIMSR, Mullana, Ambala, ³Sr. Prof. & Head, Department of Anatomy, Pt. B. D. Sharma PGIMS, Rohtak

Abstract

Background: Facial anthropometry is a part of cephalometry that involves the measurements of face. It is very helpful in reconstructive surgery, forensic medicine, orthodontics and to find ethnic differences by defining various shapes of face. Facial features of every individual are different even in siblings. These features vary according to gender, racial and biological group, ecological and geographical region. Anthropometry can also be used for estimation of stature, where it cannot be measured directly such as spinal or lower limb deformity or any other neuromuscular disorder. So, the present study attempted to find that is the facial parameters are reliable in estimation of stature in Haryanvi jaat population and also aims formulate regression equation for estimation of stature in the same.

Methods: A sample of total 300 adult Haryanvi jaatsof age group 20 to 60 years was taken for the present study. Out of them 150 were females and 150 weremales. Anthropometric measurements of face and stature were taken on each subject.

Conclusion: All the studied facial parameters showed positive significant correlation with stature (p<0.01) both in males and females So, it can be concluded that facial anthropometry is a reliable tool for stature estimation in Haryanvi population.

Key words: Anthropometry, Facial, Haryanvi, Identification, Stature.

Introduction

Identification of every human being is unique. This identification can be done by various methods like physical examination, fingerprinting, DNA printing and anthropometry etc. Facial anthropometry is a part of cephalometry that involves the measurements of face. It is very helpful in reconstructive surgery, forensic medicine, orthodontics and to find ethnic differences by defining various shapes of face¹. Males comprises large stature, more prominent cranial and facial features, greater muscularity and strength as compared with females. These differences are not visible in infants, children and sub adults but are noticeable in adult stage due to the effect of hormonal changes occurring at puberty. These features vary according to gender, racial and biological group, ecological and geographical region². Anthropometry can also be used for estimation of stature, where it cannot be measured directly such as spinal or lower limb deformity or any other neuromuscular disorder³. Its the most applied, non-invasive and inexpensive method of measurement to assess the size, proportions of the human body.⁴ Stature can also provide circumstantial and presumptive identification of individual. It is a good indicator of growth and development and may be used in clinical settings for health and nutrition research.In the past,

Corresponding Author:
Ms. Monika Rathee,
Demonstrator, Department of Anatomy, Pt. B. D. Sharma PGIMS, Rohtak,
Email: monikarathee786@gmail.com
estimation of stature from various body parts like bones, fingerprints, skulls, upper and lower limbs, hand and foot has been achieved by many researchers with varying degree of accuracy. Many studies showed that there presents a definite biological relationship between stature and facial parameters\(^5\). So, the present study attempted to find the reliability of facial parameters in estimation of stature in Haryanvi jaat population and also aims to formulate regression equation for estimation of stature in the same.

**Material and Method**

A sample of total 300 adult Haryanvi jaats of age group 20 to 60 years was taken for the present study. Out of them 150 were females and 150 were males. Written consent was taken from every subject. Measurements of stature, Bigonial diameter, morphological facial length and physiognomic facial length were taken on each subject according to the landmarks and procedure recommended by Nath S\(^6\) and Krishan & Kumar\(^7\).

Type of Study: Community based Anthropometric study

Sampling Method: Purposive sampling method

Instruments Used: Anthropometric rod, Spreading caliper and Sliding caliper.

The taken anthropometric measurements are described as: -

1. **Stature (S):** It is the vertical distance between the horizontal surface (where the subject stands straight upright and barefooted) and the highest point on the vertex on the head. The subject’s head was kept in Frankfurt Horizontal Plane.
2. **Bigonial diameter (BD):** It is the maximum breadth of the lower jaw between two gonion points on the angle of mandible.
3. **Morphological facial length (MFL):** It is the straight distance between nasion and gnathion.
4. **Physiognomic facial length (PFL):** It measures the straight distance between trichion and gnathion.

Statistics Done: Mean, standard deviation, Karl Pearson’s correlation coefficient and derivation of regression equation using Statistical Package for Social Sciences (SPSS).

**Results and Discussion**

Table 1 showing the descriptive statistics i.e mean, standard deviations, minimum and maximum values for bigonial diameter, morphological facial length, physiognomic facial length and stature measurements in adult Haryanvi males and females respectively. The values of all parameters are higher in males than females.

<table>
<thead>
<tr>
<th>measurements in (cm)</th>
<th>Gender</th>
<th>mean</th>
<th>S.D</th>
<th>minimum</th>
<th>maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bigonial Diameter</td>
<td>Male</td>
<td>10.68</td>
<td>0.559</td>
<td>9.20</td>
<td>12.00</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>9.92</td>
<td>0.559</td>
<td>8.26</td>
<td>11.46</td>
</tr>
<tr>
<td>Morphological Facial Length</td>
<td>Male</td>
<td>11.16</td>
<td>0.676</td>
<td>9.76</td>
<td>13.53</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>10.41</td>
<td>0.646</td>
<td>8.03</td>
<td>13.66</td>
</tr>
<tr>
<td>Physiognomic Facial Length</td>
<td>Male</td>
<td>17.93</td>
<td>1.046</td>
<td>15.76</td>
<td>20.30</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>16.75</td>
<td>0.959</td>
<td>13.43</td>
<td>19.16</td>
</tr>
<tr>
<td>Stature</td>
<td>Male</td>
<td>172.14</td>
<td>6.547</td>
<td>151.40</td>
<td>192.00</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>156.07</td>
<td>6.061</td>
<td>141.46</td>
<td>177.00</td>
</tr>
</tbody>
</table>
Table 2: Pearson Correlation Coefficients between stature and facial measurements

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Gender</th>
<th>Pearson Correlation Coefficient (r)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>0.278</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.324</td>
<td>0.000</td>
</tr>
<tr>
<td>Bigonial Diameter</td>
<td>Male</td>
<td>0.278</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.324</td>
<td></td>
</tr>
<tr>
<td>Morphological Facial Length</td>
<td>Male</td>
<td>0.354</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.228</td>
<td>0.005</td>
</tr>
<tr>
<td>Physiognomic Facial Length</td>
<td>Male</td>
<td>0.304</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.210</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Table 2 showing significant correlation of bigonial diameter, morphological facial length, physiognomic facial length with stature (p<0.01) in both sexes.

Table 3: Regression equations for estimation of stature from facial measurements

<table>
<thead>
<tr>
<th>Regression Equation</th>
<th>Standard Error of Estimate (SEE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>Height= 137.36+3.255(BD)</td>
<td>6.31</td>
</tr>
<tr>
<td>Height= 138.87+3.426(MFL)</td>
<td>6.14</td>
</tr>
<tr>
<td>Height= 137.99+1.904(PFL)</td>
<td>6.25</td>
</tr>
</tbody>
</table>

Table 3 showing the regression equations to calculate stature from facial measurements i.e. from bigonial diameter (BD), morphological facial length (MFL) and physiognomic facial length (PFL) by substituting the values of facial measurements in their respective equations.

Table 4: Studies showing comparison of mean values of facial parameters of present study with the mean of previous studies.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Authors</th>
<th>Study Population</th>
<th>Mean Bigonial Diameter</th>
<th>Mean Morphological Facial Length</th>
<th>Mean Physiognomic Facial Length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>2.</td>
<td>Krishna and Babu8</td>
<td>South Indian Population</td>
<td>-</td>
<td>-</td>
<td>11.26</td>
</tr>
<tr>
<td>5.</td>
<td>Yadav AB et al11</td>
<td>Indian Population</td>
<td>9.91</td>
<td>9.04</td>
<td>11.33</td>
</tr>
<tr>
<td>7.</td>
<td>Sahni et al13</td>
<td>Northwest Indian Population</td>
<td>10.64</td>
<td>10.26</td>
<td>11.25</td>
</tr>
<tr>
<td>8.</td>
<td>Kumar M and Gopichand14</td>
<td>Haryanvi Bania Population</td>
<td>11.45</td>
<td>10.33</td>
<td>11.07</td>
</tr>
</tbody>
</table>
Table 4 showing the comparison of results of present study with similar available studies on different populations which states that mean values of facial parameters i.e. bigonial diameter, morphological facial length and physiognomic facial length are higher in males as compared to females. The value of all facial parameters is different in all studies that reveals that these parameters vary in different populations.

Table 5. Studies showing p- values of facial parameters of present study and previous studies.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Authors</th>
<th>Study Population</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bigonial Diameter</td>
</tr>
<tr>
<td>1.</td>
<td>Present Study</td>
<td>Haryanvi Jaat Population</td>
<td>p&lt;0.00</td>
</tr>
<tr>
<td>2.</td>
<td>Shah et al9</td>
<td>Gujarati Population</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>3.</td>
<td>Yadav et al11</td>
<td>Indian Population</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>4.</td>
<td>Agnihotri et al12</td>
<td>Indo-Mauritius Population</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>5.</td>
<td>Kumar &amp; Gopichand14</td>
<td>Haryanvi Bania Population</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

*Insignificant

Table 5 showing p- values of facial parameters of present study and previous studies. It is evident that the facial parameters i.e. bigonial diameter, morphological facial length and physiognomic facial length showed positive significant correlation with stature in present study and in previous studies. Except the morphological facial length of Gujarati population studied by Shah et al9 which is insignificant. So, these parameters can be used for estimation of stature in Haryanvi jaat population effectively.

Conclusion

All the studied facial parameters showed positive significant correlation with stature (p<0.01) both in males and females So, it can be concluded that facial anthropometry is a reliable tool for stature estimation in Haryanvi population.

Acknowledgement : I would take this opportunity to express my sincere gratitude to my esteemed supervisor and co-supervisor for guiding this work with interest. I would like thank Dr. Kamal Singh and Dr. Aarti for their motivation, encouragement, assistance and constant support provided by them. There is no source of support and conflicting interest.

Ethical Clearance- Taken from Institutional Ethical Committee of Maharishi Markandeshwar Deemed University, Mullana, Ambala, Haryana

Source of Funding- Self

Conflict of Interest - Nil

References


6. Nath S. Anthropometry-the measurement of body size, Shape and form. 1993;32.


Stature Estimation and Formulation of Regression Equation from Ear Anthropometry in Haryanvi Population

Monika Rathee¹, Jaswinder Kaur², Suresh Kanta Rathee³
¹Demonstrator, Department of Anatomy, Pt. B. D. Sharma PGIMS, Rohtak. Mob: 8901569985, ²Professor, Department of Anatomy, MMIMSR, Mullana, Ambala, ³Sr. Prof. & Head, Department of Anatomy, Pt. B. D. Sharma PGIMS, Rohtak

Abstract

Background: Human beings are unique in number of physical characteristics like shape and form of an individual. These characteristics are influenced by a wide range of racial, ecological, psychological, geographical, gender and biological variations. Human external ear is often overlooked during anthropometric measurements. The structure of human ear is so much variable that even two ears are different in same individual and it also varies according to age, sex, race and ethnic group. Authentic anthropometric data of ear for a particular population is necessary for designing of ear products for occupational environments, to find out the age, sex and stature of an individual in that population. This data on ear measurements can also be helpful in forensic and medico-legal cases for personal identification of an individual. The main objective of the study is to find any relationship between ear parameters and stature and also to formulate regression equation for estimation of stature in Haryanvi adult Jaats.

Methods: The present study was conducted on 300 healthy Haryanvi Jaats (150 females and 150 males) aged 20 to 60 years. Measurements of ear length, ear breadth and stature were taken on each subject.

Conclusion: Ear length and ear breadth are not significantly correlated with stature (p>0.05) in both the sexes except ear breadth which is significantly correlated with stature in males (p<0.05).

The present study concluded that stature cannot be estimated from ear length and ear breadth in Haryanvi population.

Key words: Anthropometry, Measurements, Parameters, Stature.

Introduction

Human beings are unique in number of physical characteristics like shape and form of an individual. These characteristics are influenced by a wide range of racial, ecological, psychological, geographical, gender and biological variations. Human external ear is often overlooked during anthropometric measurements. Anthropometric landmarks are described to measure the dimensions of external ear¹. People often feel embarrassed and depressed who have abnormal structure of ears that may be due to accidental or natural. To rectify the auricular defects like missing parts or inappropriate size, surgery is needed. For that, the data regarding bilateral position on face and normal auricular dimension in different ethnic groups is required. The structure of human ear is so much variable that even two ears are different in same individual and it also varies according to age, sex, race and ethnic group². Authentic anthropometric data of ear for a particular...
population is necessary for designing of ear products for occupational environments, to find out the age, sex and stature of an individual in that population. This data on ear measurements can also be helpful in forensic and medico-legal cases for personal identification of an individual. Stature prediction is required in people whose height cannot be measured due to neuromuscular disorders, lower limb or spinal deformity. For them it can be measured through anthropometry. For estimation of stature various body dimensions like arm span, hand and foot length and different cranio-facial parameters are used because they exist a definite biological relationship with stature. Agnihotri et al. did not find any significant relationship between stature and ear parameters but Laxman and Abdelaleem & Abdelbaky etc reported a significant relationship. Very few studies are there to estimate the stature from ear parameters. so, the present study attempted to find any relationship between ear parameters and to formulate regression equation for estimation of stature.

**Material and Method**

The present study was a community based anthropometric study conducted on 300 healthy Haryanvi Jaats (150 females and 150 males) of age group 20 to 60 years. Purposive sampling method was used to collect the samples. The purpose of the study was explained and consent from all the subjects were taken.

Along with stature, ear length and ear breadth were measured from healthy Haryanvi jaats without any craniofacial deformity by anthropometric rod and sliding caliper. The measurements were taken according to the landmarks and procedure recommended by Nath S and Krishan & Kumar. The taken anthropometric measurements are described as:

5. **Stature (S):** It is the vertical distance between the plane (where the subject stands barefooted) and the highest point on the vertex on the head. For measurement of stature, the subject was asked to stand straight upright on horizontal surface with arms hanging at sides and shoulders relaxed. The subject’s head was kept in Frankfurt Horizontal Plane and shoulder blades, buttocks and heels touching the measurement surface.

6. **Ear length (EL):** The straight distance between the superaurale (sa) and subaurale (sba).

7. **Ear breadth (EB):** The straight distance between the two most lateral points of the ear. One lies at the base of the ear and other lies at the helix.

Statistical analysis like mean, standard deviation using Z-test, Karl Pearson’s correlation coefficient and derivation of regression equation using Statistical Package for Social Sciences (SPSS) was done.

**Results and Discussion**

Table 1 shows the descriptive statistics i.e mean, standard deviations, minimum and maximum values for ear length, ear breadth and stature measurements in adult Haryanvi males and females respectively. All the parameters are higher in males than females.

<table>
<thead>
<tr>
<th>Measurements in (cm)</th>
<th>Gender</th>
<th>Mean</th>
<th>S.D</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>6.54</td>
<td>0.484</td>
<td>5.63</td>
<td>7.96</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>6.26</td>
<td>0.425</td>
<td>5.36</td>
<td>7.56</td>
</tr>
<tr>
<td>Ear Length</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>3.41</td>
<td>0.248</td>
<td>2.76</td>
<td>4.16</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>3.26</td>
<td>0.316</td>
<td>2.66</td>
<td>5.26</td>
</tr>
<tr>
<td>Ear Breadth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stature</td>
<td>Male</td>
<td>172.14</td>
<td>6.547</td>
<td>151.40</td>
<td>192.00</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>156.07</td>
<td>6.061</td>
<td>141.46</td>
<td>177.00</td>
</tr>
</tbody>
</table>
Table 2 shows that ear length and ear breadth are not significantly correlated with stature (p>0.05) in both the sexes except ear breadth which is significantly correlated with stature in males (p<0.05).

**Table 2: Pearson Correlation Coefficients between stature and ear measurements**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Gender</th>
<th>Pearson Correlation Coefficient (r)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear Length</td>
<td>Male</td>
<td>-0.001</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.221</td>
<td>0.44</td>
</tr>
<tr>
<td>Ear Breadth</td>
<td>Male</td>
<td>0.194</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.063</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Significant (p<0.05)*

Table 3 shows the regression equations to calculate stature from cephalic measurements i.e. from ear length (EL), ear breadth (EB) by substituting the values of cephalic measurements.

**Table 3: Regression equations for estimation of stature from ear measurements**

<table>
<thead>
<tr>
<th>Regression Equation</th>
<th>Standard Error of Estimate (SEE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>Height = 172.23+(-0.14) EL</td>
<td>6.56</td>
</tr>
<tr>
<td>Height = 154.68+ 5.114(EB)</td>
<td>6.44</td>
</tr>
<tr>
<td>Height = 136.31+ 3.154 (EL)</td>
<td>6.56</td>
</tr>
</tbody>
</table>

Table 4 shows the comparison of results of present study with similar available studies on different populations which states that mean values of ear length and ear breadth are higher in males as compared to females. The value of ear length and ear breadth is different in all studies that reveals that these parameters vary in different populations.

**Table 4. Studies showing comparison of Mean Ear Length and Mean Ear Breadth of present study with the mean of previous studies.**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Authors</th>
<th>Study Population</th>
<th>Mean Ear Length</th>
<th>Mean Ear Breadth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
</tbody>
</table>
Table 4. Studies showing comparison of Mean Ear Length and Mean Ear Breadth of present study with the mean of previous studies.

<table>
<thead>
<tr>
<th>Study Reference</th>
<th>Population Type</th>
<th>Mean Ear Length</th>
<th>Mean Ear Breadth</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. Ekemini &amp; Ekanem11</td>
<td>Nigerian Population</td>
<td>5.89</td>
<td>5.65</td>
</tr>
<tr>
<td>13. Laxmi el al15</td>
<td>Northern Indian Population</td>
<td>6.20</td>
<td>5.73</td>
</tr>
<tr>
<td>15. Sharma N17</td>
<td>Northern Indian Population</td>
<td>5.43</td>
<td>5.49</td>
</tr>
<tr>
<td>16. Verma et al18</td>
<td>North- East Indian Population</td>
<td>6.15</td>
<td>5.75</td>
</tr>
<tr>
<td>17. Verma et al18</td>
<td>North- West Indian Population</td>
<td>6.37</td>
<td>5.89</td>
</tr>
<tr>
<td>18. Mustapha el al19</td>
<td>North-Western Nigerian Population</td>
<td>5.48</td>
<td>5.49</td>
</tr>
<tr>
<td>19. Taura MG et al12</td>
<td>Nigerian Population</td>
<td>6.03</td>
<td>5.95</td>
</tr>
<tr>
<td>20. Agnihotri et al6</td>
<td>Indo-Mauritius Population</td>
<td>6.17</td>
<td>5.69</td>
</tr>
</tbody>
</table>

In the present study, ear length and ear breadth are not significantly correlated with stature (p>0.05) in both the sexes except ear breadth which is significantly correlated with stature in males (p<0.05). Agnihotri et al6 also finds that ear breadth is not significantly correlated with stature in Indo-Mauritius population but ear length is found to be significant. The other studies done by Laxman K7, Abdelaleem & Abdelbaky8, Ekemini & Ekanem11 and Taura MG et al12 shows that there is significant correlation between the stature and ear parameters of different population (p<0.05).

Conclusion
The present study concluded that stature cannot be estimated from ear length and ear breadth in Haryanvi population as these parameters are not significantly correlated with stature (p>0.05). Further it is believed that large sample size should be examined to validate the findings of this study and to make a definite conclusion.

Ethical Clearance - Taken from Institutional Ethical Committee of Maharishi Markandeswar Deemed University, Mullana, Ambala, Haryana

Source of Funding - Self

Conflict of Interest - Nil

References


9. Nath S. Anthropometry-the measurement of body size, Shape and form. 1993;32.


Therapy of Human Chorionic Gonadotropin (hCG) Hormone in Dairy Cattle that Have Repeat Breeder on Pregnancy Rate in Tulungagung, East Java, Indonesia

Muhammad Aulia Rahman¹, Sri Pantja Madyawati², Oky Setyo Widodo³, Pudji Srianto², Trilas Sardjito², Rimayanti²

¹Student, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, ²Lecturer, Department of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, ³Lecturer, Department of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

This study aimed to prove that giving hCG injection at the time of artificial insemination and five days after artificial insemination in dairy cows with repeat breeder can cause pregnancy. This study employed 30 dairy cows with repeat breeder. All samples of dairy cows were divided into two treatments, P1 using hCG at a dose of 100 IU given intramuscularly at the time of artificial insemination and P2 using hCG at a dose of 100 IU intramuscularly given five days after artificial insemination. The results were analyzed using the Chi-Square test. Analysis using Chi-Square showed that all treatments caused pregnancy (100%). In summary, hCG injection at the time of artificial insemination and five days after artificial insemination in dairy cows experiencing repeat breeding can cause pregnancy.

Keywords: Artificial Insemination, Dairy Cows, hCG, Pregnancy, Repeat Breeder

Introduction

Most of the dairy cows kept in Indonesia are FH cows, the production of FH cow milk can be increased, among others, by increasing reproductive efficiency¹,²,³. Reproductive efficiency is very important for raising dairy cows. Reproductive efficiency is also a parameter that shows the ability of livestock to become pregnant and produce offspring⁴.

Low reproductive efficiency in dairy cows indicates reproductive problems, one of which is repeat breeder with cases of ovulation failure on day 21 and cases of implantation failure on day 28. Cows that experience repeat breeders are generally characterized by long calving intervals (18-24 months), low conception rates (<40%), and high service per conception (>3)⁵,⁶,⁷.

Repeat breeder is the occurrence of female cows that are mated more than 2 times and do not experience pregnancy with an average age of 3-7 years who have given birth with the cow healthy, sexually mature, normal lust cycle and not experiencing pregnancy⁸. The factors that cause repeat breeders are fertilization failure and early embryo death⁹. KUD Tani Wilis is one of the largest KUDs in Indonesia with good management in managing the business/main unit of dairy cows. In 2018, there was the latest data from the Reproductive Engineering Assistant (ATR) in the KUD Tani Wilis, the number of repeat breeder cases was 20%, a decrease of 10% from the previous year, which means that there were 1,385 cows that experienced repeat breeders or around 115 cows per month. The high number of repeat breeders that occurs in the KUD Tani Wilis area can cause losses to breeders. Therefore, in this study continued the handling of these reproductive disorders, namely repeat breeders with cases of ovulation failure on day 21 and cases of implantation failure on day 28, by administrating the hormone human chorionic gonadotropin (hCG) at IB and five days after AI, to treat ovulation failure. on day 21 and failure of implantation on day 28 in order...
to cause pregnancy events in the KUD Tani Wilis area, Tulungagung, East Java, Indonesia.

Method

Materials

This study used a sample of 30 female Friesian Holstein (FH) dairy cows with an average age of 3-7 years who have had children. The cows are healthy, sexually mature, normal short and long lust cycles, do not experience pregnancy, 15 cows as treatment during artificial insemination with cases of ovulation failure on day 21 and 15 as treatment five days after artificial insemination with cases of implantation failure on day 28. Treatment using the hCG hormone which is injected intramuscularly at a dose of 100 IU 1 mL/head/im.

Selection of FH Cows

Selection of cows in lust, reproducing actively and not experiencing pregnancy. Cows in the 2nd AI (repeat breeder) who still show symptoms in the normal heat cycle with cases of ovulation failure on day 21 and cases of implantation failure on day 28.

Artificial Insemination (AI)

Artificial insemination is carried out on cows that have repeated heat breeder using a straw owned by the inseminator, namely the Friesian Holstein cow. Insemination is carried out once after a dairy cow is in heat.

Treatment

Cows that experience repeat breeder will be given hCG hormone at a dose of 100 IU 1 mL/head/im to cows that have repeat breeders. The cows will be divided into two groups P1 and P2, each group consisting of 15 FH cows. Group P1 was injected with 100 IU hCG 1 mL/head/im at the time of artificial insemination with cases of ovulation failure on day 21 and group P2 was injected with 100 IU hCG 1 mL/head/im at five days after artificial insemination with cases of failure of implantation on day 28.

Pregnancy Examination

Pregnancy examinations are carried out by inseminator officers who have PKB certificates and veterinarians on duty at KUD Tani Wilis, Tulungagung, East Java, Indonesia. PKB will be carried out 90 days after IB, the goal is that the enlarged uterine cornua containing placental fluid is palpable and prevents miscarriage.

Data Analysis

The data obtained were tabulated, to analyze the incidence of pregnancy the Chi-Square test was presented.

Results and Discussion

This study was conducted to determine the success of giving the hCG hormone during artificial insemination and five days after artificial insemination given to FH dairy cows with repeat breeder with cases of ovulation failure on day 21 and cases of implantation failure on day 28 at KUD Tani Wilis, Kabupaten Tulungagung. The samples were divided into 2 types of treatment, namely 15 FH dairy cows that were treated with the hCG hormone injection during artificial insemination and 15 FH dairy cows which were given the hCG hormone injection treatment five days after artificial insemination.

The incidence of pregnancy in FH dairy cows that experienced repeat breeder cases and were injected with the hCG hormone at the time after artificial insemination and five days after artificial insemination by rectal palpation method examination can be shown in Table 1 below.

<p>| Table 1. Percentage of Pregnancy in Friesian Holstein (FH) Dairy Cows with Repeat Breeder that has been Injected with the hCG Hormone. |
|-----------------|--------|-----------------|</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Pregnancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>15</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>P2</td>
<td>15</td>
<td>15 (100%)</td>
</tr>
</tbody>
</table>

Note:
P1: Administration of the hCG hormone when IB 100 IU 1mL/head/im.

P2: Administration of the hCG hormone for five days after IB 100 IU 1 mL/head/im.

The results of the test using the Chi-Square 2x2 show that the Chi-Square test cannot be done because pregnancy is constant. This is because the first treatment and the second treatment showed a rate of 100% at the time of pregnancy examination. From these results, it shows that the hCG hormone can increase the incidence of pregnancy in dairy cows that experience repeat breeders.

![Figure 1. Pregnancy diagram of FH Dairy Cows experiencing repeat breeders who have been injected with the hCG hormone.](image)

In the 21st day case, Fertilization failure is the main factor causing repeat breeder. Fertilization failure factors include anatomical abnormalities of the reproductive tract, ovulation abnormalities, abnormal sperm, abnormal egg cells and reproductive mismanagement including factors that can cause fertilization failure\(^{10,11}\). Ovulation abnormalities can be caused by ovulation failure due to hormonal disorders where there is a lack or failure of the release of the LH hormone\(^{12}\). In the case of repeat breeders, failure to ovulate on day 21 often occurs in the dry season with a delay in ovulation time due to the influence of heat and weather. The administration of the hCG hormone during IB aims to meet the needs of the LH hormone in ovulation disorders and ensure the ovulation process so that there is no repeat breeder in cases of ovulation failure and also the hCG hormone given in cases of delayed ovulation in order to induce ovulation at the right time so that pregnancy occurs.

In the case of day 28, embryo mortality indicates the death of a fertile embryo until the end of implantation, which is 40 days, factors from premature embryo death include lactation which can be related to ineffective defense mechanisms of the uterus, stress during lactation and imperfect endometrial regeneration Environmental factors can also result in the mother when her body temperature increases, the factor imbalance of the hormones estrogen and progesterone can also cause premature embryo death\(^{13}\). The occurrence of early embryonic death is marked by an extension of the cycle
of lust after the last IB. The adverse effects of lactation on embryo development are thought to be related to hormonal imbalances during lactation, particularly the hormone progesterone on embryo life in the uterus. High milk production can cause the embryo in the uterus to not get enough food for its development, besides that the involution process of the uterus that is not yet complete after giving birth is also one of the causes of embryo death during lactation, the uterine wall during the implantation process so that it is easy to die of the embryo. In repeat breeder cases with failed implantation on day 28, the most cases occurred because of mild infections or subclinical endometritis which made the uterine temperature uncomfortable, therefore hCG hormone was given to keep the growth of the corpus luteum growing and accelerate the growth of the corpus luteum to produce progesterone, the hormone progesterone functions to maintain pregnancy and prevents contractions in the uterus and the fetus does not abort. The administration of the hCG hormone at five days after IB aims to increase the level of hCG in the blood. On days 5 to 9 after insemination, the concentration of interferon-τ was increased. Interferon-τ causes the endometrium to suppress PGF2α so that the corpus luteum is not regressed.

Both P1 and P2 treatments are 100% pregnant, this can be caused by several factors such as the right time of insemination, good farmer knowledge of the incidence of lust, good feed management and good cage hygiene management. Understanding livestock management and optimal breeding techniques, including fulfilling the nutritional needs of sterile labor, can maintain fertility. Pregnancy rate can be influenced by the quality and handling of semen, female fertility, time of mating, detection of estrus, and insemination techniques. The increase in the incidence of pregnancy obtained is influenced by the increase in the concentration of progesterone. The reduced concentration of progesterone in the blood can affect embryo development.

**Conclusion**

Based on the results obtained, the administration of the hCG hormone during artificial insemination and five days after AI in FH dairy cows that experienced repeat breeder short normal heat cycles (21 days) and long normal heat cycles (28 days) caused pregnancy.

**Conflict of Interest:** The author declare that they have no conflict of interest.

**Source of Funding:** None.

**Acknowledgements:** We thank Arif Nur Muhammad Ansori for editing the manuscript.

**Ethical Approval:** The study approved by the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

**References**


The Impact of the COVID-19 Pandemic on Stopping and Completing the Period of Appeal Against Judicial Rulings in Accordance with Jordanian law

Muhaned Farhan Al Taani

Assistant Professor/ Faculty of Law- Applied Science Private University – Jordan

Abstract

This study attempts to shed light on the applicability of the Jordanian Civil Trials Law No. 24 of 1988 and its amendments in light of the COVID-19 pandemic and the prevention of litigants’ access to the courts because it is an official holiday on the grounds constituting it to be a force majeure or an emergency circumstance which is considered an unexpected event. The law did not explicitly stipulate a cessation of appeal against judgments. When this impediment is present, the appellant can complete the appeal period when this impediment ceases to exist. The completion of the prescribed periods becomes in effect, while Defense Order No. 5 bridged the gap in the provisions of the Civil Trials Law in light of the country’s passage through the COVID-19 pandemic, which came suddenly being an unexpected event. The legislator was unable to address the issue of stopping and completing the appeal period against the ruling issued for its appeal within the legal period stipulated in the law. Besides, this study includes the opinions of jurists in the Civil Trials Law.

This study indicated that the event of force majeure or an emergency situation (COVID-19 pandemic) with the activation of Defense Law No. 13 of 1992 as it is pointing that completing the period of appeal with judicial rulings and the necessity of its application during the existence of the COVID-19 pandemic with the need to amend the Jordanian legislature for the legal loophole in this regard.

Keywords: COVID-19 pandemic, the period of appeal, judicial rulings, Jordanian law, Defense Law No. 13 of 1992.

Introduction

COVID-19 was first discovered on December 31, 2019, after a group of people were reported to have contracted viral pneumonia in Wuhan, the People’s Republic of China. The virus can be transmitted from one person to another, usually by close contact with the infected person, as it occurs in the context of family, work, etc1,2. Due to the spread of COVID-19 all over the world, the Jordanian Cabinet decided to announce the implementation of Defense Law No. 13 of 1992 throughout the Hashemite Kingdom of Jordan as of 3/17/2020 and under the text of Article (3) of the Defense Law, the Jordanian Prime Minister is entitled to take necessary procedures and measures to secure public safety, where the prime minister exercises his powers under written orders, including placing restrictions on the freedom of people to meet and move.

Defense Order No. (5) was issued to impose a comprehensive general ban from the period 3/18/2020 until 05/31/2020, preventing the exit of citizens, closing all court work and considering it an official holiday, stopping the periods for submitting appeals against judicial rulings issued by civil courts and moving them until after the official holiday ends and to complete it so that it becomes effective again after the end of the official holiday. As the Prime Minister issued a communiqué No. (6) issued in accordance with the provisions of Defense Order No. (5) of 2020 and stipulates that the courts, institutions, and official departments shall start their work as of May 31, 2020.
The appellant is the one who submits a list of appeals against the judgment issued by the court of the first instance to the Court of Appeal, as the Jordanian Civil Trials Law stipulated in Article (180) in Chapter 10 (Methods for Appealing Judgments) from the following: issuing the appealed judgment to be filed with the case documents after making notifications to the appealed court.

The date for submitting an appeal list to appeal the judgment issued by the court of the first instance with the judgments in front of the court starts from the day following the date of its issuance, and in the judgments issued as notable from the day following the date of its notification, unless the law stipulates otherwise Article (171) so that the appeal list is not accepted if it is not registered during the period legal time. This rule of public order enables the appellant or the court to raise what is related to the date of submitting the appeal after the prescribed legal period has passed. This legal text applies to all appeal dates for civil judicial decisions, meaning, in other words, the loss of the right to appeal a penalty that results from the failure of the opponent to appeal the judgment on the date specified in the law.

The dates for appealing judicial decisions are considered one of the procedural dates. Part of the Egyptian jurisprudence opinions refers to the term of the procedural data in the Civil Trials Law, which relates only to the period between two times, the moment of beginning and the moment of completion (Nabil, 2004). The problem exists here: is it possible to stop and complete the appeals period against the judgment issued by the court of the first instance during the legal period in light of the existence of the COVID-19 pandemic on the grounds that it is (force majeure or an emergency circumstance) based on Defense Order No. (5) or is the legal texts contained in the law sufficient, the origins of civil trials.

Electronic Trial in Jordan

The extent of modern technology in the criminal justice system in Jordan has escalated during the time of COVID-19. The use of modern technology in criminal justice in Jordan has been issued in accordance with Article 158 paragraph two of the Jordanian Code of Criminal Procedures. During the coronavirus (Covid-19) pandemic where quarantine, lockdown and self-isolation have become the new norm. To continuously guard and provide an unprejudiced administration of justice during this pandemic, it is legitimate to utilize the means of modern technology to facilitate remote court appearances in line with the criminal justice system.

Public Hearing During COVID-19

The Jordanian Constitution clearly states that courts’ sessions are public unless the court decides to conduct them in secret in order to preserve public order or morals. However, in all cases, the pronouncement of judgment is made in a public session. In the same vein, the Jordanian Code of Criminal Procedures addresses the principle of public hearing when it asserts that the trial is conducted publicly unless the court decides to conduct it in secret, in order to preserve public order or morals. However, in all cases, juveniles or a certain group of people can be prevented from attending the trial. In light of those as mentioned above, it can be noticed that the principle of public hearing has been dealt with in both the Jordanian Constitution and the Jordanian Code of Criminal Procedures. Therefore, such principle should be followed in all trials unless otherwise provided. Additionally, both legislations affirm that courts sessions and trials should be conducted publicly unless they contradict with public order or morals.

In light of the above, it can be argued that the technology of videoconferencing – as one of the means of modern technology - satisfies the requirements of the principle of public hearing as the hearing will be screened. Hence, the public can still follow the proceedings if they are allowed into the courtroom.

Results and Discussions

The law specifies dates for procedures with the aim of ensuring that litigation guarantees are not wasted and giving litigants an adequate opportunity to prepare a list of appeals against a judgment issued by a civil court.
The litigants cannot agree to violate the provisions of the law related to the dates for the appeal by appealing during the legal periods, such as agreeing to file the appeal after the legal period has passed or agreeing to find new legal periods that contradict what the law stipulated. Therefore, the general provisions for challenging judicial rulings in the Civil Trials Law must be taken into consideration.

Where Article (178) of the Civil Trials Law states the following point:

1. The period for appealing the appeal shall be thirty days for judgments ending the litigation unless a special law provides otherwise.

Whereas the Jordanian Court of Cassation decided that it is necessary to respect the deadlines for appeal by appealing during the legal period, that this is a waste of the time of the courts and judges and that it delays the execution of judgments, and that is a departure from the conduct of the profession of the lawyer who is considered a helper to the judiciary and strengthens it (Judgment No. 923/2000 on 4/24/2000).

It is observed that the rule is that the judge may not amend the appeal deadlines set by the law, either more or less, as they achieve stability for the parties to the case, but it seems that in certain circumstances, the law can recognize the judge with the exception of modifying the procedural dates, including the extension of the deadline due to an official holiday; where Article 23 of the Civil Trials Law states that: "(Notwithstanding what is stated in any other law: The dates specified by the month or year shall be calculated according to the Gregorian calendar unless the law stipulates other than If the last date coincides with an official holiday, it is extended to the first working day after that.)"

For example, with the existence of emergency conditions in the country, the Jordanian Court of Cassation decided that the last day of the appeal period coincided with (snowfall, road closures, and irregular working hours in official departments). The deadline for appeal extends to the next day, and the submitted appeal is within the legal period (The law “Judgment” No. 3119 of 2003 Court of Rights Cassation).

The Civil Trials Law stipulates that:

1) Article (122) (The court shall order the suspension of the lawsuit if it deems that the judgment in its merits is suspended on the adjudication of another matter upon which the judgment depends. As soon as the reason for the suspension is removed, any of the litigants may request to proceed with the case).

2) Article (123) (A lawsuit may be suspended based on the litigants’ agreement not to proceed with it for a period not exceeding six months from the date of the court’s approval of their agreement).

3) Article (174) (If one of the parties to the lawsuit dies, or if it is decided to declare him bankrupt, or if he has lost the capacity for litigation during the appeal dates, the judgment shall be communicated to whoever legally takes his place).

There is no judicial application in Jordan according to the Civil Trials Law that decides to complete the period of appeal with the appeal in the period between the issuance of the judgment and its appeal if force majeure or a sudden accident (such as the COVID-19 pandemic) is achieved so that the period that ended from the date of submitting the appeal and starting from the date that this bar is removed.

On the other hand, the Jordanian jurisprudence believes that the legislator has canceled the status of suspending the case by virtue of the law from the provisions of the current Civil Trials Law No. 24 of 1988 and its amendments; and that what came from a ruling related to stopping the case in the Civil Trials Law is by court order or by agreement of the litigants.
Accordingly, the Jordanian judiciary and legislator did not resolve the issue of stopping and completing the period of appeal against a judgment issued by the Court of First Instance in the event of force majeure or an emergency situation, including the COVID-19 pandemic during another period, so that the period is completed by starting the first working day after the end of the impediment and completing the remaining period during the suspension to the original appeal period.

According to Defense Law No. (13) of 1992 - and based on Defense Order No. (5) and as of 3/18/2020:

The validity of all periods and deadlines stipulated in the legislation in force shall be suspended, whether they are statute of limitations, lapse, failure to hear a lawsuit, or periods for taking any of the litigation procedures in all types of courts in the Kingdom, public prosecution departments, arbitration bodies, enforcement departments, wages authority and any conciliation and mediation council. Disciplinary and others who exercise competencies similar to those of these councils, even if these periods are among the periods to which the suspension does not apply.

The validity of the suspended periods, based on the Prime Minister’s decision, shall continue to be carried out by institutions, official departments, and courts.

The Jordanian Prime Minister’s Communication No. (6) was issued in accordance with the provisions of the Defense Order for the year 2020, as follows:

(As of 5/31/2020, institutions, official departments, and courts begin their work, and all periods and dates suspended under Defense Order No. (5) of 2020 will be completed.)

In the implementation of this, the Jordanian Court of Cassation decided (to accept the appeal submitted on 6/18/2020 within the legal period, as it was decided to suspend the calculation of appeal periods as of 3/18/2020 under Defense Order No. (5) of 2020, while it was decided to complete the calculation of the periods on 31/5/2020, based on Communication No. (6) issued based on the aforementioned Defense Order).

Case No. (Case: 2773/2020).

Consequently, Defense Order No. (5) issued under the Defense Law was decisive in its stipulation of completing the calculation of an appeal period against a judgment issued by the Court of First Instance during the period it decided in the case of force majeure or the exceptional circumstances that the world witnessed in the COVID-19 pandemic and this is what the Jordanian courts decided in adhering to the defense order, applying it to deadlines and completing the period of appeal against judicial decisions.

Conclusion

The current research shows the difference in how to clarify the time limits for submitting an appeal to challenge judicial rulings and to complete them after the impediment is removed in the Jordanian courts in the event of force majeure or exceptional circumstances. The Civil Trials Law and related judicial applications in Jordan has not resolved the issue of stopping and completing the periods for submitting the appeal in an appeal against a judicial ruling suspended due to the COVID-19 pandemic, which is expressed as (force majeure or an exceptional circumstance) and thus completing the validity of the remaining period for the end of the appeal period after the impediment is removed. This constitutes a legislative deficiency in the provisions of the Civil Trials Law that dealt with cases of stopping the deadline for appeal periods for reasons related to the death of the convicted person, to lose the capacity to litigate, or to the disappearance of the capacity of the person who was engaged in the judicial litigation, and in light of this there were many jurisprudential opinions in determining the concept of stopping and completing the deadline for appeal in Judicial judgments when there is force majeure or an emergency situation, and the Jordanian legislator must expand in addressing this legislative deficiency in accordance with certain legal standards. Defense Order No. (5) came in light of the COVID-19 pandemic, decisive in the issue of stopping the validity and completion of the deadlines for appealing judicial rulings. It has imposed on judicial applications in the COVID-19 period. In terms of commitment to what was stipulated, and the obligation to set and complete the scheduled appeal dates as imposed by them and obligate the courts to adhere to them.
The Jordanian legislator has to expand the provisions of the Civil Trials Law to address such exceptional circumstances in the future, accordingly:

1) The Civil Trials Law did not provide for the suspension and completion of the remaining period for appealing judicial decisions in emergency circumstances or force majeure, such as the COVID-19 pandemic.

2) The defense law has been decided according to Defense Order No. (5) the issue of stopping and completing the period of appeal against judicial rulings due to the legislative deficiency in the Jordanian Civil Procedure Law in Articles (23,122,123,174).

3) The application of the Jordanian courts and their commitment to the defense law in accordance with Defense Order No. (5) on calculating and completing the remaining appeal period according to the judicial rulings.

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Reference

Unanticipated Ligature Strangulation: A Case Report

Mukul Sharma¹, Kimi Soumya Padhi², Manas Ranjan Sahu³, Utkal Keshari Suna², Govinda Balmuchu²

¹Senior Resident, ²Junior Resident, ³Associate Professor, Department of Forensic Medicine & Toxicology, All India Institute of Medical Sciences, Bhubaneswar

Abstract

Strangulation is a form of mechanical asphyxia by compression of the neck with an outside force either by a ligature or hands, in which a constricting force other than the weight of the body is directly applied. Such deaths are generally considered homicidal. Here we present a case of 60-year-old female, who got trapped in the rice huller machine with the saree she was wearing and accidentally got strangled.

Key words: Mechanical asphyxia, Occupational hazard, Farm equipment, Autopsy, Strangulation

Introduction

India is an agricultural country, where agricultural workers play an essential role. They operate animal-drawn equipment, tractors, power tillers, self-propelled and power-operated machines. The population of agricultural workers in the country is about 242 million, of which 50% are female workers¹. Thus, there is a significant role of farmworkers in the country’s agriculture. Attention needs to be given to their safety and occupational health issues to have higher productivity and fewer accidents. Strangulation is a form of mechanical asphyxia by compression of the neck with an outside force either by a ligature or hands, in which a constricting force other than the weight of the body is directly applied². Such deaths are generally considered homicidal unless proved otherwise³. Here we present a case with compression of the neck and crush injuries, where the victim got accidentally strangled in a rice huller machine.

Case Report

A 60-year-old female, collecting husk from the rice huller machine, got trapped in it with the saree she was wearing which resulted in accidental strangulation along with near total amputation of the left upper limb. She succumbed to the injuries on her way to AIIMS, Bhubaneswar, where she was declared as brought dead. Medico-legal autopsy was conducted on the same day of her death. On external examination, a friction abrasion with contused margins was present over the anterior aspect of the neck, starting at a point that was 11 cm below the left mastoid and running downward in an oblique fashion crossing the thyroid cartilage and reaching on the other side of the neck at a point which was 10 cm below the right angle of the mandible (Figure 1). The maximum width of the abrasion was 4 cm which was at a point adjacent to the left side of the thyroid cartilage, and the total circumference of the abrasion was 21 cm. A layered dissection of the neck revealed contusions in the subcutaneous plane and strap muscles of the neck corresponding to the external mark of compression. The thyroid cartilage, hyoid bone, carotid, and jugular vessels were intact. The deceased had sustained a crush injury of the left upper arm with visible humerus bone (Figure 2). A laceration (17 cm x 8 cm skull deep) was present over the scalp in the right temporal region, starting at a point, 12 cm above the right mastoid, moving anteriorly and reaching at a point which was 7 cm medial to right parietal eminence. Margins of the laceration were contused.

Dr. Kimi Soumya Padhi:  
Junior Resident  
Email: kimi.soumya@gmail.com  
Department of Forensic Medicine and Toxicology, 3rd Floor, Academic Block, AIIMS, Bhubaneswar
(Figure 3). There was a grazed abrasion (38cmx 18cm) over the left back involving the inferior axillary area of the left side. On opening the thoracic cavity, chest wall on the left side was contused. The body of the sternum was fractured in the middle with a substernal hematoma of 60mg. On the left side, 2nd to 6th ribs were fractured in the midaxillary line and lung was collapsed with multiple perforations, the left pleural cavity contained one liter of fluid blood. The cause of death was opined to be hemorrhage and shock arising out of multiple injuries sustained, which could be fatal even if present alone in the ordinary course of nature.
Discussion

The accidental strangulation associated with agriculture happens due to entrapment of wearing apparel like stoll, gumcha (towels), and saree which are common in Indian wear in the crop thrashers, rice huller machines, etc. Zine et al\(^4\) reported ligature strangulation by Indian garment saree caught by crop thrasher with injuries to neck and avulsion laceration of scalp, which is quite similar to our findings in the reported case. Parchake et al\(^5\) presented a case of accidental ligature strangulation around the neck, which described the possible mechanism could be because of the rotating motion and pulling action of machineries. Arun et al\(^3\) emphasized on adoption of fitting a guard to prevent entanglement of clothing in moving belts as well as avoiding loose apparel while working in close proximity. Souza et al\(^6\) described the mechanism of death depends on two factors: the amount of resistance made by the victim and the amount of force of the moving machine. Verma et al\(^7\) mentioned strangulation can occur accidentally if someone moves into the vicinity of a moving object without caution. In the present study the findings are consistent with the studies mentioned above.

Conclusion

Education and training to the population involved in agricultural practices dealing with various types of types of machinery is an essential requirement to prevent occupational hazards. Encouragement for the use of safety gear like helmets, body fit clothing, rubber boots, etc. The concern for us here is that the vicinity of these operating equipment should be taken care of in order to avoid preventable accidents.

Conflict of Interest: None

Source of Funding: None

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References

Self-Administration of Vecuronium by a Medical Student: An Autopsy Case Report

Mukul Sharma¹, Biplab Rath², Naveen A¹, Manas Ranjan Sahu³
¹Senior Resident, ²Junior Resident, ³Associate Professor Department of Forensic Medicine and Toxicology, AIIMS Bhubaneswar

Abstract
During COVID-19 pandemic the frontline healthcare workers have been at risk the most. Long working hours, emotional stress from not seeing families for days together takes a toll on the psychological well-being of a person. This leads to increase incidence of self-harm amongst the individuals. Vecuronium bromide is a muscle relaxant, easy availability of such drugs amongst the medical professionals is a matter of great concern. Monitoring and cautious dispensing of them should be done. Here we report a case of self-administration of vecuronium by a medical student. The purpose of documenting this case is to create awareness among healthcare workers to keep a watch on mental health of fellow colleagues. Psychological support groups in medical college hospitals are the need of hour.

Key words: Vecuronium, Self-Administration, Medical Student, COVID-19

Introduction
Vecuronium is a mono-quaternary ammonium compound. It works by competitively inhibiting acetylcholine at post junctional nicotinic cholinergic receptor sites. It is used worldwide as muscle relaxants prior to intubation and surgical procedures. Though not available as over-the-counter medication, but it is easily available in the operation theatres worldwide. The easy availability and simple administration technique make it a potential drug of abuse amongst the medical and paramedical staff. COVID-19 times have been emotionally stressful for all people¹. Healthcare professionals are not an exception. This has led to widespread use of easily available drugs. Vecuronium is a steroidal, mono-quaternary ammonium compound acting as a non-depolarising muscle relaxant. It competitively inhibits acetylcholine at post junctional nicotinic cholinergic receptor sites. It has intermediate duration of action, so used widely during intubation. Patient recovers about 95% within 45 minutes to 65 minutes. It is available as a lyophilised powder. Adverse drug reactions are rare and seen in 1% of the cases². It is mainly excreted via bile and urine. Due to causation of the respiratory insufficiency caused by vecuronium, it should be administered cautiously and only by experienced doctors and nurses. The respiratory depression is the main cause of death in cases of toxicity. Here we present autopsy findings of a case of self-administration of vecuronium by a medical student.

Case Report
We received a case of a 26-years-old medical student with an alleged history of vecuronium injection. On investigation of crime scene, it was found that the room was locked from inside and a syringe along with an empty vial of vecuronium (Brand NEOVEC-10) was retrieved. Upon taking history, we came to know that the deceased had completed his duty at COVID-19 ward
following which he contracted COVID-19. This had caused a great emotional trauma and was depressed for which he was taking psychiatry consultation.

External examination of the deceased showed that the face was congested. The conjunctiva of both the eyes was deeply congested. Nail beds were bluish. Seminal fluid discharge was seen from the penile tip. Multiple injection puncture marks were seen over the left cubital fossa.

On dissection, the lungs were deeply congested and oedematous. (Fig. 1). Right lung weighed 1200 grams and the left lung weighed 880 grams. The stomach contained 500 grams of partially digested food materials with no appreciable odour. Mucosa was deeply congested and Submucosal haemorrhage was present diffusely. All the internal organs were congested. Skin along with subcutaneous tissue taken from punctured wound site, blood and routine viscera comprising of stomach, small intestine, liver and half of each kidney were collected and sent for chemical analysis at State Forensic Science Laboratory.

Chemical analysis of the skin along with subcutaneous tissue revealed presence of vecuronium which was also found in blood. The technique used for determination was High Performance Liquid Chromatography. The routine preserved viscera report was negative for presence of any toxic compounds.

The final opinion regarding cause of death was stated as respiratory failure as a result of self-administration of Vecuronium.
Discussion

COVID-19 pandemic times have been stressful to all, but the frontline healthcare workers have been the most at risk. Long working hours, emotional stress from not seeing families for days together takes a toll on the psychological well-being of a person\(^1\). This has led to increase incidence of self-harm amongst the individuals. Vecuronium is an easily available drug to the healthcare workers which leads to its misuse \(^3\)-\(^4\). Very few cases have been reported in literature regarding suicides by vecuronium and most of them are of healthcare workers\(^4\)-\(^5\). Post-mortem quantitative analysis of vecuronium and its active metabolite concentration is important for arriving at the conclusion\(^6\). Though suicides using vecuronium is uncommon, but suicides by using similar compounds have been stated in literature\(^7\)-\(^8\).

This case during the COVID-19 times gives an insight about the stress both emotional and physical which a health care worker endures while performing his duties. There is now a dire need of psychological support system to be in place for the healthcare workers to prevent such instances. The easy approachability of the drugs should be controlled. The need for prompt treatment and research into safer alternatives is the need of the hour.

Conflict of Interest- None

Source of Funding- None

Ethical Clearance: Taken from Institutional Ethical Committee, AIIMS Bhubaneswar. The identity of the deceased was not revealed in the manuscript.

References

Copper Beaten Skull: An Incidental Finding in Autopsy

Mukul Sharma1, Sasank Shekhar Maharik2, Sarthak Aeron3, Biplab Rath4, Manoj Kumar Mohanty5

1Senior Resident, Department of Forensic Medicine and Toxicology, AIIMS Bhubaneswar, 2Assistant Professor Department of Forensic Medicine and Toxicology IMS and SUM Medical College, Bhubaneswar, 3Senior Resident, Department of Forensic Medicine and Toxicology, AIIMS Jodhpur, 4Junior Resident, 5Professor and HOD, Department of Forensic Medicine and Toxicology, AIIMS Bhubaneswar

Abstract

The Sutures in the human skull are zones of separation between skull bones, which also allows the skull bones to grow in a fashion along with increase in size of brain, early closure of sutures (craniosynostosis) may lead to increase in intracranial tension and it pushes the soft skull outward which leads to undulating patterns formation in inner plate of skull called as silver beaten or copper beaten skull. Here we document an incidental finding of copper beaten skull pattern in a case of road traffic accident brought to Mortuary of AIIMS Bhubaneswar.

Keywords: Copper beaten skull, silver beaten skull, skull sutures, autopsy.

Introduction

Variations and anatomical anomalies of skull are usually related to primary maldevelopment of the brain.1 ‘Copper-beaten’ skull refers to the projecting convolutional marks found in inner table of multiple bones of the skull. Underlying cause is thought to be related to increased intracranial pressure resulting from such processes as craniosynostosis, obstructive hydrocephalus and/or intracranial masses.2 The growing brain yields a constant pulsatile pressure on the soft cranium, creating a gyral pattern on the inner table. This appears as ‘copper-beaten skull’ on plain skull X-rays. We report an incidental finding of Copper Beaten Skull which we came across during autopsy of an road traffic accident case brought to mortuary of AIIMS Bhubaneswar.

Case Report

We received a case of Road Traffic Accident victim who had died during treatment six hours before the autopsy. On taking history from the relatives, we came to know that he was mentally retarded since childhood. On day of incidence he was travelling as a pillion rider when the motorcycle met with the accident. He suffered head injuries and died 7 days after.

Upon external examination, corpse of a 18 year old male with evident dwarfism .The total body length was 136cm and 52kg in weight. There was subconjunctival haemorrhage on left side. Evidence of bleeding from right ear in form of dried blood was present. All the teeth had braces to fix them in shape. Stapled surgical incision mark was present on left parieto-temporal region underneath of which sub-scalpal hematoma was present. Multiple fractures of facial bones were present. There was Multiple abrasions and contusions were present over the body. There was a palpable left femur fracture.

Upon removal of the skull cap, we noticed multiple gyral impressions over the inner table of the skull bones. The impressions were also present over the skull base. There was comminuted fracture of the frontal bone

Corresponding author:
Dr. Mukul Sharma:
Senior Resident, Department of Forensic Medicine and Toxicology, 3rd floor, Academic Block, AIIMS, Bhubaneswar, Email- mukul.med@gmail.com
and roof of left orbit. Skull base was fractured in right middle and posterior cranial fossas. Left frontal lobe was lacerated. There was transection of brainstem. The cause of death was attributed to head injury and its complications.

Figure: On opening the skull, the inner table of skull showing - COPPER BEATEN pattern.

Discussion

Copper beaten appearance of the skull is the least common appearance found in craniosynostosis of evolving skull.\textsuperscript{1,3} The pattern was firstly presumed to have been caused from chronically raised intracranial pressure but currently widely thought to be a reproduction of normal brain growth on the inner table, without pathological significance.\textsuperscript{3} The markings are most prominent during periods of rapid brain growth that is between age 2 - 3 years and 5 - 7 years. They become less apparent after about 8 years of age.\textsuperscript{4}

Diffuse, severe beaten-copper pattern is an indicator of chronic elevated intracranial pressure, which is more common in patients with craniosynostosis.\textsuperscript{5,6} Van Der
Meulen et al. concluded that the occurrence of the copper-beaten skull pattern did not significantly affect long-term intelligence levels⁴. Though, there could be a negative effect on more subtle areas of development, like reading, spelling and behaviour.⁴ These convolutional patterns should be distinguished from Lückenschädel (lacunar skull), which is due to mesenchymal dysplasia of calvarial ossification. It is characterised by many oval lucencies of the inner table and diploic space. These are generally present at birth and are related with meningocoele/myelomeningocele/encephalocoele, spina bifida, cleft palate and Arnold Chiari II malformation.⁶ It is most prominent in the parietal and occipital bones. Seldom these findings are normal and disappear by 6 months of age.¹ Incidental finding of copper beaten skull in adult without pathological ICP rise is rare.⁷

Though an incidental finding we cannot wholly disagree on the fact that the copper beaten appearance of the inner table of skull presents lot of surfaces to the brain to have friction during a trauma to head like in case of head injury. This could be maximising the trauma caused to the brain. The hypothesis put forth need more studies for correlation.

**Conflict of Interest-** None

**Source of Funding-** None

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**References**

Effects of Ascorbic Acid on Insulin Resistance in Hyper Insulinemic and Euglycemic Persons

Muntadher H. Dawood¹, Taha H. Al-Yasiri²

¹ Lect., Department of Pharmacology and Toxicology, College of pharmacy, misan University, Iraq,
² Lect., Department of Sciences Basic College of Education, University of Misan Misan, Iraq

Abstract

This experiment was conducted in the city of Amara in southern Iraq at Al-Sadr Hospital. The study aimed to evaluate the effect of administering Ascorbic Acid on 50 diabetic patients who had insulin resistance after they were diagnosed clinically. 25 patients were treated with Ascorbic acid at dose 500 mg twice a day for 12 days and the others 25 were considered as control.

The study group demonstrated a significant decrease in the fasting patient’s blood sugar (F.B.S), insulin and fasting blood, and Insulin resistance at the end of 12 weeks (P<0.05) as compared to baseline measurements. The reduction in F.B.S, Fasting Insulin, and Insulin resistance was significantly reduced in the ascorbic acid group at week 12 of the study relative to the control group (P <0.05).

Keywords: Ascorbic acid, Insulin, diabetic, Blood sugar, Fasting

Introduction

Insulin resistance is characterized by weakness of the biological responses for target tissue, mainly adipose tissue, liver and muscle, to insulin stimulation. Insulin resistance has inhibited elimination of glucose, resulting in a compensatory rise in the insulin production from beta-cell and results hyperinsulinemia. [1][2] Hyperglycemia, hypertension, dyslipidemia, visceral adiposity and hyperuricemia, elevated inflammatory signs and impairment of the endothelial function, and prothrombic state can result in the metabolic sequel of insulin resistance which lead to metabolic syndrome, diabetes mellitus type 2 and non-alcoholic liver fat (NAFLD). [3,4]

Type 2 diabetes is the primary result of insulin resistance (T2DM). Resistance to insulin is thought to before T2DM production by 10 to 15 years. high level in the production of endogenous insulin results from growth compensatively of Insulin resistance which is associated with weight gain that occurs with an increase in the level of anabolic hormones (endogenous insulin) is associated with insulin resistance in turn, aggravate insulin resistance [5] This harmful cycle continues until the beta-cell function of the pancreas is unable to produce the insulin that meets the demand caused by insulin resistance, leading to hyperglycemia. With a The constant discrepancy observed between the demand for insulin from the body’s cells and the production of insulin, blood sugar levels elevate to levels compatible with type 2 diabetes mellitus T2DM. [6]

Vitamin C is an antioxidant vitamin with water-soluble properties and an essential cofactor in the synthesis of carnitine, collagen and catecholamine metabolism and serves to absorb dietary iron. A person cannot synthesize or produce vitamin C; Therefore, they can only get it through food such as eating fruits and vegetables. [7]

Corresponding Author.
Muntadher H. Dawood
mntzrhwn2@gmail.com
Citrus fruits, bananas, and green leafy vegetables such as onions and potatoes are a rich source of vitamin C, which is fully absorbed from food in the small intestine, and its absorption decreases with increasing concentration in the lumen of the intestine.[8] The residues of Proline amino acid that are found on procollagen allow vitamin C to be hydroxylated, making it possible to form a triple-helix for mature collagen. [9]

Skin integrity, blood vessels, bone, and mucous membranes, are impaired by the absence of a healthy triple-helical structure. Scurvy is the sequel that results from vitamin C deficiency which presents with hematological abnormalities, hemorrhage and hyperkeratosis [10]

**Aim of Study**

The aim of the trial was to evaluate the effects of vitamin C administration on insulin resistance parameters in subjects with indications of increased insulin resistance.

**Materials and Methods**

**Study design:**

The current research was performed at Al-Sader Teaching Hospital on 50 individuals with hyperinsulinemia and hyperglycemia (31 males, 19 females) aged 30-65 years. The patients were clinically diagnosed as having insulin resistance by the doctor. Criteria for the HOMA model-based diagnosis of insulin resistance.

In order to determine the effectiveness and ability of the medication used (vitamin C) in the study to change the habit of changing due to insulin resistance to the correct normal and to understand the true values of the research criteria, another 20 patients should be considered, in addition to (50) patients.

**Patients:**

In this pilot study, 25 patients were treated with ascorbic acid and changed lifestyle and diet control for 12 weeks. (500 mg), twice daily plus 12 weeks with dietary control and lifestyle changes.

**Sample Collection and Preparation:**

Blood samples were obtained from all patients and healthy people after about 12 hours of fasting by taking a sample of venous blood (10 ml), before starting drug therapy (vitamin C) and (at zero) and after 12 weeks of treatment and then changes in standards examined, blood patient samples were collected in spatial tubes, and after being centrifuged with the device at (3000) rpm for 10 minutes at 4 °C.

In Eppendorf tubes, the plasma fraction obtained was divided into two sections and stored frozen before analysis was conducted.

**Measurements:**

**Fasting blood sugar Level (FBS):**

Using a ready-made kit for this reason, serum glucose level was assessed according to the technique [11]. Based on glucose enzymatic oxidation to form hydrogen peroxide and glucuronic acid, accompanied spectrophotometrically at 505 nm by the subsequent reaction with phenol and formation of quinonimine. The results obtained were expressed as mg / dL, which corresponded to a normal glucose solution, which was treated with the same treatment.

**Serum Insulin levels:**

The Demeditec insulin ELISA, based on the sandwich concept, is an ELISA solid phase.

The microtiter holes are coated on the insulin molecule with antibody monoclonal directed toward a partially on site of antigenic with enzyme conjugate in the coated holes. A portion of the patient’s sample containing internal insulin was incubated with anti-insulin antibodies linked with amino acid (biotin). The unbound conjugated antibodies were then washed from the hopper after they were incubated. Through the second incubation period cycle the Streptavidin-Peroxidase-Enzyme linked to the antibody of the biotin.

The mounting of the horseradish peroxidase (H.R.P) complex is relative to the sample insulin concentration.
The strength of the color obtained after adding the substrate solution is in proportion to the amount of insulin concentration in the patient blood sample. The binding potential was expressed as uU/ml. The HOMA Model is a very simple and practical method for assessing insulin resistance and for testing insulin sensitivity and represents a type of glucose reaction. This assay was then used to determine stable fasting glucose and insulin levels for a variety of insulin-resistant cell function groups. Both primary HOMA and modified HOMA2 are thought to be a feedback loop from the liver to the cell.

[12,13]

Insulin-dependent HGP controls glucose concentrations, while the levels of Insulin to glucose depend on the reaction of the pancreatic B cells. Deficient function of β-cell thus indicates a decreased reaction of β-cells to glucose-induced insulin secretion. Likewise, the diminished suppressive effect of insulin on HGP is expressed in insulin resistance. This glucose insulin homeostasis is defined by HOMA via a series of nonlinear equations derived empirically. For any interference in pancreatic cell activity and insulin sensitivity, the model used predicts steady levels of glucose and insulin in the blood. (14) For a fasting plasma analysis study that includes a constant and tests glucose (fasting plasma glucose; FPG) and insulin (fasting plasma insulin; FPI) the IR approximation equation has been simplified the product of FPG×FPI/HOMA-IR = (glucose × insulin)/405 is the index of the IR.

It tests the concentration of insulin in uU/ml and glucose in mg/dl. A normalizing factor is the 405 constant, i.e. a normal FPI of 5 uU/mL 81 mg/dl normal FPG usual for a ‘normal’ healthy person = 405. Therefore, HOMA-IR =1 for a person with ‘normal’ insulin sensitivity.

Statistical Analysis

The dual t-test was used to compare the values of the result that taken from each group before and after treatment of ascorbic acid administration, and separate control t-tests were used to compare the values obtained for all patients and healthy subjects. In several comparisons to compare between groups in a variables differences before and after 12 weeks of treatment, post-hoc LSD variance analysis (ANOVA) was used. The data is provided in the form of means and standard deviations (SD).

Using a two-tailed test, P<0.05 was considered statistically relevant for all statistical analyses. Statistical data analysis was conducted using software version 16.0 of the Statistical Program for Social Sciences.

Results

Comparison of insulin resistance subjects and healthy subjects with respect to various parameters:

Mean± SD was determined for F.B.S, Fasting Serum. Insulin and Insulin reluctant, respectively, in apparently stable and patient groups, as shown in the following table. The baseline characteristics of the population of healthy and prediabetic patients were compared using the unpaired t-test. showing substantial differences in tolerance to F.B.S, Fasting S. Insulin and Insulin (p<0.001). As defined in the table.

As compared to baseline tests, the research group reported a substantial lowering in F.B.S, Fasting Insulin, and Insulin Resistance in the 12 weeks of the end of experiment (P0.05). At week 12 of the trial, the ascorbic acid group had a significantly lower F.B.S, Fasting Insulin, and Insulin resistance than the control group (P0.05).
Table (1): The effect of research therapy on the study group after 12 weeks of therapy (fasting blood sugar, fasting insulin and insulin resistance) and multiple comparisons of the change from baseline

<table>
<thead>
<tr>
<th>Group parameters</th>
<th>Control</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ±SD</td>
<td>mean ±SD</td>
</tr>
<tr>
<td>F.B.S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>119.22</td>
<td>118.30</td>
</tr>
<tr>
<td>12week</td>
<td>108.30*</td>
<td>88.71**</td>
</tr>
<tr>
<td>ΔF.B.S</td>
<td>-10.92</td>
<td>-29.59a</td>
</tr>
<tr>
<td>Fast.In</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12.32</td>
<td>12.08</td>
</tr>
<tr>
<td>12week</td>
<td>11.11*</td>
<td>8.51**</td>
</tr>
<tr>
<td>ΔFast.In</td>
<td>-1.21</td>
<td>-3.57a</td>
</tr>
<tr>
<td>In.resi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.68</td>
<td>3.46</td>
</tr>
<tr>
<td>12week</td>
<td>2.96*</td>
<td>1.76**</td>
</tr>
<tr>
<td>ΔIn.resi</td>
<td>-0.72</td>
<td>-1.70a</td>
</tr>
</tbody>
</table>

* = statistically significant (P<0.05) difference in the paired t-test after 12 weeks relative to the baseline.

** = statistically significantly important (P<0.001) difference in the paired t-test after 12 weeks relative to the baseline.

Figure (1): Fasting Insulin before and after 12 weeks of the study treatment
Figure (2): Fasting Insulin before and after 12 weeks of the study treatment

Figure (3): Changes in Fasting Insulin from the baseline to 12 weeks of the study
Discussion

As compared to baseline measures at the end of 12 weeks, the experiment showed a substantial lowering in F.B.S, Fasting Insulin, and Insulin Resistance (P0.05). Vitamin C is an important water-soluble micronutrient. It is usually used traditionally for the prevention and treatment of scurvy. Vitamin C called sorbate or ascorbic acid has a universal role in both plants and animals. New fruits and vegetables are the main dietary sources of vitamin C. Based on many factors, vitamin C has been proposed to be helpful in reversing MetS-related abnormalities. Body mass index (BMI), body fat percentage and waist circumference were inversely correlated with plasma vitamin C concentration.\textsuperscript{15}

Important reductions in debits (blood glucose ) 16.5, BP 18.25, TG and LDL-C 18.5 resulted from supplementation with vitamin C. Moreover, vitamin C is a potent antioxidant since it serves as a reducing agent that prevents oxidation of other compounds.\textsuperscript{16}

Vitamin C scavenges harmful free radicals by contributing electrons, resulting in the stable and non-reactive ascorbyl radical.\textsuperscript{17}

Vitamin C’s ability to reduce oxidative stress has been documented in previous studies. Vitamin C affects neutrophil chemotaxis in response to inflammatory mediators, improves neutrophil microbe phagocytosis, and promotes macrophage neutrophil clearance, both of which help to reduce inflammation.\textsuperscript{18,19}

Conclusions

It is easy to infer that vitamin C administration will enhance glycemic control with beneficial effects on insulin as a result. resistance in hyper-insulin and hyperglycemic individuals, according to the findings presented in this report.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required
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Relation between Level of Some Immunological Markers and Liver Functions with Respiratory Bacterial Infection in Sheep

Mustafa Ghazi Saeed¹, Bashar Sadeq Noomi², Entedhar Rifaat Sarhat³

¹MSc. Student, ²Asisst.prof., Dept. of Microbiology, Collage of Veterinary Medicine, University of Tikrit, Tikrit, Iraq, ³Prof., Department of Basic Medical Science, Dentistry College, University of Tikrit, Tikrit, Iraq

Abstract

Sheep pneumonia remains an important cause of morbidity and mortality worldwide. The aim of the study report to determine Interleukine-6, IL-10, and liver function test, and kidney function test of sheep suffering from respiratory disease. Blood samples were collected from the diseased and healthy sheep during field study, for biochemical analysis. The results of 50 diseased animals were compared with results of 50 healthy animals. Data of selected biochemical parameters implicated a significant increase in Interleukin-6, alanine aminotransferase and aspartate aminotransferase, and Gamma glutamyltransferase in the diseased group compared to the healthy sheep’s. On the other hand, a significant decrease was seen in the levels of IL-10, lactate dehydrogenase and total protein in the diseased group compared to the control. The results of this study could conclude that respiratory infections in sheep were associated with significant biochemical and immunological alterations which upon understanding can provide us with rapid diagnostic tools for pneumonia process in sheep and thus lead to better management and proper treatment. Future research is warranted to determine the response of individual animals to this disease and to describe the metabolic changes which occur during the course of respiratory diseases.

Keywords: Sheep, Pneumonia, blood, clinical biochemistry

Introduction

Sheep represent an important aspect of livestock in Iraq and the Arab world, the number of sheep in the Arab world is more than 105 million, while the number of sheep in Iraq is estimated at about (9) million (¹). It is a great wealth that deserves attention, care and development. Sheep constitute a major source of meat in the Arab world about 33.3% of the total meat, as is the case in developed countries. In addition to being an important source of 16.74% milk, leather and wool (¹). The diseases caused by many microorganism has a negative role in limiting the prosperity and growth of livestock in most countries of the world, including Iraq. This is due to the economic losses resulting from the death of large numbers of animals and obstruction of their normal growth and the decrease in their production, as well as the costs of control and treatment (²). Bacterial infections are widespread among sheep flocks widely, and at the forefront of this comes the infection of respiratory infections due to open breeding of sheep flocks provides direct and indirect contact among them and other animals for the transmission of these infections between different animals, and epidemiological and environmental factors play an important role in this field (³). Many epidemiological surveys indicated that sheep are exposed to many infections especially those that infected the respiratory system (⁴). These injuries also affect animal health, represented by weight loss, lack of milk production, poor wool, poor birth control and decreased reproductive efficiency, as well as economic damage from veterinary health services and the effort exerted for that (⁵). Consequently, infection with these germs can cause severe anemia in affected animals, loss of appetite, weight loss and a decrease in wool growth that in some causes leads to the animal’s death. Young animals are more susceptible to infection while larger
animals are susceptible to resistance to some types of disease\(^6\).

Because of absence of a comprehensive study on the epidemiology and infection of respiratory infections in sheep in the Tikrit province, the purpose of this study was to determine Interleukine-6, IL-10, and liver function test, and kidney function test of sheep suffering from respiratory disease.

**Materials and Methods**

In the current study we used sheep that suffers from respiratory signs represented by coughing, sneezing, and respiratory secretions in the same time the temperature, pulse and respiratory speed has been measured and other pathological signs associated with respiratory injuries such as enlarged lymph nodes or joints, diarrhea or others were recorded. 60 ewes were used which were divided into two groups. The first group consisted of 30 healthy ewes that were considered as a control group, while the second group consisted of 30 ewes with respiratory diseases that represented the studied group.

**Blood samples**

Blood samples were obtained from the jugular vein of healthy and infected sheep using test tubes that do not contain anticoagulants to study some biochemical parameters in the serum. It was left at room temperature for 20 minutes, then a centrifuge was used at 3000rpm for 10 minutes for the purpose of separating the serum and then kept in the Apendorf tubes for the purpose of conducting biochemical analyzes.

The IL-6, and IL-10 were measured by using the commercial enzyme-linked immunosorbent assay (ELISA) kits. Serum AST, ALT TP, albumin, GGT levels were measured by spectrophotometric kit.

**Statistical Analysis**

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Independent t test was used to assess significant differences between means. P < 0.05 is considered statistically significant.

**Results**

In Tables 1, 2 & 3 values of serum biomarkers are expressed as mean and mean of standard error (mean ± SEM) of the measured biochemical and immunological parameters in healthy and diseased sheep.

The obtained results revealed that a significant increase in IL-6 162.49±16.04 vs 71.42±6.16 pg/ml: <0.01 values while decreased in IL-10 (3.24±0.42 vs 5.39±0.65 pg/ml: <0.01) were observed in the diseased group compared to the healthy group as shown in Table (1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Serum</th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>71.42±6.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>5.39±0.65</td>
<td>3.24±0.42</td>
<td></td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The mean value of ALT, AST, LDH, and GGT were significantly increased (P<0.01) (127.58±4.72 vs 84.82±4.91:<0.01), ALT(37±3.5 vs 20.13±2.21:<0.01), (61.07±5.23 vs 19.43±2.14:<0.01) whereas LDH(113.78±7.78 vs 781±49.86:<0.01) significantly reduced in diseased pneumonic sheep than healthy ones (Table 2).
Table 2: Means (± SE) of ALT, AST, LDH, and GGT for two sheep groups (diseased and healthy).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Serum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diseased</td>
<td>Control</td>
</tr>
<tr>
<td>AST</td>
<td>127.58± 4.72</td>
<td>84.82±4.91</td>
</tr>
<tr>
<td>ALT</td>
<td>37± 3.5</td>
<td>20.13±2.21</td>
</tr>
<tr>
<td>LDH</td>
<td>113.78±7.78</td>
<td>781±49.86</td>
</tr>
<tr>
<td>GGT</td>
<td>61.07±5.23</td>
<td>19.43±2.14</td>
</tr>
</tbody>
</table>

There was a significant decrease (P<0.01) in the concentration of total proteins in infected (5.08±5.74 g/dI) as compared with healthy (9.11±0.59) and decrease in albumin (2.74± 0.45 vs 3.97±0.20 g/dl) (P<0.01) in diseased pneumonic sheep than healthy ones (Table 3).

Table 3: Means (± SE) of total proteins, albumin for two sheep groups (diseased and healthy)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Serum</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diseased</td>
</tr>
<tr>
<td>Total proteins (g/dl)</td>
<td>9.118±0.59</td>
<td>5.08±0.57</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.74± 0.45</td>
<td>3.97±0.20</td>
</tr>
</tbody>
</table>

Discussion

Cytokines are a heterogeneous group of soluble small polypeptides with a wide range of inflammatory, metabolic and immunomodulatory properties and tissue homeostasis, that play a critical role in mediating inflammatory processes and tissue homeostasis underlying pneumonia\(^{(7,8)}\).

Serum biochemical alterations in respiratory diseases were common and might display reasonably predictable changes in response to inflammation. In this regard, the results of the our study revealed higher serum levels of IL-6 in the diseased group versus control group, suggesting the implication of systemic inflammation in diseased groups.

Interleukin-6 (IL-6) is a multifunctional inflammatory cytokine produced in response to tissue damage and infections. Multiple cell types including fibroblasts, keratinocytes, mesangial cells, vascular endothelial cells, mast cells, macrophages, dendritic cells, and T and B cells are associated with the production of this cytokine. After targeting its specific receptor\(^{(9,10)}\),

In an animal trial, IL-6 was shown to affect megakaryocytopoiesis, leading to bigger platelets released into the circulation\(^{(11)}\). The mechanism of action of IL-6 on platelet size in pneumonia is unclear.

Interleukin 10 (IL-10) is considered an immunoregulatory cytokine produced by monocytes, macrophages, B and T lymphocytes, brain cells such as neurons and microglia that capable of inhibiting proinflammatory responses. Despite the previous fact,
IL-10 is a pleiotropic cytokine that can exert either immune-stimulatory or immunosuppressive properties on many cells\textsuperscript{(12-14)}. In the present study, the IL-10 in affected sheep in farm (A) showed significant decrease which was similar to Fernández et al.\textsuperscript{(15)} who reported a significant interaction between the microorganisms and cytokine markers that leads to significant decrease in IL-10 in diseased group.

Liver is one of the largest organs in the body. It has many important metabolic functions. Liver tissue has a relatively large amount of enzymes activity and produces inflammatory cytokines\textsuperscript{(16,17)}. Data obtained from the present study implicated a significantly high serum enzymatic activity of AST, ALT, ALP in the diseased group. These changes could be changes in the antioxidant abilities that occurred in liver, may be attributed to cellular damage in the liver alters their transport function and membrane permeability, leading to leakage of enzymes from the cells. For it, the marked release of AST and ALT from liver cytosol into circulation refers to the extensive damage of hepatic tissue membranes\textsuperscript{(18,19)}, which attributed to dysfunction of various organs including liver due to hepatic degenerative and necrotic changes caused by bacterial infection and toxins. Similar to findings observed by Hassan HY\textsuperscript{(20)}, El-Deeb et al.\textsuperscript{(21)}, and Donia et al.\textsuperscript{(22)}. Furthermore, a significant increase in the ALP may be resulting from the elevated biliary pressure and acute cell necrosis caused the release of ALP from its membranes bound site and its entry into blood\textsuperscript{(23)}. LDH is one of the potential variables presented in the literature as a possible indicator of lung damage. Increased LDH activity in sheep in the diseased group probably resulted from increased respiratory rate and muscle work during prolonged duration or severe cases of respiratory disease\textsuperscript{(24)}. Similar finding were recorded by Nagy et al.\textsuperscript{(25)}.

As markers of renal function, serum concentrations of creatinine and blood urea were measured. Creatinine is a breakdown metabolite of creatine phosphate in muscle. It is eliminated by kidneys, mostly via glomerular filtration and partly via secretion at proximal tubule. Therefore, creatinine clearance could be used to estimate the glomerular filtration rate (eGFR) by measuring serum creatinine (SCr), urine creatinine, and volume. Additionally, SCr can be a reflection of muscle mass and diet\textsuperscript{(26)}. Blood urea nitrogen (BUN) is a common marker of kidney function, and hence its blood concentrations vary according to change in glomerular filtration rate (GFR). BUN levels are increased by the catabolism of lean body mass and lower levels of BUN may be a marker of the cessation of catabolism, which is certain for respiratory muscle recovery and a well-known predictor of weaning\textsuperscript{(27)}.

The results revealed that the mean values of serum creatinine and urea blood urea concentrations were significantly higher in the diseased ewes compared to control. The obvious implication of this observation is that, the high level of serum urea has been attributed to excessive tissues protein catabolism associated with protein deficiency and could be explained also by the accelerated catabolism of body protein and could result as a response to infection. The present study demonstrate that the increasing level of serum creatinine in diseased group than control group 1.35&1.09 mg/dl respectively. High creatinine is indicative of poor protein and amino acid metabolism that can lead to impaired renal function or might be attributed to kidney dysfunction after infection, which would be promoted by a reduction in the glomerular filtration rate\textsuperscript{(28,29)}. Creatinine is formed by the degradation of phosphocreatine for energy release in the skeletal muscle\textsuperscript{(30)}.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** Obtained from Institutional ethical committee

**References**


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A Protocol of Low Dose Chest CT to Verify Suspected COVID-19 infection

Muthana Hussien Mohan¹, Mahmood Radhi Jobayr², Salam Mohmad Joori¹

¹Research Scholar, Medical City Complex, X-Ray Institute, Baghdad, Iraq, ²Professor, Middle Technical University (MTU), College of Health and Medical Technology, Dept. Radiology Technology, Baghdad, Iraq

Abstract

Chest CT scanning is significant in verifying COVID-19 infection in patients with clinical symptoms, although CT scanning uses ionizing radiation. So we aim to investigate the demonstration of relevance and accuracy of chest tomography using a low-dose protocol compared to the standard auto-modulation protocol of 50 – 210 mAs. Moreover, to assess whether any distinguishing signs in patients might help determine the severity of the respiratory infection from COVID-19. A standard auto-modulation protocol of 50 – 210 mAs was performed for all patients (120 patients), Rotation time 0.5 second. Of the 120 patients whose CT scan showed signs of COVID-19 infection, 47 patients aged over 50 years were selected. Low dose CT scanning (30 mAs) was performed on these patients immediately. Of the 120 patients over the age of 50 with COVID-19 according to a PCR underwent a CT chest, only 47 showed signs of COVID-19. When compared to standard-dose, low-dose demonstrated excellent sensitivity in detecting typical findings of COVID-19 (coefficient of significance (C.S) = 0.98–0.99, P-values < 0.05 one reader and P-values < 0.01 two readers). The mean effective dose values were 6.32±2.82 and 1.45±0.29mSv in the stander and low-dose, respectively. A low dose protocol can be used with high reliability and accuracy to reduce the ionizing radiation risks in the state of reproducible assessment. On the other hand, we emphasize there are indicators with high reliability of a relationship between infection with COVID-19 and its severity and the work nature, environment, age, body mass index (BMI) and Patient Health Status.

Keywords: COVID-19, Chest CT, Computed tomography, Low dose.

Introduction

This research was performed in an Iraqi hospital following the outbreak of COVID-19, which has surpassed the highest number of cases since disease registration began in Iraq (500000). As it is known, the first cases of COVID-19 patients were recorded in Wuhan, China, in December 2019. Since then, the disease has spread like a pandemic in various countries of the world. The number of confirmed cases has reached more than eighty million cases all over the world, according to the statistics of the World Health Organization, and the number is constantly increasing¹. It is possible to limit the rapid spread of the COVID-19 and reduce deaths to a minimum through a set of measures represented by early detection of infection, appropriate intervention and commitment to appropriate preventive measures ². Unenhanced chest CT may be considered for early diagnosis of viral disease, though “real-time polymerase chain reaction (RT- PCR)” detection of viral nucleic acid remains the standard of reference. Real-time verse transcription-polymerase chain reaction (RT-PCR) is
the primary basis for diagnosing COVID-19\(^{(3)}\). Some incorrect practices may lead to incorrect readings, including the sample, the adequacy of the sample, the type of the sample, and the stage of infection in which the sample is obtained \(^{(4)}\). The COVID-19 in image computed tomography (CT) appearance are similar to those of viral pneumonia \(^{(5)}\), with ground-glass opacity and uniformity in peripheral distribution being the most common findings \(^{(6)}\). Precise diagnosis of Chest CT-based viral pneumonia may indicate Isolation and has a major role to play in the treatment of suspected SARS-CoV-2 in patients Infection, particularly when there is no scientifically validated recovery therapies About COVID-19. Early conversations indicated that recommended diagnostic modality for CT should be about COVID-19. The use of CT, however, for diagnosing COVID-19 is controversial. As a result of the large radiation dose received on CT scans is of great concern to communities. Also, there are relatively few cases in the literature that demonstrate detailed CT characteristics of COVID-19 \(^{(7-8)}\). The purpose of this study was to statement the importance of low dose computed tomography of the chest and evaluate the diagnostic accuracy to detect lung lesions related to COVID-19, using a low-dose chest CT protocol compared with standard protocol. Moreover, to assess whether any discriminatory signs in patients might help determine the severity of the respiratory infection from COVID-19.

**Patients and Methods**

This study included 120 patients who have clinical features (such as fever and/or disorder of the respiratory system) suspected of having COVID-19 for the period from October 1 to December 30, 2020, at the X-ray Institute / Medical City Complex / Iraq-Baghdad. All the suspected patients were tested by RT-PCR tests conducted at Medical City Complex / Baghdad that throat and nose swab specimens were obtained and considered as COVID-19-positive cases. Patients with positive RT-PCR tests were selected and were over 50 years old. Patients under the age of 50 were excluded, to reduce the risks associated with exposure to ionizing radiation, which is higher in younger subjects \(^{(9,10)}\). Only 47 patients, over the age of 50, with an average age of 57.63, participated in the analysis, of whom 31 were males and 16 were females who were exposed to an additional, reduced dose of Multi-Detector CT (MDCT). A 64-row detector multislice helical CT system was used to acquire the CT chest (Aquilion CXL- TSX-101A/Q, Toshiba, Japan) capable of generating 128 slices per rotation. Assessment of image quality was undertaken by three experts (Consultant Radiologists within X-ray Institute / Medical City Complex); images from the two modalities, i.e. protocol A, and protocol B, were qualitatively evaluated to ensure that the quality criteria for diagnostic radiographic images.

**CT scanning protocols**

A simulation experiment was performed using a standard protocol (Protocol A: 120 kVp, automatic modulation ranging from 50 – 210 mAs), in addition, four proposed protocols to choose the appropriate protocol (Protocol B: 120 kVp – 30 mAs, Protocol C: 120 kVp – 25 mAs, Protocol D: 120 kVp – 40 mAs, and Protocol E: 100 kVp – 30 mAs). As shown in Figure 1, where the experts agreed that (Protocol B: 120 kVp – 30 mAs) as shown in figure 2 could give clear results and high diagnostic accuracy compared to the other proposed protocols.
Figure 1: A simulation experiment for five protocols: (a) Protocol A: standard protocol, (b) Protocol B: 120 kVp – 30 mAs (c) Protocol C: 120 kVp – 25 mAs (d) Protocol D: 120 kVp – 40 mAs (e) Protocol E: 100 kVp – 30 mAs

Result

First of all, our study included one hundred twenty patients with common symptoms that showed PCR that they had the COVID-19, of which 47(39.2%) patients, with an average age of 57.63 years were diagnosed with COVID-19 by CT imaging.

Table (1) shows the age groups of the patient according to gender, Body Mass Index, blood groups, severity disease, and chronic disease. The age group (50-55) included the highest percentage of males 15(31.9%) and females 9(19.2%), with the highest percentage of 14(29.8%) of overweight for body mass index. This group also included the highest percentage of 11(23.4%) of blood group O+, the severity of mild symptoms 13(27.6), and non-chronic disease 14(29.8%).
Table 1: Distribution of Age groups of the patient (Low dose n=47) according to gender, Body Mass Index, blood groups, the severity of disease and chronic disease.

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>(50 – 55)</th>
<th>(56 – 60)</th>
<th>(61 – 65)</th>
<th>(66 – 70)</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>Male</td>
<td>15(31.9%)</td>
<td>4(8.5%)</td>
<td>5(10.6%)</td>
<td>7(14.9%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>9(19.2%)</td>
<td>5(10.6%)</td>
<td>1(2.1%)</td>
<td>1(2.1%)</td>
<td></td>
</tr>
<tr>
<td>Body Mass Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>Under weight</td>
<td>0</td>
<td>1(2.1%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1(2.1%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Over weight</td>
<td>14(29.8%)</td>
<td>3(6.4%)</td>
<td>4(8.5%)</td>
<td>4(8.5%)</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>9(19.2%)</td>
<td>5(10.6%)</td>
<td>2(4.3%)</td>
<td>4(8.5%)</td>
<td></td>
</tr>
<tr>
<td>Blood groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>O+</td>
<td>11(23.4%)</td>
<td>5(10.6%)</td>
<td>2(4.3%)</td>
<td>1(2.1%)</td>
<td></td>
</tr>
<tr>
<td>O-</td>
<td>0</td>
<td>0</td>
<td>2(4.3%)</td>
<td>1(2.1%)</td>
<td></td>
</tr>
<tr>
<td>AB+</td>
<td>3(6.4%)</td>
<td>0</td>
<td>1(2.1%)</td>
<td>1(2.1%)</td>
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</tr>
<tr>
<td>A+</td>
<td>6(12.8%)</td>
<td>1(2.1%)</td>
<td>0</td>
<td>3(6.4%)</td>
<td></td>
</tr>
<tr>
<td>B+</td>
<td>4(8.5%)</td>
<td>1(2.1%)</td>
<td>1(2.1%)</td>
<td>2(4.3%)</td>
<td></td>
</tr>
<tr>
<td>B-</td>
<td>0</td>
<td>2(4.3%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Severity of disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>Mild</td>
<td>13(27.6)</td>
<td>4(8.5%)</td>
<td>4(8.5%)</td>
<td>5(10.6%)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>8(17.1)</td>
<td>3(6.4%)</td>
<td>0</td>
<td>1(2.1%)</td>
<td></td>
</tr>
<tr>
<td>Sever</td>
<td>3(6.4%)</td>
<td>2(4.3%)</td>
<td>2(4.3%)</td>
<td>2(4.3%)</td>
<td></td>
</tr>
<tr>
<td>Chronic disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>Diabetic</td>
<td>3(6.4%)</td>
<td>1(2.1%)</td>
<td>1(2.1%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>5(10.6%)</td>
<td>3(6.4%)</td>
<td>1(2.1%)</td>
<td>2(4.3%)</td>
<td></td>
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<tr>
<td>Renal disease</td>
<td>2(4.3%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Non</td>
<td>14(29.8%)</td>
<td>5(10.6%)</td>
<td>4(8.5%)</td>
<td>6(12.8%)</td>
<td></td>
</tr>
</tbody>
</table>

**Radiation dose analysis**

When we compare the volume CT dose index (CTDI), which is a standardized calculation of CT scanner radiation dose production and a common tool to estimate patient radiation exposure from a CT procedure, the instrument automatically measured (8.95mGy) in the standard dose CT scan and (2.21mGy) in the low dose CT scan. This implies that patient radiation exposure will be reduced by 40% - 85% when we decrease the mAs automatic modulation from (50-210) to 30 mAs.
Figure 3: Chest CT scan of a 54-year-old man with COVID-19; (a) Standard-dose image, (b) the low-dose image

Figure 3a, standard-dose CT scan, showing multifocal ground-glass opacity of the chest predominantly in the peripheral and anterior regions associated with an interlobular septal thickening. Whereas Figure 3b shows a low-dose CT scan for the same patient. All experts agreed to diagnose the COVID-19.

Figure 4: Chest CT scan of a 56-year-old man with COVID-19; (a) Standard-dose image, (b) the low-dose image.

Figure 4a, standard-dose CT scan, showing demonstrated bilateral ill-defined ground glass opacities scattered in both lungs predominantly posterior and peripheral regions associated with interlobular septal thickening “crazy” paving image suggesting COVID-19. Figure 4b, CT scan image using the low-dose, the image with same standard dose markers indicative of COVID-19 infection.

Tables 2 and 3 show the Wilcoxon signed-rank test, the comparison between the reading of the three radiologists according to diagnostic image quality and anatomical structures. We observe statistically significant
differences between the reading of the three radiologists (Rad.1, Rad.2 and Rad.3) according to the diagnostic image quality of the standard doses and the lower doses of the number of patients as positive and negative ranks.

This means that the rank descriptions between the three radiologists’ readings were statistically significant differences [P=.046 (S), P=.001(HS) and P=.001(HS)].

Table 2: Wilcoxon signed-rank test for comparing quality standard dose and quality low dose

<table>
<thead>
<tr>
<th>Compared Related Samples</th>
<th>Ranks Description</th>
<th>Number of patients</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rad1 diagnostic image quality standard dose – Rad1 diagnostic image quality low dose</td>
<td>Negative Ranks</td>
<td>0</td>
<td>.00</td>
<td>.00</td>
</tr>
<tr>
<td></td>
<td>Positive Ranks</td>
<td>5</td>
<td>3.00</td>
<td>15.00</td>
</tr>
<tr>
<td></td>
<td>Ties</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rad2 diagnostic image quality standard dose – Rad2 diagnostic image quality low dose</td>
<td>Negative Ranks</td>
<td>0</td>
<td>.00</td>
<td>.00</td>
</tr>
<tr>
<td></td>
<td>Positive Ranks</td>
<td>12</td>
<td>6.50</td>
<td>78.00</td>
</tr>
<tr>
<td></td>
<td>Ties</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rad3 diagnostic image quality standard dose – Rad3 diagnostic image quality low dose</td>
<td>Negative Ranks</td>
<td>0</td>
<td>.00</td>
<td>.00</td>
</tr>
<tr>
<td></td>
<td>Positive Ranks</td>
<td>7</td>
<td>4.00</td>
<td>28.00</td>
</tr>
<tr>
<td></td>
<td>Ties</td>
<td>40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Comparing between Reading according to Diagnostic image quality

<table>
<thead>
<tr>
<th>Significance</th>
<th>Rad.1</th>
<th>Rad.2</th>
<th>Rad.3</th>
</tr>
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<tbody>
<tr>
<td>Diagnostic Image quality</td>
<td>2.236</td>
<td>3.464</td>
<td>2.646</td>
</tr>
<tr>
<td>Z-test</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P-Value</td>
<td>.025</td>
<td>.001</td>
<td>.008</td>
</tr>
<tr>
<td>coefficient of significance (C.S)</td>
<td>P&lt;0.05 (S)</td>
<td>P&lt;0.01 (HS)</td>
<td>P&lt;0.01 (HS)</td>
</tr>
</tbody>
</table>
Table 4 shows the mean value and standard deviation of body mass index according, mAs standard-dose, mAs low dose, DLP1, DLP2, CTDIvol1 and CTDIvol2 of the normal body mass index level group. The mean and standard deviation were (30.28±6.00) for body mass index, (1034.77±376.69) was the mean and standard deviation of mAs standard dose, while (428.62±39.75) of mAs low dose. Also, the mean and standard deviation of DLP1 was (316.34±141.33) for standard dose but (72.56±14.93) for low dose (DLP2). The mean and standard deviation of CTDIvol1 was (8.95±4.06) for standard dose but (2.21±0.94) for low dose (CTDIvol2). Using the tissue weighting factor of the chest (WT = 0.02), the effective dose value in both protocols, the standard dose and the low dose, were 6.32±2.82 mSv and 1.45±0.29 mSv respectively. The effect of body mass index on mAs standard-dose, mAs low dose, DLP1, DLP2, CTDIvol1 and CTDIvol2 was a highly significant difference (P< 0.01 HS).

<table>
<thead>
<tr>
<th></th>
<th>Mean ±Std. Deviation (mGy)</th>
<th>t-test</th>
<th>P-Value</th>
<th>C.S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index</td>
<td>30.28±6.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standar dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLP1</td>
<td>316.34±141.33</td>
<td>14.114</td>
<td>.000</td>
<td>P&lt;0.01 (HS)</td>
</tr>
<tr>
<td>CTDIvol1</td>
<td>8.95±4.06</td>
<td>25.711</td>
<td>.000</td>
<td>P&lt;0.01 (HS)</td>
</tr>
<tr>
<td>mAs Standard dose</td>
<td>1034.77±376.69</td>
<td>18.470</td>
<td>.000</td>
<td>P&lt;0.01 (HS)</td>
</tr>
<tr>
<td>Low dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLP2</td>
<td>72.56±14.93</td>
<td>17.133</td>
<td>.000</td>
<td>P&lt;0.01 (HS)</td>
</tr>
<tr>
<td>CTDIvol2</td>
<td>2.21±0.94</td>
<td>30.914</td>
<td>.000</td>
<td>P&lt;0.01 (HS)</td>
</tr>
<tr>
<td>mAs Low Dose</td>
<td>428.62±39.75</td>
<td>70.079</td>
<td>.000</td>
<td>P&lt;0.01 (HS)</td>
</tr>
</tbody>
</table>

**Discussion**

In the assessment of patients complaining of chest disorders, CT scanning of the chest is a vital imaging modality, since it can verify COVID-9, detects a wide range of chest diseases with high precision, and is important for the care of those patients. Therefore several experiments have been performed to reduce the radiation dose as low as possible. In this study, the low-dose protocol used a tube current setting (30mAs) and automatic modulation ranging from (50-210) mAs was used in the standard dose protocol. The image quality evaluation was divided into two categories: the diagnostic and related radiological results and the clarity of COVID-19 significant detection\(^{(9)}\).

The results of our study showed that COVID-19 diagnostic and related radiological characteristics can be clearly and adequately visualized on low-dose protocol scans without any noticeable difference from the standard-dose protocol, although there was some increase in noise that did not have a significant effect on
image quality. There were no large variations between the two protocols given by the 1st and 2nd reviewers in the total and individual scores and only minor differences in the scores given by the 3rd reviewer. The results show that the number of differences between findings in low-dose and findings in standard-dose MDCT scans either did not vary or were even less than the number of differences between all reviewers, depending on the features considered, when defining diagnostic image quality and the clinically important and significant anatomical structures of the chest. When we compare the volume CT dose index (CTDI), which is a standardized radiation dose performance calculation of the CT scanner and a typical method of estimating patient radiation exposure from a CT procedure, the instrument automatically measured (8.95mGy) in the standard dose CT scan and (2.21mGy) in the low dose CT scan. This means that patient exposure to radiation will be decreased by (40-85)% when we decrease the mAs from (50-210) to (30).But this does not reliably quantify the patient’s absorbed dose, which depends on the body mass index$^{[10]}$.

The protocol for a low-dose chest CT scan can be of particular importance in determining the severity of infection with COVID-19 and using it in the follow-up of patients to determine the appropriate treatment. Of 120 patients with COVID-19 infection according to PCR, a CT scan using the standard dose protocol showed only 47(39%) infection when compared to PCR. The possible explanation for this could be that patients are examined early for infection, that is, at the onset of symptoms, since the CT test depends on the signs that appear on the lung. This differentiation with PCR could give an important indication that CT imaging has not played a role in the effective triage of potential COVID-19 patients, particularly during the early stages of COVID-19 infection. Using a low effective radiation dose CT (1.45±0.29 mSv) protocol the 47 patients were subjected to comparison with the standard dose (6.32±2.82 mSv) protocol.

**Conclusion**

In conclusion, we emphasize that COVID-19 diagnostic and related radiological characteristics can be clearly and adequately visualized on low-dose protocol scans (120 kVp – 30 mAs) without any noticeable difference from the standard-dose protocol (120 kVp – Automatic modulation ranging from 50 – 210 mAs), despite there was some increase in noise that did not have a significant effect on image quality. On the other hand, it was emphasized that the absence of radiological signs by tomography does not mean that there is no injury. So, we emphasize can be utilized low-dose protocol and a reproducible assessment particularly in patients with symptoms moderate and severe. We also indicate a possible relationship between infection with COVID-19 and its level of severity, the patient’s environment, work, and health status.

**Ethical Clearance**: Taken from Middle Technical University ethical committee

**Source of Funding**: Self

**Conflict of Interest**: Nil

**References**


Enhancement of Coping Patterns among Parents of Children with Attention Deficit Hyperactivity Disorder

Nabila Atallah Attia Moawad¹, Ghada Mohamed Mourad², Fatma Ata³

¹PhD, ²Professor, ³Assistant Professor of Psychiatric Mental Health Nursing Department, Faculty of Nursing, Ain Shams University

Abstract

Background: Families of children with ADHD encounter greater difficulties such as family conflict, negative parent-child relationship, higher rates of parent stress and ineffective coping. The aim of study: was to enhancement of coping patterns among parents of children with ADHD. Study design: a quasi-experimental design was utilized to conduct this study. Setting: This study was conducted at Pediatric Out-Patient Clinic at El-Abbassia hospital for psychiatric and mental health. Method: convenient sample of 50 parents were chosen for conducting this study. Data collection tools: (1) Socio demographic and clinical characteristics data for children and their parents. (2) Parental stress scale. (3) Ways of coping Questionnaire. Results: the present study revealed that there were highly statistically significant differences between pre- and post-program of parental stress regarding level of stress, there were highly statistically significant improvement between post-program compared to pre-program regarding coping patterns of the parents have children with ADHD. Conclusion: parents of children with ADHD had high level of stress, which decreased after implementation of the educational program with a highly statistical significant difference. In addition to the current study revealed that after the intervention there were improvements in coping patterns of parents. Recommendations: Continuous education programs and counseling are important to improve parents’ adjustment toward care of their ADHD children.

Keywords: Parents of children with ADHD, Stress, Coping patterns, Educational program

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a common neurodevelopmental disorder with an estimated worldwide prevalence of approximately 5% (¹). It is the most common childhood behavioral disorder, affecting 5–7% of school-aged (²) children characterized by a persistent pattern of inattention and/or hyperactivity/ impulsivity that interferes with functioning and/or development (²).

Parents having children with ADHD had higher levels of conflict and low levels of family organization.

Parents of children with ADHD are also more experience problems in their relationship with their partner or spouse and greater mental health problems themselves. Additionally, parents of children with ADHD are more likely to experience disagreements about child- rearing and have higher rates of marital conflict, separation, and divorce than parents of children without ADHD (³).

The response of parents to the stresses of raising their child with ADHD depends on a wide variety of factors influencing their ability to cope, such as their interpretation of the crisis event, the family’s sources of support, resources, and family structure. Depending upon which type of strategy is used; one form of coping patterns can be more effective than another. Characteristics of personality of the family members,
their financial status, educational level, problem-solving skills, and spirituality influence a family’s ability to cope. Additionally, strong marital relationship and social support also help determine parental adjustment(4).

Coping patterns identified two main types: adaptive coping methods (e.g., information seeking and problem solving) and palliative coping strategies (e.g., efforts to deny, minimize, or escape the stressful situation). Adaptive coping patterns are directly aimed at coping with the source of stress, whereas palliative patterns indirectly help reduce a person’s awareness of the stress(5).

Maladaptive coping can adversely affect physical and emotional health. Using of maladaptive coping strategies for challenging behavior constitutes a risk for parental stress (6). So, parenting interventions are recommended for ADHD (7).

Nurse have important role in raising the level of awareness about ADHD through help parents understand the reason of the diagnostic process, the process of treatment, and the importance of follow-up to re-evaluate their child’s case and make sure that the diagnosis and treatment are appropriate over time. (8).

Significance of the Study
The behavior of children with ADHD is often perceived by parents as challenging to manage, and research has shown that parents of children with ADHD experience more stress than do those of children without disabilities(9). Parents of children with ADHD need support often they are frustrated that there are much stress, which make them feel emotionally, physically, financially, and perhaps spiritually stressed and upsetting, therefore they can learn more about the disorder to help them to reduce their blame and guilt about the children problem and to cope accepting their children with ADHD. So, this study designed to achieve long-term benefits through appropriate practice for parent caring ADHD child, to enhance their coping patterns.

The aim of the study
The study aimed to enhancement of coping patterns among parents of children with ADHD.

This aim achieved through:
1- Assessing level of stress among parents of children with ADHD.
2- Assess coping patterns among parents of children with ADHD.
3- Accordingly designing and implementing an educational program for enhancing coping patterns among parents of children with ADHD.
4- Evaluate the effect of the educational program on the coping patterns among parents of children with ADHD.

Research Hypothesis
The educational program will have positive effect on enhancement of coping patterns among parents of children with ADHD.

Materials and Method
The study design
A quasi-experimental design (one group pre/posttest) was used in the current study.

Setting
This study was conducted at Pediatric Out-Patient Clinic at El- Abbassia hospital for psychiatric and mental health.

Subject of the study:
Sample type: A convenience sample. Sample size: The sample was chosen as the number of available parents of children with ADHD and the parents of the present study included (50) parents who meet the following criteria: -A- For parents: Both sexes (mothers and fathers). All ages. Parent wholiving in the same dwelling and responsible for caring ADHD child. B- For children: Males and females.Ages from 6 to 12
years (school age). Free from any physical disease and without mental retardation or neurological disorders.

Tools of data collection:

1- Socio-demographic and clinical characteristics data sheet for children and their parents: This tool was designed by the researcher, it included (13) items for socio-demographic data divided into two parts which included: part one was used to identify personal characteristics of parents as: age, sex, marital status, job of parents, level of education, and monthly income. Part two was developed to personal and clinical characteristics of ADHD children: age, sex, and diagnosis, number of sibling, family birth order, and family history of ADHD.

2- Parental stress scale (Berry and Jones, 1995): This scale was developed by(7). After adaptation of this scale by researcher, it consists of 24 items answered by parents before and after the program. The scale was divided into three subscales as: psychological stress, physical stress, and social and financial stress. Scoring system: Scoring system was done using three points Likert scale ranging from Zero to 2 respectively: “agree=2, uncertain=1, and disagree = Zero”. Items 1,5,6,10,11 and 18 were reverse scored as “agree= zero, uncertain= 1, disagree= 2”. Parents with a total score reach or more than 60% were considered to have high level of stress and those with less than 60% were considered to have low level of stress.

3- Ways of coping (8): This scale was developed by(9) after adaptation of this scale by researcher; it consists of 50 items answered by parents before and after the program, the questionnaire was divided into three parts: Patterns of avoidance adaptation, patterns of seek support, and patterns of effective adaptation. Parents are presented with three graded responses: used, used somewhat, and not used.

Scoring system: Scoring system was done using three points Likert scale ranging from Zero to 2 respectively: “used =2, used somewhat=1, and not used = Zero”. Items 1,2,3,4,5,6,7 ,9,10,11,12,13,14,15,17,18 and 30 were reverse scored as used = Zero, used somewhat=1, and not used = 2”. Subjects with a total score reach or more than 60% were considered to have Positive coping and those with less than 60% were considered to have Negative coping.

Operational design

The operational design consists of (the preparatory phase, implementing phase, evaluating phase, limitations of the study, and ethical considerations). This study was conducted from October 2019 to Mars 2020, in Cairo, Egypt.

1- Preparatory phase:

Validity of the tool: A panel of five experts in three different specialties: Psychiatric nursing, Pediatric nursing and Public health nursing validated the tool. They assessed the tool for relevance, comprehensiveness, and clarity. The tool was finalized based on their comments and suggestions.

Reliability of the tool: The reliability of the tools was assessed through measuring their internal consisted by Cronbach’s Alpha coefficient test, and re-testing. Tool reliability test carried out on ten parents having children with ADHD, and after two weeks carried out re-testing on another ten of parents. To achieve the criteria of trust worthiness of the tool reliability, it was proved to be good. The validity and reliability process was done during the period first of October 2019 until end it.

Pilot study: A pilot study was carried out on 10% of the studied parents to test the clarity and applicability of the tools, and the feasibility of the research process. The pilot study was also used to estimate the time required to respond to the questionnaire. The pilot study was excluded from the study results.

2-Implementing Phase:

Fieldwork

The actual fieldwork for the process of the data collection has consumed three months and half started on beginning of half of December 2019 and was completed
by the end Mars 2020, through the following steps:

**First step:** Before starting the data collection, the researcher met with parents having children with ADHD after introducing herself, explained the nature and purpose of the study to seek participants’ co-operation, emphasizing that all collected information is strictly confidential, then oral approval consent was obtained from them before the intervention method was applied. Additionally, set time table for sessions.

**Second step:** Interviewing sessions were implemented twice / week Saturday and Tuesday during afternoon period (9:30 Am to 11:30 Am) from 40 - 45 minutes according to the parents understanding and span of attention. The parents were divided into 4 groups and each group didn’t exceed 13 parents. Collection of data as begun with the socio-demographic questionnaire, and parents need about 20-25 minutes to complete the questionnaire. After distributing the tools, the researcher explained the aim and objectives to them, while the researcher was present to assure that all questions were completed.

**Third Step:** The teaching sessions were conducted in a classroom located at the ground floor of the psychiatric hospital. The classroom was quiet, well ventilated, well furnished, and had adequate lighting and adequate spacing for implementing educational program activities. The program content and its objectives were developed by the researcher in the form of (14) sessions to be covered in (10) hours, (4 Theoretical hours and 6 Practical hours).

Every session of the program has general and specific objectives, the researcher was used different teaching methods and media such as; lecture, and open-group discussions. In addition, the researcher also used the role play, demonstration, re-demonstration, real life situations, colored posters and handouts.

**The program content was as follows:**

**Introductory Session (1):** During this initial session the researcher explained the aim of the program, determined the place of meeting, the time table, and administered the pre-assessment. During this session the parents fill in data collection tools (pretest).

**Session (2):** The focus of this session was to provide an overview about Meaning, symptoms, causes and problems caused by such disorder and treatment of Attention deficit hyperactivity disorder.

**Session (3):** The focus of this session was to provide an overview about Meaning, sources and effects of stress.

**Session (4):** The focus of this session was to provide an overview about Meaning and Types of Patterns of Coping.

**Session (5):** The focus of this session was to provide tips for stress management and how to deal with it.

**Session (6):** The focus of this session was to provide Critical problem solving techniques to management the range of difficult behavior.

**Session (7):** The focus of this session was applying steps of deep breathing technique.

**Session (8):** The focus of this session was applying Progressive muscles relaxation techniques.

**Session (9):** The focus of this session was to apply Guided imagery technique.

**Session (10):** The focus of this session was to provide Skills of how to stop negative thoughts and promoting the art of self-dialogue.

**Session (11):** The focus of this session was promoting positive feeling towards children and replacing negative feeling with positive ones.

**Session (12):** The focus of this session was to improve daily communication with the child and apply the skills of good communication, and nonjudgmental acceptance of self and child.

**Session (13):** The focus of this session was to apply the methods coping with the task of give orders, increasing
child attention and punishment of the child.

**Evaluation Session (14):** This session was the termination of the educational program and final evaluation and fill in data collection tools (posttest).

**3-Evaluation phase:** Evaluation of outcome of the program was carried out by the researcher immediately after implementation of the program (post-test) by using the same study tools that have been used in pretest to estimate the enhancement of coping patterns among parents of children with ADHD.

**Ethical considerations:**

The ethical research considerations in this study include the following:

1. A written initial approval was obtained from the research ethical committee at the faculty of nursing, Ain Shams University.

1. Individual oral consent was obtained from each participating parents after explaining the nature and benefits of the study.

2. The researcher cleared the objectives and aim of the study to participating parents.

3. The researcher maintained anonymity and confidentiality of participating parents.

4. Participating parents were allowed to choose to participate or not in the study, and given the right to withdraw at any time from the study without giving reasons.

**Statistical Analysis**

Data collected were checked for accuracy and completeness and were coded and entered into statistical package for social science (SPSS) software version 20. Describe of quantitative variables as mean and SD. Description of qualitative variables as number and percentage. Description of qualitative variable of between pre & post program through chi-square and P value were used. Measure, including frequency; percentages, arithmetic mean and standard deviation were presented and r-Pearson tests for Correlation.

**Results**

**Table 1:** reveals that, three quarters of children (76%) were in the age group 6–9 years with a mean age of 8.43 ± 1.90, and more than three quarters of children sex (80%) were male and 20% were female. Regarding to child diagnosis 88% was Combined Type (ADHD). According to number of siblings were (44%) of children had two of sibling, and less than half (42%) of the child arrangement between siblings were second, 38% were first. According to the presence of ADHD in family slightly three quarters (74%) of the sample have not.

**Table 2:** clarifies that, there are highly statistically significant differences between pre-and post-program of parental stress regarding level of stress, “P-value <0.001”.

**Table 3:** clears that, there are highly statistically significant improvement between post-program compared to pre-program regarding coping patterns of the parents have children with ADHD, “P-value <0.001”. 
Table 4: Reports that, there was positive correlation between Pre-post of total score of stress and total score of ways of coping among parents and statistically high significant.

Table (1): Socio-demographic and clinical characteristics of ADHD child (N=50).

<table>
<thead>
<tr>
<th>Items</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6&gt;9 Years</td>
<td>38</td>
<td>76%</td>
</tr>
<tr>
<td>9&gt;12 Years</td>
<td>12</td>
<td>24%</td>
</tr>
<tr>
<td>Mean± SD 8.43 ± 1.90</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>80%</td>
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<td>20%</td>
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<tr>
<td>ADHD</td>
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<td>88%</td>
</tr>
<tr>
<td>HD</td>
<td>3</td>
<td>6%</td>
</tr>
<tr>
<td>ADD</td>
<td>3</td>
<td>6%</td>
</tr>
<tr>
<td>Number of siblings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>One</td>
<td>18</td>
<td>36%</td>
</tr>
<tr>
<td>Two</td>
<td>22</td>
<td>44%</td>
</tr>
<tr>
<td>More than Two</td>
<td>9</td>
<td>18%</td>
</tr>
<tr>
<td>Family birth order</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>19</td>
<td>38%</td>
</tr>
<tr>
<td>Second</td>
<td>21</td>
<td>42%</td>
</tr>
<tr>
<td>Third or more</td>
<td>10</td>
<td>20%</td>
</tr>
<tr>
<td>Family history of ADHD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td>26%</td>
</tr>
<tr>
<td>No</td>
<td>37</td>
<td>74%</td>
</tr>
</tbody>
</table>

SD: Standard deviation
Table 2: Comparison between pre-program and post program of parental stress regarding level of stress (N= 50).

<table>
<thead>
<tr>
<th>Level of stress among parents having children with ADHD</th>
<th>Pre program</th>
<th>Post program</th>
<th>X2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Low</td>
<td>12</td>
<td>24.0%</td>
<td>33</td>
<td>66.0%</td>
</tr>
<tr>
<td>High</td>
<td>38</td>
<td>76.0%</td>
<td>17</td>
<td>34.0%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0%</td>
<td>50</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

**p-value <0.001 HS

Table 3: Comparison between pre and post-program of coping patterns among parents having children with ADHD regarding their total coping patterns (N= 50).

<table>
<thead>
<tr>
<th>Total coping patterns among parents having children with ADHD</th>
<th>Pre program</th>
<th>Post program</th>
<th>X2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Negative</td>
<td>30</td>
<td>60.0%</td>
<td>16</td>
<td>32.0%</td>
</tr>
<tr>
<td>Positive</td>
<td>20</td>
<td>40.0%</td>
<td>34</td>
<td>68.0%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0%</td>
<td>50</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

P-value>0.05 NS; *p-value <0.05 S; **p-value <0.001 HS

Table 4: Correlation between Pre-post total score of Parental Stress and total score of ways of coping among parents having children with ADHD (n=50).

<table>
<thead>
<tr>
<th>Items</th>
<th>Total score of coping</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre program</td>
</tr>
<tr>
<td>Total score of stress</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>.607**</td>
</tr>
<tr>
<td>p-value</td>
<td>0.001**</td>
</tr>
<tr>
<td>N</td>
<td>50</td>
</tr>
</tbody>
</table>

**p-value <0.01 HS    r-Pearson Correlation Coefficient
Discussion

The current study aimed to enhance the coping patterns among parents of children with ADHD through the Implementation of the educational program. In the present study, the findings showed that, more than half of parents were in the age group 31-40 years with a mean age of 37.81 ± 6.25. This result is similar to that of (9) in study entitled “Relation between Attention Deficit Hyperactivity Disorder among School Age Children and Parent’s Stress and Sense of Competence” who found that, about half of parents were between the ages 31-40 years old. While this finding was in contrast with (10) who stated that age of mothers of ADHD children was ranged between 20-30 years.

The findings of the present study reveals that, majority of the studied parents have children with ADHD were mothers, and the majority of parents were married. These findings may be due to the care given is often expected performed by mothers because the mothers traditionally take more responsibility of children care, observe any behavioral changes in their children and spend a part of their effort and time to meet the demands of medical treatment and they are caring their child such as, periodical visits to psychiatric clinic, giving medication on time and follow up the child condition. Moreover, it reflects the strong emotional ties between mothers and their children.

The finding of the present study reveals that, more than half of parents in the studied sample had Intermediate education level while illiterate parents in the studied sample were lowest percentage. Based on the present study finding, this might be due to the fact that ADHD as a disease is difficult to be noticed by uneducated parents. Meanwhile, this generally the education might have a positive impact on the care and management of the ADHD child.

The finding of the present study indicates that, more than half of the parents were not working and more than three quarters of them had enough monthly income, these results might be due to different reasons; unemployment, unavailable job, low level of education, inability to work. Or due to that majority of the parents were mothers, this means that they preferred to stay at home to taking care of their children.

Concerning Socio-demographic and clinical characteristics of children with ADHD the present study findings show that, about three quarters of children were in the age group 6>9 years with a mean age of 8.43 ± 1.90, It might be due to that this age characterized by pervasive developmental changes and ADHD begin in early childhood and the main complain in this age is impulsivity, hyperactivity and lack of attention in duties of schools.

The finding of the present study reveals that, about more than three quarters of children sex were male. This might be due to the prevalence of ADHD more common in the boys than girls. In addition, the girls may also develop the symptoms of ADHD at a later age than boys and girls sometimes show symptoms of ADHD in less obvious ways, such as being inattentive. This result was supported by Harold, (2018) in study entitled “Prevalence of attention deficit hyperactivity disorder” who found that, the majority of the studied samples were boys while the minority was girls.

Concerning the types of ADHD, the current study showed that, the majority of the children included in the study had combined type (ADHD), while the minority was predominantly inattentive type and Hyperactive – Impulsive type. This result was supported by (11) who conducted a study about the prevalence of attention-deficit/hyperactivity disorder in Fayoum city (Egypt) among school age children found that the combined ADHD subtype was the most prevalent.

In this respect, (12) found that, in a sample of Egyptian children, the distribution of the subtypes of ADHD was combined type the first, then hyperactivity predominant type and lastly attention deficit predominant type.

The findings of the present study revealed that, slightly more than three quarters of parents had a negative family history of ADHD, while less than one quarter had positive family history of ADHD. Based on the finding of
the current study, high negative family history in Egypt may be due to that most of parents do not say to anyone about the status of their children for their thinking it is a stigma for going to psychiatry hospital.

The findings of the present study clear that, less than half of the child arrangement between siblings are second. Based on the finding of the current study, high positive family history in Egypt may be due to no tendency for families to stop reproduction after the diagnosis of an affected child.

The findings of the current study revealed that, there was reduction in the total level of stress post program implementation as regards low stress (pre 24% vs. post 66%), high stress (pre 76% vs. post 34%).

This result may be due to that the educational program sessions helped parents to know sources and effects of stress, replace negative emotion with positive ones, and improve their emotional status that can lead to reduce the emotion of over involvement and reduce level of stress, by relaxation technique such as deep breathing exercise, progressive muscle relaxation and effective coping with negative thought and emotion.

This result also came in harmony with a study was conducted by (13) in study entitled” Development of an Executive Function Training Program for Preschool and School-aged Children with ADHD” who noted that, lower scores of parenting distress were found for parents of children that participated in the executive function training program than parents of the controls.

The finding of the present study represents that, after the intervention there were improvements in coping patterns of parents as regards Negative patterns (pre 60% vs. post 32%), positive patterns (pre 40% vs. post 66%). These results might be due to the educational program helped the parents to know more about ADHD, ways of effective communication and coping skills to dealing with their children and their problems. This led to that the parents had positive patterns to deal with their children and stress.

This finding also was consistent with the study by (14) about” Treatment effects of combining social skill training and parent training in Taiwanese children with attention deficit hyperactivity”. He found that after the program enhancement of positive parenting and release parenting stress in order to decrease negative and ineffective discipline.

The present study represented that there was highly statistical significant correlation between total score of Parental Stress and their total score of ways of coping during pre- post of program implementation (P≤ 0.001).

The result of the current study could be due to Parental Stress and ways of coping among parents of children with ADHD are correlated to each other. Parents who have high level of stress cannot cope effectively. This result nearly in agreement with (15) (22) who showed that there was a significant relation between parental stress level and coping strategies.

Limitation of study

Difficulties faced in gathering groups of parents to attend the program and the necessary preparation regarding time and place. In addition, some parents’ withdrawal from the study before program implementation completely (5 parents) and they were replaced by other parents.

Conclusion

In the light of the current study, it can be concluded that, parents of children with ADHD had high level of stress, which decreased after implementation of the educational program with a highly statistical significant difference (P≤ 0.001). In addition to the current study revealed that after the intervention there were improvements in coping patterns of parents with a highly statistical significant difference (P≤ 0.001).

The study recommendations

- Continuous education programs are important to improve parents’ adjustment toward care of their ADHD children.
- A hotline must be available to solve immediate problems of parents having children with ADHD.

- Counseling clinics for parents of children with ADHD are needed to minimize their burdens and inform them about the coping strategies for dealing with their children and to ensure an effective response to the needs of the ADHD children and their families.

- Further researches about the social stigmatization among parents of children with ADHD are important in support of the parents.

- Strengthening the role of sources of support (family, teachers, media and social networking), and maintaining its positive role

**Ethical Clearance:** The study was approved from ethical and research committee faculty of nursing Ain Shams University, Egypt.

**Source of Funding:** Self-funding

**Conflict of Interest** – Nil

**References**


12- Hussein S.,Relation between Attention Deficit Hyperactivity Disorder among School Age Children and Parent’s Stress and Sense of Competence. Master thesis, Minia University. 2019


14- Moawad, N., Stress and Coping Patterns among Parents of Children with Attention Deficit Hyperactivity Disorder,


Knowledge, Attitude, Action, Ability to Pay (ATP), and Willingness to Pay (WTP) Young Adults in Fulfilling the Need for Oral Hygiene Maintenance

Nadya Savira Giyansyah¹, Nadia Chairony¹, Indira Arella Harianto¹, Ulfie Rahmah Aliyah², Aryo Dwipo Kusumo³,⁴

¹Undergraduate Student of Dental Health Science, ²Graduate Student, of Dental Health Science, Department of Dental Public Health, Faculty of Dental Medicine, Universitas Airlangga, Indonesia, ³Graduate Student of Health Administration and Policy Department, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia, ⁴Staff of Department of Dental Public Health, Faculty of Dental Medicine, Universitas Airlangga, Indonesia

Abstract

Background: One of the preventive efforts that can be performed to avoid dental and oral health problems is by maintaining oral hygiene. Currently, there are various tools for maintaining dental and oral hygiene that are sold in the market. Purpose: To reveal the knowledge, attitude, action, Ability to Pay (ATP), and Willingness to Pay (WTP) of young adults in fulfilling the need for maintenance of oral hygiene. Methods: This research was a descriptive research type. The sample size was determined as 100 people, targeting the group of young adults aged 20-29 years who have worked or earn. A cross tabulation test was conducted between the ATP and WTP variables with demographic characteristics, namely age and work experience. Results: Based on the age frequency of ATP sufferers, those aged 20-29 years are able to meet the needs of oral hygiene at most at a cost of Rp. 51,000- Rp. 100,000, and the age frequency with the most WTP at a cost of Rp. 141,000 - Rp. 160,000. Based on the length of time working with the ATP, most of the oral hygiene needs are Rp. 51,000-Rp. 100,000. Meanwhile, the frequency of working with WTP is the maximum cost of Rp. 141,000 - Rp. 160,000. Conclusion: According to the research results, it can be concluded that the level of knowledge, attitude, and action of young adults in fulfilling the needs for maintenance of oral hygiene is good.

Keywords: knowledge, attitude, action, ability to pay, and willingness to pay, young adults, oral hygiene.

Introduction

Teeth are part of the body that play an important role in performing several functions such as chewing, speaking, and maintaining the shape of the face. Hence, WHO defines oral health as a state free from oral and facial diseases, throat cancer, infections, and sores in the mouth, gum and periodontal tissue disease, and disorders that limit an individual’s capacity to chew, bite, smile, speak, and psychosocial well-being[1]. Dental and oral health are not only about dental health, but also related to the tissues that support them, such as the gingiva, alveolar bone, all oral mucosal tissues, the tongue, and lips[2,3].

The dental and oral health situation in Indonesia according to the results of the 2018 Basic Health Research stated that 45.3% of the population experienced cavities / damaged / sick teeth, 19% experienced tooth loss either due to extraction or self-loss, 10.4% experienced loose
teeth, and 4.1% of the teeth were filled. Meanwhile, as many as 14% of oral health problems as a whole had swollen gums / abscesses due to cavities. Tooth and mouth disease is a disease that can affect all age groups from children to adults. Based on data compiled by the Central Statistics Agency in 2019, the proportion of adolescents and young adults in Indonesia aged 15-24 years is 83.58% of the total population. Pain or other discomfort that is felt due to disruption of oral health will certainly have a negative impact on one’s productivity.

Oral and dental health are absolutely closely related to oral hygiene. Oral and dental problems such as caries, bleeding gums, bad breath, plaque, and tartar build-up can occur due to lack of maintenance of oral hygiene. Currently, there are many various tools for maintaining oral hygiene that are sold in the market including toothbrushes, toothpaste, mouthwash, dental floss, toothpicks, and miswak. The various choices of tools for maintaining oral hygiene have differences in use, age, and socioeconomic targets.

Based on previous research, it was found that there was a significant relationship between the work of the head of the family and the DMFT index. Work is related to the level of income or level of expenditure. The lower the level of income and expenditure, it is assumed that the lower the ability to perform oral hygiene maintenance efforts such as the purchasing power of toothpaste and toothbrush. In other words, a person’s purchasing power for oral hygiene maintenance facilities is highly dependent on Ability to Pay (ATP) and Willingness to Pay (WTP). Thus, the purpose of this study is describing the knowledge, attitude, action, ability to pay (ATP), and willingness to pay (WTP) of young adults in fulfilling the need for maintenance of oral hygiene.

**Material and Method**

This type of research was a descriptive study with a quantitative approach. The population in this study was a group of young adults who have worked and were earning. The young adult group was people aged 20-29 years. The research instrument was an online questionnaire with the Google Form tool which was distributed through social media platforms to the target sample group community. Data collection procedures: distribution of questionnaires via social media (Line, WhatsApp, Instagram, and Twitter), Subjects filling out, and answering questionnaires, questionnaire data will enter the database, data processing. The data obtained from the results of filling out a questionnaire in a group of young adults in Surabaya city used descriptive analysis. Descriptive analysis of the frequency in this research was conducted on the results of respondent data based on demographic characteristics, variables of knowledge, attitude, and action, products purchased to fulfil the needs of oral hygiene, ATP, and WTP. Cross tabulation analysis in this research was conducted on age characteristics with ATP and WTP variables as well as length of work with ATP and WTP variables.

**Result**

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>44</td>
</tr>
<tr>
<td>Female</td>
<td>56</td>
</tr>
<tr>
<td><strong>Latest Education</strong></td>
<td></td>
</tr>
<tr>
<td>Senior High School</td>
<td>10</td>
</tr>
<tr>
<td>Diploma/Bachelor</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 1 Frequency distribution of study subjects based on demographic characteristics
Table 1 showed the frequency of each demographic characteristic of the respondents in this research. Based on this table, it can be concluded that the number of female respondents was more than male respondents. The latest education frequency analysis showed that the majority of respondents have the latest education at the diploma / bachelor level. Meanwhile, based on the analysis of the frequency of types of work and length of work, it was found that most of the respondents worked as general employees with the majority working experience for less than one year.

Graph 1 Type of product purchased
Based on graph 1, the results showed that most young adults buy toothbrushes, fluoride toothpaste, and mouthwash to fulfil their oral hygiene needs. Meanwhile, products that were rarely purchased are interdental brushes, miswak, and toothpaste without fluoride.

Graph 2 Frequency of knowledge in young adults

Graph 2 showed that most young adults have good knowledge in fulfilling oral hygiene needs, 87%, 13% have sufficient knowledge, and none have poor knowledge.

Graph 3 Frequency of attitude in young adults

Graph 3 showed that most young adults have a good attitude in fulfilling oral hygiene needs, 87%, 12% have a
fairly good attitude, and only 1% have a poor attitude.

Graph 4 Frequency of action in young adults

Graph 4 showed that most of the young adults had good actions in fulfilling oral hygiene needs by 74%, 25% had good enough actions, and only 1% had poor actions.

Table 2 Frequency of Age with ATP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ATP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;50.000</td>
<td>51.000-100.000</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-23 years old</td>
<td>N 28</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>% 40%</td>
<td>38.6%</td>
</tr>
<tr>
<td>24-26 years old</td>
<td>N 3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>% 15.8%</td>
<td>47.4%</td>
</tr>
<tr>
<td>26-29 years old</td>
<td>N 1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>% 9.1%</td>
<td>36.4%</td>
</tr>
<tr>
<td>Total</td>
<td>N 32</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>% 32%</td>
<td>40%</td>
</tr>
</tbody>
</table>

Based on table 2, respondents aged 20-23 years were able to fulfil the needs of the most oral hygiene at a cost of <IDR 50,000. Meanwhile, at the age of 24-29 years, they can afford to pay Rp. 51,000 - Rp. 100,000.
Table 3 Frequency of age with WTP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WTP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;20000</td>
<td>21000-40000</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-23 years old</td>
<td>N</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>2.9%</td>
</tr>
<tr>
<td>24-26 years old</td>
<td>N</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>10.5%</td>
</tr>
<tr>
<td>26-29 years old</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>N</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>4%</td>
</tr>
</tbody>
</table>

Based on table 3, respondents aged 20-29 years were at most willing to fulfil their oral hygiene needs at a cost of Rp. 141,000 - Rp. 160,000.

Table 4 Frequency of work experience with ATP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ATP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;50,000</td>
<td>51,000-100,000</td>
</tr>
<tr>
<td>Length of Work</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>N</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>42%</td>
</tr>
<tr>
<td>1-3 years</td>
<td>N</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>29%</td>
</tr>
<tr>
<td>&gt; 3 years</td>
<td>N</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>10.5%</td>
</tr>
<tr>
<td>Total</td>
<td>N</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>32%</td>
</tr>
</tbody>
</table>

Regarding a length of work <1 year, they were able to fulfil the needs of the most oral hygiene at a cost of <Rp 50,000. Meanwhile, respondents who worked for 1 year to > 3 years were able to pay Rp. 51,000 - Rp. 100,000.
Table 5 Frequency of work experience with WTP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WTP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;20000</td>
<td>21000-40000</td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>N 2</td>
<td>1</td>
</tr>
<tr>
<td>% 4%</td>
<td>2%</td>
<td>20%</td>
</tr>
<tr>
<td>1-3 years</td>
<td>N 2</td>
<td>3</td>
</tr>
<tr>
<td>% 6.5%</td>
<td>6.5%</td>
<td>0%</td>
</tr>
<tr>
<td>&gt; 3 years</td>
<td>N 0</td>
<td>0</td>
</tr>
<tr>
<td>% 0%</td>
<td>0%</td>
<td>5.3%</td>
</tr>
<tr>
<td>Total</td>
<td>N 4</td>
<td>2</td>
</tr>
<tr>
<td>% 4%</td>
<td>2%</td>
<td>11%</td>
</tr>
</tbody>
</table>

Based on table 5, respondents with length of work < 1 year to > 3 years were at most willing to fulfil oral hygiene needs at a cost of Rp. 141,000 - Rp. 160,000.

**Discussions**

Oral hygiene is the most significant factor in preventing disease and maintaining oral health. The best way to improve oral hygiene is by practicing oral hygiene using products sold in the market\(^{[11]}\). Along with the development of the dental industry, various dental and oral care products are widely available in the market which include toothbrushes, toothpaste, mouthwash, dental floss, and teeth whitener. Choosing the right product can improve health and prevent diseases of the teeth and mouth. Hence, people must be able to determine products that suit their needs and perform regular maintenance to get maximum results\(^{[11]}\). According to Awais et al. (2019), there are several factors that affect a person’s oral health, including education, employment, social class, and income\(^{[12]}\).

Based on data analysis regarding the knowledge aspect, it was found that 87% of respondents had a good level of knowledge. This can be explained by the theory that the more old a person is, the higher the maturity level of a person in thinking\(^{[13]}\). A person’s age affects their perceptive power and mind-set. As you get older, your perception and mind-set will also develop, thus the knowledge you get will be better\(^{[14]}\).

A high level of knowledge is also followed by good attitudes and actions that the respondents have. This can be seen from the results of data analysis in the attitude domain, where 87% of respondents had good attitude scores. Knowledge and good attitudes are also applied through good actions, where in this study 74% of respondents got good action scores. This is in line with the theory which says that the knowledge that a person acquires will then lead to an inner response in the form of
attitudes towards the object he already knows\textsuperscript{[14]}. Good action is also influenced by socio-psychological factors, one of them is attitude\textsuperscript{[15]}. Attitude is a very important factor because attitude determines a person’s tendency to act. Good knowledge will influence taking the right attitude, thus it will be willing to start taking the right action as well\textsuperscript{[16,17]}

Based on the results of the cross tabulation test between age and ATP and WTP, respondents aged 20-23 years were able to fulfil the needs for oral hygiene maintenance of <50,000, while respondents aged 24-29 years were able to fulfil the needs for oral hygiene maintenance at a cost of Rp. 51,000 - Rp. 100,000. This can be explained by the theory that the more mature a person is, the higher one’s strength at work\textsuperscript{[13]}, thus the higher the income one gets. The amount of income earned certainly affects a person’s ability to fulfil their daily needs, including the need for maintaining oral hygiene.

In regard to the WTP, respondents aged 20-29 years are at most willing to fulfil the needs for oral hygiene maintenance at a cost of Rp. 141,000 - Rp. 160,000. When compared between ATP and WTP, a condition occurs where ATP <WTP, meaning that the respondent’s desire to buy oral hygiene maintenance products is greater than their ability. It is natural for people with low ATP to have a high WTP because the WTP value is determined by the user’s psychological considerations\textsuperscript{[18]}

According to the results of the cross tabulation test between work experience and ATP and WTP, ATP or the ability to pay the costs for those who have 1 year of work experience to>3 years is higher than work experience <1 year. This is because work experience affects the income used in calculating ATP. The higher the work experience, the income earned will increase. Income is directly proportional to ATP. This is as same as the greater the income and the smaller the frequency of purchasing goods, the value of ATP will increase\textsuperscript{[19]}. This means that young adults in all work experiences have the same awareness of the costs they want to spend to fulfil dental health products\textsuperscript{[20]}. Therefore, it is sufficient to fulfil the needs of health and dental hygiene such as toothbrushes, toothpaste, dental floss, and mouthwash at a cost of Rp. 141,000 - Rp. 160,000.

**Conclusions**

According to the research results, it can be concluded that the level of knowledge of young adults in fulfilling the needs for maintenance of oral hygiene is good (87% of respondents). The attitude of young adults in fulfilling the need for maintenance of oral hygiene is good (87% of respondents). Besides that, the action of young adults in fulfilling the need for maintenance of oral hygiene is good also (74% of respondents).

The ability of adolescent adolescents in Ability to Pay (ATP) and Willingness to Pay (WTP) based on age who have a higher level of ability in maintaining oral hygiene is aged 24-29 years. The ability of young adults in Ability to Pay (ATP) and Willingness to Pay (WTP) based on length of work which has a higher level of ability in maintaining oral hygiene is 1 year to> 3 years of work, while WTP in all work experiences is the same.

**Conflict of Interest:** None

**Source of Funding:** Self-Funding

**Ethical Clearance:** Approved

**References**

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Dental Health Care for Older Persons with Diabetes by Caregivers in Ubon Ratchathani

Namphet Tungyingyong¹, Ruengsin Thuennadee², Niruwan Turnbull³

¹Doctoral Degree Student, Faculty of Public Health, Mahasarakham University, Mahasarakham, 44150, Thailand, ²National Health Security Office Region 10, Ubon Ratchathani, 34000, Thailand, ³Associate Professor at Public Health, Faculty of Public Health, Mahasarakham University, Mahasarakham, 44150, Thailand

Abstract

Background: Ubon Ratchathani province has seen the phenomenon of the population aging since 2010 and the number of the elderly continues rise each year. According to the assessment of oral health, most of the elderly suffer from Gingivitis and Periodontitis, and have lost their teeth as a result of tooth decay and periodontal diseases. Methods: This study design was a research and development study aimed to explore the process of dental health caregiving for older persons with diabetes by caregivers in Ubon Ratchathani province. Proportional to the estimation of the population, the sample size was 403 elderly patients for contextual study from June 2016 to July 2018. Data was collected using questionnaires with a reliability of 0.8, and analysis was conducted using statistical tools, number, percent, mean, standard deviation, independent sample t-test and paired sample t-test. Results: The finding demonstrated that the participants, similarly, needed care from caregivers, the role of caregivers, health promotion and prevention, while treatment was less of a need. Comparisons of evaluation results from both groups considered impacts on their oral health, on the Daily Performance of their Lives, as well as their Knowledge of and Attitude towards oral care, demonstrated a statistically significant difference (p-value = <0.001). Elderly patients without caregivers suffered largely from loose teeth, showing little knowledge and misunderstanding of tooth brushing techniques, believing that the harder they brush the better it is for their dental health. As for behavior, both groups showed no statistically significant difference (p-value = 0.09) and were rated to be at the same medium level. Conclusion: The finding can be applied for consistency of information from current such as the service was easy to access, the development of the potential and skills of the elderly patients with diabetes and their involved, as well as follow-up for strength and teamwork.

Keywords: caregiver, patients with diabetes, dental health, elderly, oral care model

Introduction

Ubon Ratchathani province has seen the phenomenon of the population aging since 2010 and the number of the elderly continues rise each year. In 2015, the total number of the elderly in the province was 205,698 – or 11.1% of the city’s population. Of this number, 56.2% are in early old age, 30.4% are in middle old age, and 13.3% are in late old age. According to the assessment of Activities of Daily Living (ADL),¹ the samples were divided into three groups: Group 1 (social-oriented, 93.1%), Group 2 (home-oriented, 5.02%), and Group 3 (bed-oriented, 1.86%). Most of the elderly are experiencing chronic diseases: 164,689 of them (80.0%) have diabetes and 162,205 of them (78.8%) have high blood pressure. According to the assessment of oral health, most of the elderly suffer from Gingivitis and Periodontitis, and have lost their teeth as a result of tooth decay and periodontal diseases. This leaves 52.7% of them with fewer than 20 teeth and only 55.8% with at least 4 occlusal pairs: one permanent tooth with another,
or permanent tooth with a denture. This has undermined their ability to chew food and has a negative impact on their quality of life. It was also found that 2.1% of the elderly were experiencing malnutrition. As many of the elderly have chronic diseases such as diabetes that slow down the healing process of wounds and bruises, have a lack of knowledge in self-care, and no caregiver in their families, it is important that caregivers be assigned to help educate the elderly on how to appropriately practice self-care and dental care.

Consequently, Ubon Ratchathani Provincial Health Office has worked to equip local caregivers with the necessary knowledge and skills through a 70-hour training program which includes both lecture and practice hours enabling caregivers to become more capable of taking care of the elderly. However, in practice, different groups of patients with diabetes, such as the home-oriented and the bed-oriented, may pose different challenges leading to the necessity for different methods of care. Therefore, it is asserted that there is a need for oral and dental care from caregivers among elderly patients with diabetes in Ubon Ratchathani province, as well as to compare the impacts of oral health on the Daily Performance of their Lives, knowledge, attitude and behavior regarding oral care among elderly patients with diabetes, with or without caregivers, requires further examination.

**Objective**

The objective of this study was to explore the process of dental health caregiving for older persons with diabetes by caregivers in Ubon Ratchathani province.

**Material And Methods**

**Research model and sample**

This research an action research study was carried out using a purposive sampling to selected elderly with diabetes patients in Ubon Ratchathani province to participated in this study. The participants of this study were examined 164,689 elderly patients with diabetes in Ubon Ratchathani province. Of this number, 43,454 patients had caregivers while 121,235 did not. Proportional to the estimation of the population, the sample size of the elderly patient’s group with a caregiver was 403 patients and there were 245 patients in the elderly patient’s group without a caregiver.

**Data collection**

The model by Kemmis and McTaggart study (Kemmis & McTaggart, 2005) was used to answer the question and explore the new practice of dental health caregiving for older persons with diabetes. The research comprises two phases, each of which consists of 4 phases: 1) planning, 2) action, 3) observation, and 4) reflection. In the first cycle, the planning phase included studying the context of dental health care for older persons, Oral Impact on Daily Performance (OIDP Index), designation of the working team, meeting of the team, and introducing the data into the action phase. The action phase simply followed the plan. The dental health care manual for older persons with diabetes was made. A workshop training was provided to older persons with diabetes, their relatives, and caregivers. The observation phase involved patient visit for enhancing empowerment of the working team. Finally, the reflection phase involved the considering the lesson learned from the above steps. In the second cycle, the working team had a meeting to make a plan and introduced the data into the action phase. In the action phase, the working team followed the action plan and provided a specific skill training program for older persons with diabetes, updated the data, and gave feedback for them on a monthly basis. In the observation phase, the working team visited the patients for enhancing empowerment of the working team. Finally, the reflection phase involved the considering the lesson learned from the above steps in the second cycle. From the implementation of the entire cycle, the key success factors can be summarized into 9 activities as follows: 1) designation of the research team and participant selection, 2) investigation of dental health care situation among older persons with diabetes, 3) introducing the data from the study as the background knowledge in order to determine the dental health care method for older persons with diabetes, 4) the workshop training for making the action plan, 5) trial of the resultant model in the area and developing.
the lesson, 6) development of the data processing system and reflection of the lesson learned, 7) expanding the specific skill training for dental health care, 8) modifying the caregiving system for older persons with diabetes and dental health problems to cover all patients, and 9) monitoring for enhancing empowerment of the working team.

Regarding the key success factors, it was found that having systematic planning and collaboration among the community leader, local administrative organization, and relatives of older persons with diabetes, caregivers, and dental professionals is of paramount important. This includes availability of the updated and complete data on dental health of older persons with diabetes, dental service reorientation, reference system from caregivers, increased access to the service of older persons with diabetes, increased social connection (Line Application), skill and potential development for the relatives of older persons with diabetes so that they can perform self-care, and enhancing empowerment. Community leaders and local administrative organization play a prominent role because they can enhance empowerment of the community for dental health care for older persons with diabetes. According to the qualitative research, the new method of dental health care giving for older persons with diabetes was developed from the key success factors. See Figure 1 for the “DDS CG Ubon Ratchathani Dental Health Care”.

**Human research ethics**

The study has been approved by the Human Research Committee in the University of Mahasarakham. On 7 February 2561, PH002/2017, based on the declaration of Helsinki (Declaration of Helsinki) and good clinical research practices.

**Data analysis**

SPSS version 16 for Winders were used for the data analysis. Descriptive statistics mainly frequency, percent, mean, and standard deviation (SD), max.-min., were used to summarize elderly with diabetes’ demographic characteristics Oral Impact on Daily Performance (OIDP), impact, knowledge, attitude, and behavior between with and without caregivers. Independent sample t-test was used for compared OIDP indices, knowledge, attitude, and behavior between elderly patients with and without a caregiver.

![Figure-1: The method of DDS CG for Ubon Ratchathani Dental Health Care](image)
Results

Data on the demographic characteristics of the participants

Elderly patients in both groups: with and without caregivers demonstrated that most of them are female (68.2% and 66.3% respectively), age ranged between 60 and 69 (72.1% and 68.2% respectively), already married (78.0% and 76.9% respectively), with primary education (89.7% and 88.0% respectively), working on the farm (79.8% and 79.3% respectively), with an income of less than 17,000 baht (95.9% and 95.8% respectively), with an average income of 4,335.92 baht and 4,854.84 baht respectively, have been given care by any caregivers for a year, 63.3% of elderly patients with caregivers have received news and information from public health officials, 95.9% of elderly patients with caregivers and 95.8% of elderly patients without caregivers have had Diabetes for less than 21 years, have no dentures (81.6% and 80.9% respectively), are socially-oriented - 78.4% and 81.1% respectively.

The need for oral care from caregivers to elderly patients with diabetes

Elderly patients in both groups: with and without caregivers demonstrated that most of them needed caregivers to teach them about nutrition and oral health (98.4% and 97.5%), how to maintain oral health (96.3% and 95.5%), and thirdly they needed caregivers to teach them how to conduct oral health check-ups by themselves (94.7% and 93.1%) (Figure 2). In figure 2, the results demonstrated that elderly patients in both groups: with and without caregivers needed the caregivers to conduct oral check-ups on them (82.4% and 81.4% respectively), needed the caregivers to record their oral health for them (63.7% and 61.5% respectively), needed oral care from the caregivers (92.7% and 91.3% respectively), needed to gain more knowledge regarding nutrition for good oral health (98.4% and 97.5% respectively), needed the caregivers to teach them how to clean their dentures (75.5% and 77.9% respectively), to teach them how to correctly brush their teeth (76.6% and 77.4% respectively), needed the caregivers to teach them how to use accessories for oral health care (69.0% and 69.2% respectively), needed the caregivers to teach them how to conduct oral health check-ups by themselves (94.7% and 93.1% respectively), needed to learn healthy cooking from the caregivers (71.4% and 72.7% respectively), needed the caregivers to teach them oral cleaning (96.3% and 95.5% respectively), need implant replacement teeth (46.9% and 48.4% respectively), needed scaling of teeth (60.8% and 58.1% respectively), needed tooth extraction (35.9% and 38.2% respectively), and needed teeth filling (38.4% and 42.9% respectively).

![Figure-2: The need for oral care from caregivers of diabetes elderly patients with or without caregivers](image-url)
Data on the Oral Impact on Daily Performance (OIDP Index)

(Both groups) The group of elderly patients without caregivers showed an average OIDP index at the medium level (Mean = 2.06, S.D. = 0.39), which was higher than that of the group with a caregiver – which stood at a low level (Mean = 1.08, S.D. = 0.16). The group without caregivers also experienced a greater incidence of loose teeth and had more difficulty taking in food than the group with caregivers. Thus, the latter had experienced a better quality of life than the former.

Data on knowledge level

Oral health care data showed that elderly patients both without and with caregivers, have a medium level such as knowledge (11 – 15 points) at 43.67% and 66.94% respectively. It was also noted that the former was at the low-to-medium level of such knowledge while the latter was at the medium-to-high level. This is partly because caregivers may have provided advice to the latter group to a certain extent allowing them to have a better knowledge of oral care than the group without a caregiver.

Data on attitude

Both groups regarding oral care by the type of activity, showed that both elderly patients without and those with caregivers have an appropriate Attitude with average points standing at the medium level (Mean = 1.73, S.D. = 0.36; Mean =1.83, S.D. = 0.48, respectively). The types of activity that saw a better Attitude from both groups, than other types of activity were eating, smoking, and chewing. Both groups agreed that they should brush their teeth after eating, while smoking and chewing a betel nut may pose a health risk to their gums (Mean = 1.86, S.D. = 0.66; Mean = 1.96, S.D. = 0.59, respectively).

Data one behavior

Data regarding Oral Care by type of activity, showed that both groups of elderly patients without and those with caregivers had a medium level result (Mean = 2.10, S.D. = 0.67; Mean = 2.33, S.D. = 0.46, respectively). The reason for the type of activity that saw better behavior from both groups, than other types of activity, is the risk of catching oral diseases (Mean = 2.35 and S.D. =0.33; mean = 2.43 and S.D. = 0.45, respectively). For all types of activity, elderly patients without caregivers scored lower than the elderly patients with caregivers.

Comparisons of Oral Impact on Daily Performance (OIDP) as well as knowledge, attitude and behavior regarding Oral Care

Among elderly patients with diabetes, it was shown that those patients with a caregiver had a better quality of life than those without, with a statistically significant difference of 0.01 and the difference between average points of both groups was 8.11 points (95% CI=7.69-8.52). Those patients with a caregiver also had a better Knowledge of Oral Care than those without, with a statistically significant difference of 0.05 and the difference between the average points of both groups was 0.58 points (95% CI=0.03-1.13), as well as a better attitude towards Oral Care with a statistically significant difference of 0.01 and the difference between the average points of both groups was 1.12 points (95% CI=0.42-1.83), they also had better behavior’s in Oral Care with a statistically significant difference of 0.05 and the difference between the average points of both groups was 0.72 points (95% CI=0.13-1.58) as shown in the table 1.
Table-1: Average points on the behavior of both groups regarding Oral Care by type of activity

<table>
<thead>
<tr>
<th>Aspect</th>
<th>With caregivers (n=245)</th>
<th>Without caregivers (n=403)</th>
<th>Mean Diff.</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
<td></td>
</tr>
<tr>
<td>Impact</td>
<td>17.06</td>
<td>3.14</td>
<td>8.95</td>
<td>1.34</td>
<td>8.11</td>
</tr>
<tr>
<td>Knowledge</td>
<td>11.90</td>
<td>0.19</td>
<td>12.48</td>
<td>2.46</td>
<td>-0.58</td>
</tr>
<tr>
<td>Attitude</td>
<td>18.52</td>
<td>4.86</td>
<td>19.65</td>
<td>3.66</td>
<td>-1.12</td>
</tr>
<tr>
<td>Behavior</td>
<td>36.99</td>
<td>6.02</td>
<td>37.71</td>
<td>4.15</td>
<td>-0.72</td>
</tr>
</tbody>
</table>

Oral Impact on Daily Performance (OIDP Index) and comparison between OIDP indices, knowledge, attitude, and behavior of older persons with diabetes who have caregivers and those who do not have caregivers.

The study revealed that older patients with diabetes who do not have caregivers have the OIDP Index at medium level (mean=2.06 and S.D=0.39) while those who have caregivers have the OIDP Index at low level (mean=1.08 and S.D=0.16). The former group suffered more oral impact on daily performance than the latter group. Those who do not have caregivers have odontoseisis problem which affects their chewing more than the other group. It is safe to say that older persons with diabetes who have caregivers have better quality of life than those who do not have as shown in tables 2.

Table-2: Average OIDP Index sorted by the type of impact

<table>
<thead>
<tr>
<th>Types of impact</th>
<th>Older persons without caregivers (n=403)</th>
<th>Older persons without caregivers (n=245)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>Physical impact</td>
<td>2.19</td>
<td>0.41</td>
</tr>
<tr>
<td>Psychological impact</td>
<td>2.13</td>
<td>0.48</td>
</tr>
<tr>
<td>Social impact</td>
<td>1.88</td>
<td>0.67</td>
</tr>
<tr>
<td>Overall impact</td>
<td>2.06</td>
<td>0.39</td>
</tr>
</tbody>
</table>
Comparision between OIDP indices, knowledge, attitude, and behavior of older persons with diabetes who have caregivers and those do not have caregivers

As a result of comparison between OIDP indices, knowledge, attitude, and behavior of older persons with diabetes who have caregivers and those do not have caregivers, it was found that the former group have a better quality of life than the latter with the significance level of 0.01 with the mean difference of 8.11 (95% CI = 7.69-8.52). In addition, it was also found that the former group have better knowledge on dental health care than the latter at the significance level of 0.05 with the mean difference of 0.58 (95% CI = 0.03-1.13), the former group have better attitude toward dental health care than the latter at the significance level of 0.01 with the mean difference of 1.12 (95% CI = 0.42-1.83), and that the former group have more proper dental health care behavior than the latter group at the significance level of 0.05 with the mean difference of 0.72 (95% CI = 0.13-1.58) as shown in table 3.

Table-3: Comparison between OIDP indices, knowledge, attitude, and behavior of older persons with diabetes who have caregivers and those do not have caregivers

<table>
<thead>
<tr>
<th>Topic</th>
<th>Without caregivers (n=245)</th>
<th>With caregivers (n=403)</th>
<th>Mean Diff.</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
<td></td>
</tr>
<tr>
<td>OIDP</td>
<td>17.06</td>
<td>3.14</td>
<td>8.95</td>
<td>1.34</td>
<td>8.11</td>
</tr>
<tr>
<td>Knowledge</td>
<td>11.90</td>
<td>0.19</td>
<td>12.48</td>
<td>2.46</td>
<td>-0.58</td>
</tr>
<tr>
<td>Attitude</td>
<td>18.52</td>
<td>4.86</td>
<td>19.65</td>
<td>3.66</td>
<td>-1.12</td>
</tr>
<tr>
<td>Behavior</td>
<td>36.99</td>
<td>6.02</td>
<td>37.71</td>
<td>4.15</td>
<td>-0.72</td>
</tr>
</tbody>
</table>

Change in behavior of dental health care for older persons

As a result of comparison between OIDP indices, knowledge, attitude, and behavior of older persons performing self-care before and after the development of the model are as shown in table 4. According to table 4, the knowledge, attitude, and behavior of older persons with diabetes before and after the model development are difference with the significance level of 0.001.

Table-4: Comparison of average score of knowledge, attitude, and behavior of the experiment group before and after the development of the model

<table>
<thead>
<tr>
<th>Experiment group</th>
<th>N</th>
<th>( \bar{x} )</th>
<th>S.D.</th>
<th>Mean diff.</th>
<th>95% CI Upper</th>
<th>95% CI Lower</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge</td>
<td>141</td>
<td>11.51</td>
<td>2.41</td>
<td>2.10</td>
<td>-1.67</td>
<td>-2.72</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table-4: Comparison of average score of knowledge, attitude, and behavior of the experiment group before and after the development of the model

<table>
<thead>
<tr>
<th></th>
<th>Knowledge</th>
<th>Attitude</th>
<th>Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before model development</td>
<td>141</td>
<td>19.65</td>
<td>33.76</td>
</tr>
<tr>
<td>After model development</td>
<td>141</td>
<td>22.20</td>
<td>36.11</td>
</tr>
<tr>
<td>Before model development</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After model development</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

In terms of the needs for oral care from caregivers among elderly patients with diabetes, it was found that both groups - without or with a caregiver – needed care and health promotion from caregivers, and this showed at a high level, of 53.3% and 79.6% respectively. This finding was confirmed by the study of Jeasoh study which claimed that the elderly usually needed a lot of health services – ranging from annual check-ups and appropriate physical activity, to memory exercises which should be delivered and/or monitored closely by caregivers. Moreover, the elderly need special care and home visits from caregivers. They need intimate care, especially within the family, which will help mitigate the feelings of abandonment, isolation, or neglect, that elderly people suffer from, while enabling them to become motivated and stay positive with all aspects of the health services being provided – i.e. health promotion, prevention, treatment and rehabilitation – to give them a chance to improve both their physicality and mentality. The Oral Impact on Daily Performance between the two groups, it was illustrated that the group with a caregiver had less Oral Impact on Daily Performance or a better quality of life than the group without a career, with a statistically significant difference of 0.01 (p-value = <0.001). Eating as a type of activity has the most effect of all types of activity. This finding was confirmed in a study with Watthanasan study et al. study, which claimed that daily performances of their sample – such as talking and working – are affected by oral ill health, and that many patients are experiencing difficulty in chewing their food nearly every day. Charoentanyarak et al. found that the prevalence of Oral Impact on Daily Performance was most obvious when elderly people experienced toothache with severe or extremely severe pain during eating and while conducting oral hygiene. The most common causes of such pain are toothache, tooth loss, and loose teeth, which may result from diabetes. This places patients at greater risks of being diagnosed with Periodontitis. Patients suffering from these causes of pain should be treated quickly to prevent any further complications that may also cause them to lose more teeth. Suksang et al. study found that the way that patients ate, talked, expressed their feelings through their faces, as well as the OIDP index, before and after surgery were different suggesting that corrective jaw surgery could help to improve the oral health of the patients. Turk et al. suggested that if patients could effectively control their diabetes they would have a better quality of life than those who could not. Laiteerapong et al. study argued that low levels of blood sugar in patients with diabetes may be related to a lower quality of life. In 2015, Wändell posited that patients with diabetes who have received appropriate and continued care will have a better quality of life while, suggested that the overall
quality of life may be partly improved by appropriate oral care. Comparisons of knowledge, an attitude, and behavior regarding oral care between both patient groups with diabetes showed that the group with a caregiver had better knowledge than the group without a caregiver, with a statistically significant difference of 0.05 (p-value = 0.03) and a better Attitude with a statistically significant difference of 0.01 (p-value = <0.001). But in terms of oral care behavior there was no statistically insignificant difference and no significant differences in behaviors r between them. This may result from the factors that determine everyday behaviors which include not only the level of Knowledge and Attitude, but also feelings, habits, and convenience, for instance.

Therefore, it can be assumed that elderly people with better knowledge and attitude may not have better corresponding behaviors for oral care, given that most of them (approximately 90%) showed a medium-high level of knowledge and attitude, but only 77.2% (relatively low) of them brushed their teeth twice a day. It can also be assumed that the level of knowledge is not related to eating habits that may cause tooth decay, which means that an inverse variation exists between the level of knowledge and behavior, with a statistical significance \( r = -0.164, p = 0.001 \), and that the Level of knowledge and attitude are independent to the oral care behaviour in students, which argues against the study of Muksing et al. claiming that the level of knowledge and attitude regarding self-care have a positive relationship at a low level with self-care behavior in students, with a statistical significance \( r = 0.28, p\text{-value} < 0.01 \). Watthanaasen et al. also added that the level of knowledge in oral care has a positive relationship at a low level with oral care practice, with a statistical significance \( r = 0.13, p\text{-value} = 0.046 \) while Uthon confirmed that the Level of Knowledge and Attitude have a positive relationship with oral care behavior, with a statistical significance \( r = 0.124, p\text{-value} = .019 \) and \( r = 0.172, p\text{-value} =0.001 \). Pulthong et al. claimed that when elderly people become more knowledgeable of oral care their oral care behavior improves as well, with a statistical significance, while Jitaram and Makboon study found that the level of knowledge and attitude regarding oral care for children between the ages of 3 and 5 years is related to the parents’ behavior in taking oral care of their children with a statistical significance at 0.05. Based on the relevant literature, the Level of Knowledge and Attitude may not be directly related to the behavior as there might be other factors that have come in to play in determining the behavior – including feelings, personal habits, and awareness of self-care importance.

**Conclusion**

This study concluded that both patient groups with diabetes: one with and one without a caregiver, are mostly socially-oriented (86.6% and 82.8% respectively) and require a high level of care from caregivers (53.3% and 79.6% respectively). Comparisons between these two groups have shown that in terms of Knowledge and Attitude they are different with a statistically significant difference of 0.05. However, in terms of oral care behavior they are different with a statistically insignificant difference of 0.05. In terms of oral impact on daily performance there is a statistically significant difference of 0.01. It can be implied that the socially-oriented groups have the potential to take good care of their oral health if they are taught, trained, and inspired to do so. As a result, the development of capacity and better practice of caregivers is the key to the improvement of such care for the elderly. As the findings of this research demonstrates, elderly patients with diabetes still have low levels of oral care behavior, they should be trained on how to better perform oral self-care, and their relevant medical records should be initiated, summarized, and then returned to them so as to make them better aware of the importance of oral self-care performance.

**Suggestion**

1. The administrators of the Ubon Ratchathani Provincial Public Health Office, Ubon Ratchathani, Thailand who take care of and perform all aspects of this work, such as providing support in exchanging knowledge with other agencies, encourage a mentor to supervise and monitor the operation, budget support, sufficient personnel for the operation, coordination
with relevant network parties, as well as providing various social welfare for older persons with diabetes by caregivers.

2. Executives should encourage health officer to have a forum to showcase their works or organize public benefit activities. Networking partners from all sectors join as mentors, support and advisor in organizing activities of the older persons with diabetes by caregivers.

Acknowledgment

Thank you to Niruwan Turnbul (PhD) Associate Professor of Public Health, Faculty of Public Health, Mahasarakham University, Thesis Advisor and all respondents who cooperated well in this study.

Ethical Clearance: The study has been approved by the Human Research Committee in the University of Mahasarakham. On 7 February 2561, PH002/2017, based on the declaration of Helsinki (Declaration of Helsinki) and good clinical research practices. Participants could refuse and/or leave this research at any time. The data in the evaluation forms was kept confidentially without specifying the participants’ names in the document.

Conflict of Interests: This study has no conflicts of interest.

Source of Funding: This study was supported by Ubon Ratchathani Provincial Public Health Office, Ubon Ratchathani, Thailand. And Faculty of Public Health, Mahasarakham University.

References


Endodontic Retreatment in Underfilled Root Canal of Maxillary First Molar with Chronic Periapical Abscess: A Case Report

Nanik Zubaidah¹, Kun Ismiyatin¹, Cinitra Anindya², Nindhira Puspita Sari², Singgih Harseno², Ahmad Afif Dzulfikar², Dian Dwi Pratiwi²

¹Staff, ²Resident of Conservative Dentistry Department, Faculty of Dental Medicine, Universitas Airlangga

Abstract

Background: The failure of endodontic treatment commonly caused by imperfect obturation, periodontal-periradicular lesions, untreated root canals, and some other factors. The failure may trigger the occurrence of secondary infections due to persistent bacteria. One of the abnormalities that appear after endodontic treatment is a periapical abscess. Endodontic failure can be overcome by endodontic retreatment, apical surgery, or extraction. During an endodontic retreatment, endodontic instruments are used to remove the root canal filling material and to repeat the steps of endodontic treatment to achieve the apical patency.

Purpose: This case report presents management to overcome the failure of previous endodontic treatment with nonsurgical endodontic retreatment.

Case: A 66 years old male patient came with a dull pain of tooth no 16 and uncomfortable when used for chewing since 2 months after endodontic treatment. The episodic swelling appeared since 3 months ago which then deflated. The objective examination showed a positive response to bite test and percussion test. Intraoral periapical radiograph confirmed an underfilled root canal, and a periapical radiolucency with a diffuse border on palatal root.

Case Management: Based on the patient’s history taking, radiographic, and clinical examination, endodontic retreatment was done and followed by porcelain fused to metal crown restoration.

Conclusion: Endodontic retreatment is the appropriate treatment option to overcome the failure of the previous endodontic treatment that accompanied with a periapical lesion and to preserve its function in stomatognatic system.

Keywords: endodontic retreatment, endodontic failure, chronic periapical abscess

Introduction

There are many causes of endodontic treatment failure including imperfect obturation, root perforation, external root resorption, periodontal-periradicular lesions, overfilling, the presence of a left root canal, periapical cysts, the lagging of broken instruments in the root canal, perforation of the nasal foramen and coronal leakage.

The main differences between endodontic therapy and endodontic retreatment are the necessity to remove filling materials from the root canals. A good retreatment procedure required no visible debris observed on the instrument flutes. Cases of retreatment always begin with a careful examination of the endodontic morphology of the involved tooth on pre-operative radiographs, because many times the failure of the first performed therapy is caused by a missed and untreated root canal. To be able to find all the canals of a tooth root and biomechanically treat and obturate, a knowledge of root canal morphology is needed.
Persistent microbiological infection plays an important role of endodontic failure. One of the abnormalities that appear after endodontic treatment is a chronic periapical abscess. Chronic abscesses may not cause pain or only cause mild pain. Radiographic examination often shows a diffuse area of rarefaction of bone and thickened periodontal ligament.

This case report presents management to overcome the failure of previous endodontic treatment with nonsurgical endodontic retreatment in underfilled root canal with a chronic periapical abscess.

Case Report

A 66 years old male patient came to the dental hospital of Universitas Airlangga with a dull pain of tooth no 16 and uncomfortable when used for chewing since 2 months after endodontic treatment. The episodic swelling appeared since 3 months ago which then deflated. A week ago, the throbbing pain appeared and access opening were done by the previous General Practitioner. The patient has no medical history.

The objective examination showed there was a metal crown on tooth no 16 (Fig 1A), bite test (+), percussion test (+), vitality (-), gingivitis (+). Intraoral periapical radiographic confirmed that the patient had a deficient previous root canal treatment, with inadequate obturation, a radiopaque view on the root canal from pulp chamber to apical third, and periapical radiolucency with a diffuse border on palatal root (Fig 1B).

Treatment planning was endodontic retreatment with balance force preparation technique and single cone obturation technique. The final restoration chosen was fiber post and porcelain fused to metal crown.

Under patient’s consent, endodontic retreatment started with the removal of the metal crown and the threaded post (Fig 2), then rubber dam was placed for isolation. Access opening and rewalling was performed. The removal of gutta percha using retreatment files (Dentsply Maillefer, Baillagues, Switzerland) was done, followed by taking the radiographic image to evaluate the result of the gutta percha removal. After Glide path using K-Files #10 (Dentsply Maillefer, Baillagues, Switzerland) was established, a combination of electronic apex locator (Root ZX, J. Morita Corp, Tustin, California, USA) and periapical radiographs were used.
to estimate working lengths. Biomechanical preparation was performed using Reciproc R40 (VDW, Munich, Germany) on the palatal root canal; Reciproc R25 (VDW, Munich, Germany) on the mesiobuccal and distobuccal root canals. The motor used for reinstrumentation of the root canals was the VDW Silver Reciproc (VDW, Munich, Germany). The canals were irrigated with 2.5% NaOCl and aquadest throughout these processes. Gutta point trial radiographic image was taken (Fig 3), followed by the final irrigation with 2.5% NaOCl, 17% EDTA, and 2% Chlorhexidine Gluconate. Each time the irrigation solution was replaced, it was irrigated with sterile aquadest and activated using the EDDY system. The root canal was dried with sterile paper points. Intracanal medication (calcium hydroxide paste) was applied.

Fig 2. Clinical image after the metal crown was removed (with threaded post)

Fig 3. Radiographic image

A week after the first visit, the clinical examination showed palpation test (-), percussion test (-), swelling (-), normal periodontal tissue and temporary filling was in good condition. The tooth was isolated using rubber dam, then the canals were irrigated with 2.5% NaOCl, 17% EDTA, and 2% Chlorhexidine Gluconate. Each time the irrigation solution was replaced, it was irrigated with sterile aquadest and activated using the EDDY system. The root canal was dried with sterile paper points. Single cone technique was used to fill the root canals using R40 and R25 gutta-percha (VDW, Munich, Germany) and resin based sealer (Dentsply Maillefer, Ballaigues, Swiss. The radiographic image was taken to confirm the result of the obturation on the root canals (Fig 4).

Figure 4. Obturation

On the third visit, a week after the second visit, the clinical examination showed palpation test (-), percussion test (-), normal periodontal tissue and temporary filling was in good condition. Rubber dam was placed to isolate the tooth during the removal of the gutta percha for preparation of fiber post insertion. After the fiber post was inserted, preparation for the crown was performed, and the impression of mandibular and maxillary teeth were made. Bite registration were made, and the shade selection was performed using shade guide, (Vitapan3D master, Vita Zahnfabrik, Bad Sackingen, Germany). The shade chosen was 3M-3, then the provisional crown was inserted.

Six months after the third visit, the clinical examination showed percussion test (-), normal periodontal tissue and the provisional crown was in good condition. The provisional crown then removed and replaced with the porcelain fused to metal crown (Fig 5A) using luting cement (Fuji I Glass Ionomer Luting Cement, GC Europe). The occlusion and articulation were checked using articulating paper.
Fig 5. A : Porcelain fused to metal crown insertion (Buccal view); B : Post operative radiographic image

Follow up was performed six months after endodontic retreatment, the clinical examination showed no abnormality, percussion test (-), normal periodontal tissue and the porcelain fused to metal crown was in good condition. Radiographic examination showed healing of the periapical lesion (5B).

Discussion

There are many factors that affect the success of endodontic treatment. All this time, endodontic failure has been associated to persistent infection of canals 8. Cleaning and shaping technique, and also the measurement of the working length is the key of successful endodontic retreatment 9. An inadequate obturation or overextended filling often plays a role in endodontic failure. Success rates are naturally lower for obturations which are under or overextended 10.

Based on several studies, in different European countries, there is a large number of teeth needed to be retreated because of periapical lesion appeared from poor root-canal treatment. In 2004, Friedman reported healing rates of nonsurgical retreatment range between 74% and 98% 11.

In this case report, the persistence of bacteria in the root canal occur because of inadequate obturation, resulting a periapical lesion, which was a periapical abscess. Chronic abscesses may not cause pain or only cause mild pain, which sometimes, the patients is unaware to the symptoms. Therefore, non surgical endodontic treatment is needed to overcome the failure of the previous endodontic treatment.

Since the first-line treatment option for previously treated tooth is nonsurgical retreatment 12, in this case report, those treatment was chosen. The function of retreated tooth can be maintained for a long time, even for a lifetime 9.

The reciprocating, alternating or oscillatory movement in Reciproc file system (balanced force), is a system with a single-use file 13,14. Using the reciprocal file system to remove the previous root filling materials is the most rapid method compared with other retreatment instruments 9.

Conclusion

Endodontic retreatment is the appropriate treatment option to overcome the failure of the previous endodontic treatment that accompanied with a periapical lesion and to preserve its function in stomatognatic system.

Ethical Clearance: Nil

Source of Funding: By self

Conflict of Interest: No
References


Harlequin Ichthyosis is the Most Severe Form of the Congenital Ichthyosis; A Case Report Study

Nasim Talebi-Azar¹, Mayam Rasouli², Babak Choobi Anzali², Rasoul Goli³, Jaizeh Karimi Johani⁴, Navid Faraji³, Amireh Hassanpour⁵, Aynaz Bagherzadi³

¹Assistant Professor, Psychiatrist, Urmia University of Medical Sciences, Urmia, Iran, ²Assistant Professor, Department of Emergency Medicine, Urmia University of Medical Sciences, Urmia, Iran, ³MSc, Department of Medical-Surgical Nursing, School of Nursing and Midwifery, Urmia University of Medical Sciences, Urmia, Iran, ⁴MSc, Department of Community Health Nursing, Tabriz University of Medical Sciences, Tabriz, Iran, ⁵MSc, Department of Critical Care Nursing, School of Nursing and Midwifery, Urmia University of Medical Sciences, Urmia, Iran

Abstract

Harlequin ichthyosis (HI) is a rare and the most severe form of the congenital ichthyosis with an autosomal recessive inheritance. At birth, the HI phenotype is striking with thick hyperkeratotic plate-like scales with deep dermal fissures, severe ectropion, among other findings. Although HI infants have historically succumbed in the perinatal period related to their profound epidermal compromise, the prognosis of HI infants has vastly improved over the past 20 years. The disease might be lethal at birth and the affected babies are often premature. The present study reports a new case with HI and adds to the collective knowledge of this rare skin disorder.

Keywords: Harlequin ichthyosis, Gene, Mutation, Autosomal recessive, Case report, Iran

Introduction

Harlequin ichthyosis (HI) is a genetic disorder that results in thickened skin over nearly the entire body at birth.¹ The skin forms large, diamond/trapezoid/rectangle-shaped plates that are separated by deep cracks. These affect the shape of the eyelids, nose, mouth, and ears and limit movement of the arms and legs.² Restricted movement of the chest can lead to breathing difficulties. These plates fall off over several weeks.³ Other complications can include premature birth, infection, problems with body temperature, and dehydration. The condition is the most severe form of ichthyosis, a group of genetic disorders characterized by scaly skin.²

HI is caused by mutations in the ABCA12 gene. This gene codes for a protein necessary for transporting lipids out of cells in the outermost layer of skin. The disorder is autosomal recessive and inherited from parents who are carriers.⁴ Diagnosis is often based on appearance at birth and confirmed by genetic testing.⁵

Before birth, amniocentesis or ultrasound may support the diagnosis.⁴ Newborns with HI present with thick, fissured armor-plate hyperkeratosis. Sufferers feature severe cranial and facial deformities.⁶ The ears may be very poorly developed or absent entirely, as may the nose.² The eyelids may be everted (ectropion), which leaves the eyes and the area around them very susceptible to infection. Babies with this condition often bleed during birth. The lips are pulled back by the dry skin.⁷

Joints are sometimes lacking in movement, and may be below the normal size. Hypoplasia is sometimes found in the fingers. Polydactyly has also been found on
In addition, the fish mouth appearance, mouth breathing, and xerostomia place affected individuals at extremely high risk for developing rampant dental decay.\(^2\)

Patients with HI are extremely sensitive to changes in temperature due to their hard, cracked skin, which prevents normal heat loss. Respiration is also restricted by the skin, which impedes the chest wall from expanding and drawing in enough air.\(^8\) This can lead to hypoventilation and respiratory failure. Patients are often dehydrated, as their plated skin is not well suited to retaining water.\(^3\)

There is no cure for the condition. Early in life, constant supportive care is typically required. Around half of those affected die within the first few months; however, retinoid treatment can increase chances of survival.\(^9\) Children who survive the first year of life often have long-term problems such as red skin, joint contractures and delayed growth.\(^7\)

**Case presentation**

A 26-year-old woman, gravida 3, was admitted to Kosar Hospital in Urmia, Iran at 29 weeks of gestation because of oligohydramnios. There was no family history of harlequin ichthyosis. Ultrasound examination showed a 28 gestational weeks fetus with oligohydramnios. A male baby with HI was born via normal spontaneous vaginal delivery. Her birth weight, length, and head circumference was 2.4 kg, 43 cm, and 28 cm, respectively [see Figure 1]. Parents had a distant relation and had two other normal healthy children. Thick skin with deep fissures, general hyperkeratinization, cyanosis, flat fontanels, ectropion, immature eyes and auricles, eclabium, bradycardia, bradypnea, and moaning were noted in the physical examination. Antibiotic therapy and conservative treatments were started after admission to the neonatal intensive care unit. However, the newborn died after two weeks.

![Figure 1: The newborn with open wide mouth, abnormal eyes, and flatted nose and e](image-url)
Discussion

HI is a rare and extremely severe form of congenital ichthyosis, with an incidence of about 1 in 300000 births. Prenatal diagnosis is usually difficult because of nonspecific signs in the ultrasonographic examination and rareness of the disorder. Delivery of a child with congenital ichthyosis identifies a family at risk, and for subsequent pregnancies prenatal diagnosis can be offered. This report is a typical example of all of these issues.

Mutations in the ABCA12 gene have been reported in the majority of HI patients. This gene plays a major role in transporting lipids to cells that form the epidermis and the normal development of the skin. At birth, infants are covered with hard hyperkeratonic armor, composed of large, thick, yellowish brown, and very sticky plates. After birth, deep red fissures occur on these hard and inflexible plates that extend to the dermis, resulting in a joker-like skin. Infants with HI might have microcephaly, ectropion, and eclairbium.

External auditory meatus and nostrils appear rudimentary and immature. In addition, patients with HI have respiratory failure as a result of restricted chest expansion and skeletal deformities. Feeding problems may result in low blood sugar, dehydration, and kidney failure. In addition, temperature instability and infection would be common. Almost all these clinical features were observed in the current case.

The mortality of HI is high and most of the victims die within a few weeks of birth because of secondary complications such as infection and dehydration. However, survival contributes to the type of mutations; victims with the compound heterozygote mutation survive more than those with the homozygote mutation. In addition, advances in the postnatal treatments and cares improve the prognosis of the disease. The survival rate increases to more than 50% with early prescription of oral retinoids. The patients’ quality of life improves with supportive cares. In addition to the routine care such as checking vital signs, patients should be kept in a warm and humid incubator. Hydration should be performed. As accessing to the peripheral vessels can be difficult, an umbilical venous catheter might be needed. Taking shower twice per day, saline compresses and gentle emollients must be used to keep the skin soft and to accelerate the desquamation. Water and electrolyte disturbances must be managed as well. Environment must be cleaned up to prevent infection; hence, repeated cultures of the skin would be essential to detect the hazardous microorganisms.

Conflict of Interests: None.

Source of Funding: Self.

Ethical Clearance: The study was undertaken after gaining Motahari medical research center/ Urmia Medical University’s approval.

References


Assess the Effectiveness of Breast Crawl Technique on Selected Maternal and Neonatal Outcomes among Mothers Admitted in Labour Ward at Selected Hospitals of Kheda-Anand District, Gujarat

Neha Parmar1, Patel Sonali2, Prajapati Nilam2, Patel Urja2, Christian Richa2, Patel Radhika2, Parmar Mittal2

1Research Guide, Assistant Professor, Obstetrics and Gynecology Department, Dinsha Patel College of Nursing, Nadiad, 2Researcher, B.sc Nursing, Dinsha Patel College of Nursing, Nadiad, 387001, Gujarat.

Abstract

Background: The Investigator conducted a study to Assess the Effectiveness of Breast Crawl Technique on selected, Maternal and neonatal Outcomes Among Mothers Admitted in Labour Ward at selected hospitals of Kheda-anand District Gujarat. was carried out by researcher. The objectives of the study were: (1) To assess the effectiveness of breast crawl technique on maternal and neonatal outcome among experimental group and control group. (2) To find out the association between maternal and neonatal outcomes and selected demographic variables in experimental group. Research Design: A quasi experimental research approach was used with non-equivalent control group post test only design. The researcher used non-probability purposive sampling technique for selecting the 40 samples. Tools: Latch scale was used to assess the initiation of breast feeding, Visual analogue pain scale was used to assess the intensity of pain, and APGAR score was used for newborn assessment. Validity was assessed by 7 experts. Reliability of latch scale, Visual analogue pain scale and APGAR Score was ascertained by using Karl Pearson’s co-relation coefficient formula. Data Analysis & Results: The data will be analyzed by descriptive statistics such as Mean, Standard deviation, Frequency, Percentage. The mean score of effectiveness of breast crawl technique on initiation of breast feeding, intensity of pain, newborn assessment was higher in experimental group than the control group. So it was revealed that there was significant difference between experimental group and control group. For comparison of effectiveness of breast crawl technique on initiation of breast feeding, intensity of pain, newborn assessment in that mean score was higher and calculated “t” test value is 4.83 which is greater than tabulated value 2.09. So, that the research hypothesis is accepted and proved that there will be significant association between initiation of breast feeding, intensity of pain, newborn assessment between the experimental group and control group. The finding of the study also revealed that there was significant association between the initiation of breast feeding, intensity of pain, newborn assessment with selected demographic variables.

Key Words: Breast Crawl Technique, DF, Labour, Latch Score, Partograph, WHO, SD

Introduction

“With baby’s small head pillowed against mother’s breast and milk which is warming baby inside, Her baby knows closeness to her.”

The woman may experience stress and physical pain and danger may lurk around the corner. The goal of maternity care is a healthy pregnancy with a physically safe and emotionally satisfying outcome for mother, infant, and family. The uterus and the placenta have provided warmth, protection, nutrition, and oxygen as
well as close and continual proximity to the mother's heart and voice. After birth, the mother's body and breasts take over the function of the uterus and placenta in providing warmth, protection, nutrition, and oxygenation.

Being skin to skin with the mother is the newborn infant’s "natural habitat" - the one place where all his needs are met. Breast crawl is the most natural spontaneous and logical method of initiating breastfeeding. It is the simplest method that provides prolonged skin to skin contact and will start the first feed. Every newborn, when placed on mother’s abdomen soon after birth, has the ability to find its mother’s breast all on its own and to decide when to take the first breast feed. Breast crawl also helps mother to have good uterine contraction and fastens expulsion of placenta, decreases maternal blood loss, and prevents anemia. The promotion of early initiation of breast feeding has great potential; 16% of neonatal deaths could be saved if all infants were breastfeed from day 1 and 22% if breastfeeding was started within the first hour after birth. Improving initiating rates, breast feeding success, and reducing neonatal, infant, and under-five mortality rates and morbidity by early initiation of breastfeeding.

Need of Study

The first hour after birth has a measure influence on the survival, future health, and wellbeing of a newly born infant. The best time to start breast feeding is within 20-30 minutes of the new-born’s birth, if there are no complications with delivery. It helps to promote mother-infant bonding and immune protection to the newborn by the first breast feed. The basic needs of a baby at birth are warmth, normal breathing, mother’s milk, and protection from infection. Early initiation of breast feeding serves as the starting point for a continuum of care for mother and newborn that can have long-lasting effect on health and development.

WHO and UNICEF recommended early initiation of breast feeding which results in lower neonatal mortality. Each year approximately 4 million newborn die, mostly from preventable causes. Deaths in the neonatal period accounts 41% of all deaths in children below five years and almost 99% of neonatal deaths takes place in low and middle income countries. In which India is leading with 28% of global neonatal deaths. Evidence shows that early initiation of breast can prevent 22% of all deaths among babies below one month in developing countries. About 16% of neonatal deaths could be prevented if all infants were breastfed from day 1 and 22% if breastfed within 1st hour after birth.

Dr. Sarah Buckley studied that during breast crawl technique immediately after birth benefit the mother who release high level of oxytocin which help the uterus to contract and help in preventing excessive bleeding. Moreover, maximum benefits of early initiation of breast feeding are best achieved with breast crawl.

Objective of The Study

1. To assess the effectiveness of breast crawl technique on maternal and neonatal outcome among experimental group and control group.

2. To find out the association between maternal and neonatal outcomes and selected demographic variables in experimental group.

Hypotheses

• H1 – There will be significant difference between the breast crawl technique on maternal and neonatal outcomes in experimental group at 0.05 level of significance.

• H2 – There will be a significant association between the maternal and neonatal outcomes among mothers with selected demographic variables at 0.05 level of significance.

Material and Methods

— Research approach: Quantitative approach.

— Research design: quasi-experimental research approach, Non-Equivalent control group post-test-only design.

— Target population: mothers who are in labour in selected hospital of Kheda-Anand district, Gujarat.
— **Accessible population:** mother who deliver full term neonate without any complications.

— **Sampling technique:** non probability purposive sampling technique

— **Sample size:** 40 mothers (20 experimental group and 20 control group)

— **Data collection tool:** standardized tool was used.

1. **LATCH SCALE**” for assessment of initiation of breast feeding,

2. **VISUAL ANALOGUE PAIN SCALE”** for assessment of intensity of labour pain

3. **CLINICAL OBSERVATION CHECKLIST”** for assessment of uterus involution

4. **VIRGINIA’S APGAR SCORING SYSTEM”** for newborn assessment .

— **Data analysis:** Descriptive statistics and Inferential statistics

— **Criteria measure:** Maternal Outcome (initiation of breastfeeding, intensity of labor pain, assessment of uterus involution and neonatal outcome (APGAR Score)

**Results and Discussion**

1. Findings related to demographic variables of samples

   majority samples for age 8(2Q0%) sample were of 18-20 years, 12(30%) sample were of 21-23 years, 14(35%) samples were of 24-26 years, 6(15%) were of 27-30 years. In the stream of education 10(25%) samples were from primary education, 17(42.5%) samples were from higher education, 10(25%) samples were graduated and above, 3(7.5%) samples were illiterate. In occupation 3(7.5%) samples were government employee, 6(15%) samples were private employee, 31(77.5%) samples were housewife, 0(0%) samples were in business. In religion 23(57.5%) samples were Hindu, 6(15%) samples were Christian, 11(17.5%) samples were Muslim. In type of family 21(52.5%) samples were from nuclear family, 19(47.5%) samples were from joint family, 0(0%) samples were from single parents. In parity 17(42.5%) samples were from first parity, 16(40%) samples were from second parity, 7(17.5%) samples were from third parity, 0(0%) samples were from more than three parity. In duration of 1st stage of labour 13(32.5%) samples were from 4-6 hours, 8(20%) samples were from 7-9 hours, 16(40%) samples were from 10-12 hours, 2.5% samples were from 13-15 hours. In duration of 2nd stage of labour 21(52.5%) samples were from ½-1 hour, 15(37.5%) samples were from 1½-2 hours, 4(10%) samples were from 2½-3 hours. For gender of baby 17(42.5%) samples have male child, 23(57.5%) samples have female child. For birth weight of new born 24(60%) samples have 2500-3000 grams of child, 13(32.5%) samples have 3100-3500 grams of child, 3 (7.5%) samples have 3600-4000 grams of child.

2. Mean, mean difference, standard deviation, ‘t’ test value of experimental and control group on initiation of breast feeding

<table>
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<th>Mean difference</th>
<th>SD</th>
<th>Calculated ‘t value</th>
<th>Table ‘t’ value</th>
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<td>2.09</td>
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</table>
Above table, reveals that the mean score of the experimental group is significantly greater than the mean score of the control group. The calculated ‘t’ value (t=8.68) is greater than the tabulated ‘t’ value (t=2.09). Therefore, the null hypothesis H01 is rejected and research hypothesis H1 is accepted and it reveals that breast crawl is effective in reducing the initiation of breast feeding among the sample.

3. Mean, mean difference, standard deviation, ‘t’ test value of experimental and control group on intensity of pain.

<table>
<thead>
<tr>
<th>Intensity of pain</th>
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<th>Mean difference</th>
<th>SD</th>
<th>Calculated ‘t’ value</th>
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<td>6.68</td>
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<td>1.38</td>
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</table>

Above table reveals that the mean score of the experimental group is significantly greater than the mean score of the control group. The calculated ‘t’ value (t=4.73) is greater than the tabulated ‘t’ value (t=2.09). Therefore, the null hypothesis H02 is rejected and research hypothesis H2 is accepted and it reveals that breast crawl is effective in reducing the intensity of pain among the sample.

4. Mean, mean difference, standard deviation, t-test value of experimental and control group on newborn assessment.

<table>
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<th>Neonatal wellbeing</th>
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<th>Mean difference</th>
<th>SD</th>
<th>Calculated ‘t’ value</th>
<th>Table ‘t’ value</th>
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<td>0.98</td>
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</table>

Above table, reveals that the mean score of the experimental group is significantly greater than the mean score of the control group. The calculated ‘t’ value (t=4.83) is greater than the tabulated ‘t’ value (t=2.09). Therefore, the null hypothesis H01 is rejected and research hypothesis H1 is accepted and it reveals that breast crawl is effective in the newborn outcome among the sample.

5. Findings related to association between maternal outcome with the selected demographic variables of samples

The findings of the study revealed that there was no significant association between maternal outcome with selected demographic variables. The calculated Χ² values of the demographic variables was less than the tabulated Χ² values of the demographic variable at 0.05 level of significant. It reveals that there was no significant association between the maternal outcome with selected demographic variables in experimental group.

6. Findings related to Association between neonatal outcome with the selected demographic variables of samples

The findings of the study revealed that there was no significant association between neonatal outcome and selected demographic variables. The calculated Χ²
values of the demographic variables was less than the tabulated $X^2$ values of the demographic variable at 0.05 level of significant. It reveals that there was no significant association between the neonatal outcome with selected demographic variables in experimental group.

**Conclusion**

The following conclusions can be drawn from the present study findings:

To mark a good beginning, the process and experience of labour should not be a misery for a mother. There are variety of discomfort that women will experience during the labour. Reducing the discomfort is an important part of good nursing care. Breast crawl enhances the initiation of breast feeding early. Nurse should have a vital role in early initiation of breast feeding, reduction of labour pain and newborn assessment.

The findings indicated that the breast crawl technique was effective method in early initiation of breast feeding, reduction of labour pain and newborn assessment of mother admitted in labour at selected hospital of Anand-Kheda district, Gujarat.

**Ethical Clearance:** Taken from institutional ethical committee, DPCN, and got permission from superintendent of selected hospitals of Kheda –Anand District, dated 30/1/2020

**Conflict of Interest:** Nil

**Source of Funding:** Self

**References**


The Effect of Capitation Value of Healthcare and Social Security Agency on Service Quality at Primary Clinics

Neny Nurlaily¹, Titik Ernawati¹, FaraValeyria Irma Zain¹, Chomariyah²

¹Student of Master of Law Program, Hang Tuah University, Surabaya, Indonesia, ²Associate Professor, Faculty of Law, Hang Tuah University, Surabaya, Indonesia

Abstract

Indonesia started to apply the National Health Insurance System on January 1, 2014, and the legal entity formed to administer this program is the Healthcare and Social Security Agency. Funding for the Healthcare and Social Security Agency at primary clinic uses the capitation system where the funds are mostly used for services and operations. The use of capitation funds at the primary clinic is fully managed by itself based on the agreement. This actually creates the potential for fraud because of the lack of regulations on using the capitation funds. Fraud at the primary clinic does not really appear on the surface like a fraud in hospitals. It can be in the form of insufficient resources, facilities and service discrimination. Many studies have shown a link between capitation funds and health services. A primary clinic needs to have an audit team to evaluate whether the use of capitation funds is following the expected targets and needs.

Key Words: Healthcare and Social Security Agency, Fraud, Capitation, Primary Clinic, Quality, Services.

Introduction

Health development is integral and most important part of national development, as stated in the 1945 Constitution. Implementing health development aims to increase awareness, willingness, and ability to live a healthy life for everyone to achieve an optimal degree of public health. The success of health development plays an important role in improving service quality and competitiveness of Indonesian human resources.¹ ²

The National Health Insurance System has been implemented in Indonesia since January 1, 2014, and is planned to achieve universal health coverage by 2019. Act No. 40 the Year 2004 on the National Social Security System defines as a procedure for administering social security programs by the social security administering agency. A legal entity established to administer the health social security program is the Healthcare and Social Security Agency.³

The Healthcare and Social Security Agency has the authority to make contracts with healthcare facilities that provide individual health services, both promotive, preventive, curative, and rehabilitative, carried out by the government, regional government, and/or the community. The agency also has the duty to pay the benefits and/or costs of health services based on the provisions. ⁴

Authorized Primary Care Facilities provide non-specialized personal health services. This includes the needs for observation, diagnosis, treatment, treatment and other health services. Health service facilities as a media to carry out health efforts for the community.⁵

One of the Authorized Primary Care Facilities provided by the government is community health centres, while private services such as clinics and independent practice doctors.⁶ The Healthcare and Social Security Agency has 28,000 health service facilities tied in contracts, including 237 main clinics and 6,535 primary clinics.² The clinic receives funding from The Healthcare and Social Security Agency on a capitation basis.
Capitation is the amount of payment per month paid in advance to the Authorized Primary Care Facilities based on the number of registered participants regardless of the type and number of health services provided. The capitation rate is strongly influenced by the utilization rate of health services, and the types of health insurance packages (benefits) offered and unit costs service. The formula for calculating capitation is “the utilization rate multiplied by the unit cost”. The utilization rate is the utilization of health service facilities owned by Authorized Primary Care Facilities, expressed in percent. The utilization rate is influenced by the characteristics of the population, the service system’s nature, the benefits offered and the insurance policy. Utilization is the number of visits per 100 people in a certain population or number of visits/total population x 100%. 

Capitation is given to authorized primary care facility or Authorized Primary Care Facilities based on the number of participants served in an area. The capitation model allows the doctor or Authorized Primary Care Facilities management to clearly calculate the income, based on the number of dependent participants in the area. Research shows that the use of capitation funds in clinics is mostly to pay for services and the rest for operations.

Capitation funds for authorized primary care facilities are generally insufficient, especially for drugs and laboratory costs. The use of capitation funds by Authorized Primary Care Facilities is to provide health services, both preventive and curative services. This article aims to review the effect of the BPJS capitation value on Primary Clinic services’ quality.

**Discussion**

**The Healthcare and Social Security Agency and Capitation Fund**

Indonesia began to apply the The National Health Insurance System system in 2014 and is targeted to reach Universal Health Coverage by 2019. The legal entity established to implement this program is the Healthcare and Social Security Agency. The Healthcare and Social Security Agency has a task to pay for each benefit and/or costs of health services based on the provisions. The development of the Healthcare and Social Security Agency services must have a service quality control system and payment system for the efficiency and effectiveness of health insurance.

National Health Insurance is one part of the National Social Security mandated by the Constitution. The 5th precept of Pancasila is the reason for the birth of the amendment to the 1945 constitution article 28H, which outlines special provisions regarding the social rights of citizens which the state must guarantee, including the right to health. 

The Healthcare and Social Security Agency is to collect contributions paid by participants, then distributed on a capitation basis to optimize services. Capitation is one of the models used in payments to Authorized Primary Care Facilities in the The National Health Insurance System era. Capitation funds are distributed to contracted Authorized Primary Care Facilities by the Healthcare and Social Security Agency. The use of capitation funds at the Primary Clinic is fully managed by itself. This actually creates the potential for fraud because of the lack of regulations on using the capitation funds.

The capitation system does not rule out the fraud, for example, the primary clinic, is not following statutory provisions, manipulates claims on non-capitally paid services, receives commissions for referrals to Authorized Primary Care Facilities, charges fees from participants that should have been guaranteed in capitation fees and/or non-capitation by the stipulated standard rates, making patient referrals only to obtain certain benefits; and/or other The National Health Insurance System fraudulent actions.

The primary clinic’s standard capitation rate is Rp. 8,000.00 (eight thousand rupiahs) to Rp. 10,000.00 (ten thousand rupiahs) per participant per month. The capitation rate calculation then considers the criteria for human resources, completeness of facilities and
infrastructure, service scope, and service commitment.

The average number of capitation received by the Primary Clinic in Siswoyo’s study was 39.4 million rupiahs, increasing the number of capitation funds by 9%. This made the Primary Clinic the Authorized Primary Care Facilities with the highest increase in capitation value. The utilization of capitation funds in terms of services at Primary Clinics generally tends to be higher than doctor/dentist practices. The ratio between operational costs and services at the Primary Clinic is 45%: 55%.15

Payment for health services obtained by health workers (doctors) is based on the number of capitation funds at the primary clinic. Primary Clinic, with large capitation funds, provides health services based on attendance and performance. Primary Clinic, with a small capitation fund, provides health services on a per-patient visit basis.16

Most non-health workers get paid for services below the Regional Minimum Wage. This is influenced by education, length of work, and the number of capitation funds the clinic receives. If the amount of capitation funds is large, health and non-health workers’ salaries will also be large. The capitation funds received by Primary Clinic are also used to support health operational costs.16

Primary clinics with no collaboration with pharmacies are generally required to have pharmaceutical services with the pharmacist as the person in charge and procure drugs. In contrast, clinics with pharmacies services can use capitation funds to purchase medical devices and other health services.

Ghana is one of the countries that first introduced the capitation payment method in 2012, but there is a lot of resistance due to people’s perception of poor service quality. There are three regions have a good perception of health services, while one region has a poor perception. The regions with good perceptions are areas with large capitation funds, while one other, with poor perception, is small capitation fund area.17

**Overview of the Quality of Health Services**

Service quality is an approach to running a business to maximize organizational competitiveness through continuous improvement or its products, workforce, processes and environment. A customer-focused marketing work strategy, satisfaction assurance, teamwork and employee empowerment is required.18

The influence of service attitudes on patient interest in using health care facilities. Measurement of service quality to obtain the best reliability response where Authorized Primary Care Facilities always provides the best physical appearance services (tangibles), responsiveness, assurance, and empathy through a dynamic attitude as an overall service evaluation.19

The availability of resources, patient cooperation, and collaboration among health workers can support health service quality improvement. Supportive leadership, proper planning, education and training and effective management of resources and processes also improve medical services quality.20

Patient satisfaction is closely related to the quality of health service providers.21 Trust is an important factor for the service industry to maintain customer satisfaction. Medical service is a type of intangible product with more than one service to involve. Both medical care workers and general service workers must develop trust with patients to increase patient satisfaction.22

The four main factors contributing to the quality improvement process are leadership, including the leader’s awareness and attitude towards quality improvement, the involvement of the leader in the quality improvement process, and decision-making in budget allocation for quality improvement. The second factor is staff enthusiasm and multidisciplinary collaboration, followed by organizational culture as the third factor. The last factor is the standardization process.23

The success of improving quality in Indonesia’s existing decentralized system requires action at four levels. At the individual level, leadership tools can
create an internal quality environment and foster an organizational culture to change. Staff enthusiasm and collaboration can be sparked at the team level by involving and assigning everyone in the quality improvement process and having a shared vision of what quality should be. At the organizational level, the quality improvement must be integrated into planned activities, ensuring financial and human resources.²⁴

Quality improvement is carried out by improving the medical and non-medical aspects. Some medical aspects have a significant role in improving quality services such as waiting time, communication, clear information, straightforward administration, additional services such as a comfortable room, and drinking water. Medical aspects include training and education for health workers.²⁵

**Overview of Service Quality Based on Capitation Funds at Primary Clinic**

The capitation funding system used by the Healthcare and Social Security Agency for health financing at the Authorized Primary Care Facilities causes the number of funds received by the Primary Clinic to match the number of participants at the clinic. The more participants, the greater the capitation funds the Clinic will receive. ²⁶

The disparity in The National Health Insurance System membership occurs due to The National Health Insurance System participants (Contribution Assistance Recipients) according to the area where they live and the percentage of poor people as the The National Health Insurance System participants. This is consistent with the results of the Ministry of Health evaluation in 2015. It was said JKN participants registered at the Authorized Primary Care Facilities are not ideal.²⁶ Each Participant is entitled to receive Health Insurance Benefits in health services, such as medical benefits, accommodation and ambulance.

The capitation fee for the Primary Clinic is Rp. 8,000 (eight thousand rupiahs) to Rp. 10,000 (ten thousand rupiahs). This calculation is not influenced by how many patients go to Primary Clinic, so capitation may not be sufficient if the Primary Clinic serves patients repeatedly within a month.

The Primary Clinic’s various obligations as above are as if the Primary Clinic was being forced to carry out heavy obligations with low capitation. Doctors do have a clear legal basis in determining their service definition, but according to article 53 of Act No. 29 the Year 2004 on Medical Practice, patients who have received medical practice services have an obligation to provide compensation for the services they receive.²⁹

For example, fees can be completely waived or reduced if medical expenses are too heavy for patients with economic difficulties. This condition is not a problem for private practising doctors. Still, it is certainly different for Primary Clinic because its status is a business entity with many employees and operational needs; of course, capitation funds are vital for the survival of the Primary Clinic.

The ratio of doctors to The National Health Insurance System participants is 1: 6,765, still higher than the national standard of 1: 5,000 participants, even higher than the national average of 1: 6,708 participants. The high ratio of participants results in a decrease in the quality of service time. The number 1: 5,000 is obtained if 1 doctor serves the patient for 10 minutes per patient, with examinations for 5 hours and 25 working days, then if the length of time the doctor checks each patient who visits, ideally 15-20 minutes or 18-20.9 minutes.²⁶

Ideally, doctors’ ratio to participants is a maximum of 1: 2,500 participants if it is taken for just 20 minutes, with 15% participant visits. Clinics or Authorized Primary Care Facilities with large capitation funds have more flexibility in managing them, whereas those with small capitation funds have limitations in allocating their capitation funds. This will lead to neglect of service to some people.²⁶

Opportunities for fraud that could harm The National Health Insurance System participants in terms of the limitation of health services and access, including
discrimination to health services for participants and non-participants of JKN, limited basic service facilities, an improper work system leading to detrimental to The National Health Insurance System participants and very complicated procedures for participants to get health services.27

Weak internal controls facilitate fraud to grow and develop rapidly beside an inadequate external monitoring system. It makes fraud detection is not optimal. There are two mechanisms to prevent fraud. The first is to implement pre-employment screening, and the second is to implement mechanisms (internal controls) during the service time. An internal control system is clearly necessary for business success. A business needs to have some control to eliminate the element of opportunity.27

Discrimination is an unfair and unbalanced treatment to distinguish between individuals or groups, based on criteria, usually categorized or differentiating attributes. Theoretically, discrimination sometimes occurs through policies to reduce, destroy, conquer, move, protect legally, create cultural pluralism and assimilate other groups. In this regard, the differentiation of services and types of time for The National Health Insurance System participants is one example of discrimination.27

The service sharing system is considered valuable to employees, and it gives a positive perception of the service sharing system. Employees with the perception that the benefits they receive are not sufficient generally will try to find a better-rewarded place to work. Incentives can also affect the performance and presence of health workers at a primary clinic. Research in Banda Aceh showed that the distribution of capitation funds affects health workers’ performance in providing services. As the researchers suggest, Authorized Primary Care Facilities management can increase the distribution of capitation funds to each health worker to achieve better performance and provide health services as well as possible.28 This can be a form of service discrimination and fraud at the Primary Clinic.

Human resources are the potential to realize their role as adaptive and transformative social beings who can manage themselves and all the potentials around them to achieve balanced and sustainable life welfare. An effort to support productivity and activities needs competent and quality human resources to achieve the goals of a country or organization successfully.

Human resource skills play an important role in managing community service. The quality of human resources is one of the important factors for the continuity and progress of an organization since it can directly affect the organization’s performance. The main focus of human resource management is to contribute to organizational success. In clinics, unreliable human resources become a form of fraud.

The key to improving public and private health organizations’ performance is ensuring human resource activities support organizational efforts on productivity, service, and quality. Human Resource performance appraisal plays an important role in improving organizational performance. It is essential, especially for the services provided by human resources which support holistic health services.

<table>
<thead>
<tr>
<th>Big Capitation Funds</th>
<th>Small Capitation Funds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity is met, quality is in process.</td>
<td>Quantity and quality are not met.</td>
</tr>
<tr>
<td>High utilization by the community</td>
<td>Low utilization by the community</td>
</tr>
<tr>
<td>Low quality of record reporting</td>
<td>Better reporting quality</td>
</tr>
<tr>
<td>Overall performance increases</td>
<td>No performance increase</td>
</tr>
<tr>
<td>The provision of training is still low.</td>
<td>No training provision</td>
</tr>
</tbody>
</table>

Table 1. Difference capitation funds use by Hasan (2017)
Most of the primary clinics have constraints in infrastructure development. It is because infrastructure development costs a lot. Funds for supporting health operational costs are mostly used for infrastructure development. It is known that the unit cost of health services and medicines is a major component in calculating the amount of capitation.

The use of too-high operational costs can reduce the share of other costs utilization. For example, the use of operational costs taken from drug costs can cause the primary clinic to experience a stock out of the drug, disrupting the health service process for BPJS participants. Some small capitation fund primary clinics without pharmacies may experience losses and require bailouts to meet drug needs.

Inadequate facilities and infrastructure led to fraud; for example, The National Health Insurance System participants were sent to private laboratories for examining process. Public health facilities must provide minimum health services in a standard way. Facilities must include a general examination room, a treatment room, family planning and immunization, a dental and oral health room, a nursing room, a health promotion room, a pharmacy room, a birthing centre, a laboratory, and others. Other supporting facilities include waiting/registration rooms, consultation rooms, administration rooms, treatment rooms, action rooms, breastfeeding rooms, bathrooms, etc.

Activating internal controls can prevent the occurrence of fraud in the primary clinic. Active internal control is the most widely applied internal control applied. It is like a fence preventing thieves from entering people’s yards; however, a strong fence can still be penetrated by clever and brave thieves. Internal audit is beneficial to prevent the occurrence of greater fraud so that health services can run effectively, efficiently and integrated. The primary clinic must make clear policies and funds in conducting audits based on positive actions.29

Conclusion

The Healthcare and Social Security Agency has tasks to collect contributions paid by the participants, and then distribute it on a capitation basis to optimize services. Capitation is one of the models used in payments to Authorized Primary Care Facilities in The National Health Insurance System. Capitation funds are distributed to Authorized Primary Care Facilities which have collaborated with The National Health Insurance. The capitation system does not rule out the fraud, for example, the primary clinic, is not following statutory provisions, manipulates claims on non-capitally paid services, receives commissions for referrals to Authorized Primary Care Facilities, charges fees from participants that should have been guaranteed in capitation fees and/or non-capitation following the stipulated standard rates, making patient referrals only to obtain certain benefits; and/or other The National
Health Insurance System fraudulent actions. An internal audit team needs to be formed to oversee the use of capitation funds at the primary clinic. The capitation fund currently given to the primary clinic is considered insufficient to run the primary clinic’s operations and infrastructure, especially clinics with limited facilities, such as pharmacies and laboratories.

**Ethical Clearance:** Nil

**Conflict of Interest:** Nil

**Source of Funding:** Self-Funding

**Acknowledgement:** Nil

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Patient Satisfaction with Hospital Services, Physicians and Nurses Care of Government Hospital in Sulaimani City

Bekhal Abdalwahid Amin1, Niaz Mustafa Kamal2, Bestun Ibrahim Hama Rahim3
1Assistant Lecturer, Maternity Nursing Department, 2Lecturer, Pediatric Nursing Department, 3Lecturer, Community Health Department, Sulaimani Polytechnic University

Abstract

Background: Patients’ satisfaction is a person’s feeling of pleasure or disappointment resulting from a service’s perceived performance or outcome to his or her expectations. As this definition makes it clear, satisfaction is a function of perceived performance and expectations. This study was undertaken to evaluate patients’ satisfaction level with the hospital services and health care providers among four government hospitals in Sulaimani city. The data from 311 inpatients admitted to and stayed in the hospital for any medical condition were collected by a face-to-face interview from November 2019 to February 2020. Standardized 4-point Likert scales ranging from satisfying to dissatisfying (1 to 4 points) were used for all the 24 items in the questionnaire. Data analysis was performed using a statistical package of Stata (14. version). A frequency analysis was used for demographic data analysis. A person Chi-square test was employed to determine the association between the categorical independent variables. The participant’s mean age was 37.9 SD 13.5 years with ages ranged from 14-85 years. The majority (86.5%) of the study participants were satisfied with the hospital services and environment while a small number 42 (13.5%) of them were dissatisfied with the issue. Almost 275 (88.4%) of the patients were revealed excellent nurse care and a very small number 36 (11.6%) was showed dissatisfaction. Also, 245 (79.1%) of the patients were satisfied with doctor care. Only 65 (20.9%) of them have a negative aspect of doctor care. A significant difference was found between the level of education and patients’ satisfaction with doctor care (coefficient 0.88, \( p<0.05 \)). Besides, a significant association was found between residence and hospital services (coefficient 0.63, \( p =0.05 \)). In general, we have discovered a satisfaction level among our sample is considered high and indicates good care provided by health facilities in all hospitals. Further study required seeking the least satisfactory factor regarding doctor care.

Keywords: Satisfaction, Shar hospital, nurse, Sulaimani, patients, physician

Introduction

Patient satisfaction has been described as the value and reaction of patients towards the care they received in the hospital and the medical care was performed (1). Patient satisfaction is a central indicator of health care quality and reflects the ability of the provider to meet the patients’ needs (2). So, it is a process as much as an attitude, so it must be, monitored continuously, and frequently measured. Patient satisfaction is essential and must be taken into account in assessing the quality of health care delivered particularly when decisions are being made about changes and enhancement in services. It also is used as an instrument in determining payment rates, especially in the context of a competitive healthcare atmosphere and consumerism (3, 4). Gradually, patients’ satisfaction became an essential component of health care services quality monitoring and improvement.

Corresponding author:
Niaz Mustafa Kamal
Mobile 7701547004
niaz.Kamal@spu.edu.iq
processes (5). Patient satisfaction with nursing care is of great importance to any health care agency because nurses comprise most health care providers and they provide care for patients 24 hours a day (6). Assessing to what extent patients are satisfied with health services is clinically relevant, as satisfied patients are more likely to comply with treatment, take an active role in their care, continue using medical care services, and stay within a health provider (where there are some selections to be made) and maintain with a specific system (7). Nurses are the frontline people that patients most likely meet up with, spend the highest amount of time with and rely upon for recovery during their hospitalization. Nursing care plays a prominent role in determining the overall satisfaction of patients’ hospitalization experience (2). Furthermore, maintaining good technical as well as interpersonal skills is essential for doctors to satisfy their patients (8). Besides, the demonstration of professionalism and ethical practice are also required to meet the expectations of patients. The technical expertise of physicians is regarded as consisting of maintaining an appropriate level of experience, ability to diagnose, the performance of clinical procedures, prescribing medicine, and learning about the latest medical developments. Moreover, the success with technical procedures, treatment, and medication depends upon favorable communication with patients (9, 10). Unlike developed countries, doctors are not made to comprehend the importance of ethical practice and communication skills during medical training. Physicians working in public hospitals deal with patients of a lower socio-economic class with negligible health awareness and poor hygiene. Understanding the patients and making them understand is the big challenge with which physicians are confronted in public outpatient clinics in developed countries (10, 11). Patients’ satisfaction of their relation with their doctors is a key element in the efficiency and usage of health services and varies depending on patient characteristics. Each patient has expectations when meeting a doctor and the difference between these expectations and what he obtains represent the perception of the satisfaction (12). Many previous studies have developed and applied patient satisfaction as a quality improvement tool for health care providers. Thus, patient satisfaction is an important issue both for the evaluation and improvement of healthcare services (13). The aims of the study: first, to analyze patient satisfaction with doctor and nurse-patient interaction and relationship. Second: to determine patient satisfaction with the quality of hospital and services performed to patients during their hospital stay in Sulaimani.

**Methodology**

This cross-sectional study was conducted from November 2019 to February 2020. The study population consisted of all patients admitted to four government hospitals (Shar Hospital, Shahid Hemen Hospital, Teaching Hospital, and Maternity Teaching Hospital) during the period of study. The four hospitals are located in the center of Sulaimani City. Shar, Shahid Hemen, and Teaching hospital are provided a wide range of care and treatment to adult people. Maternity Teaching Hospital is provided a wide range of care and delivers to women. In this study, the participation of the patients was voluntary. The data from 311 inpatients admitted to and stay in the hospital for any medical condition were collected. Convenient sample size was chosen because this is conservative and adequate when the proportion of participants is unknown. Patients selected for the study included those who were at least 2 days of experience of hospitalization and aged 14 years and over. The patient was interviewed when they were in a good situation and conscious. Accompanying patients were excluded from the study. The authors personally visited the hospitals and all of the respondents (patients) after informed consent was taking were told about the aim of the study and they were encouraged to contribute. Furthermore, the authors guaranteed the privacy of the responses of the participants. However, face-to-face interviews were conducted with patients with no education and education. Each interview session took about 10 to 15 minutes and the data collection process was conducted over a period of 16 weeks.

The questionnaire was originally developed in Kurdish for a better understanding of the local people.
The questionnaire was shared with the experts to ensure face validity, revised and a pre-tested questionnaire was used in this research. A pilot study was done before administering it to a representative sample. The aim was to find out if the questions are clear enough, clearly understood, in the right order and the provided answers are sufficient and detailed enough. The fill-in time was also determined. The questionnaire consists of four sections, the first section include Sociodemographic characteristics (age, gender, residence, occupation, marital state, and education), the second section consists of 11 items on the hospital services and environment, third and fourth section each consist of 7 items on physicians and 6 items for nurses care. The study was approved by the ethics committee of the Technical Institute of Sulaimani and permission was also taken from Sulaimani Directory of health and all hospitals.

Data analysis was performed using a statistical package of Stata (14 version) after the data was entered into the Epidata (3.1). A frequency analysis was used for demographic data analysis. A person Chi-square test was employed to determine the association between the categorical independent variables. Standardized 4-point Likert scales ranging from satisfying to dissatisfying (1 to 4 points) were used for all 24 items. Internal consistency was tested using Cronbach’s alpha coefficient. Scales 1, 2, and 3 were interpreted as being satisfied Patients’ and 4 dissatisfied. Binary logistic regression was subsequently conducted to predict the factors which influence the level of satisfaction.

Results

A frequency analysis was used for the demographic data analysis and general information about the survey participants. The results of the frequency analysis or demographic assessments are presented in Table 1. This includes age, gender, education, residence, marital status, occupation, and education of the participants. One-third of the participants (83.3%) aged 25 and over. The mean age was 37.9 SD 13.5 years with ages ranged from 14-85 years. The majority of the study participants were women (81.1%). A very slight difference was found between inside and outside participants (49.8% and 50.1%) respectively. Of (80.1%) the participants were married while the rest (19.9%) was unmarried. The larger part of respondents had no employment (20.3%). However, most of them were educated (60.1%).

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Gender</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;25</td>
<td>52</td>
<td>16.7%</td>
<td>Male</td>
<td>59</td>
<td>18.9%</td>
</tr>
<tr>
<td>&lt;25</td>
<td>259</td>
<td>83.3%</td>
<td>Female</td>
<td>252</td>
<td>81.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Residence</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Marital state</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside Sulaimani</td>
<td>155</td>
<td>49.8%</td>
<td>Married</td>
<td>249</td>
<td>80.1%</td>
</tr>
<tr>
<td>Outside Sulaimani</td>
<td>156</td>
<td>50.1%</td>
<td>Unmarried</td>
<td>62</td>
<td>19.9%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Education</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employee</td>
<td>63</td>
<td>20.3%</td>
<td>Educated</td>
<td>187</td>
<td>60.1%</td>
</tr>
<tr>
<td>Unemployed</td>
<td>248</td>
<td>79.3%</td>
<td>Uneducated</td>
<td>124</td>
<td>39.9%</td>
</tr>
</tbody>
</table>
The distribution of the participants of four hospitals was presented in Figure 1. There was a similar number (32.2%) of the patients who participated from Shar and Teaching hospital. While (25.6%) was from Maternity teaching hospital and fewer patients have participated from Shahid Hemen hospital was (9%).

3.1- Patient satisfied with hospital services and environment

Analyses of patients satisfied with hospital services and environment were shown in Table 2. A large number of the patients were satisfied with four hospital services 280(90%) out of 311 patients. We noted most of the respondents were satisfied with the patient’s ward and bedding (61.1%), ward cleanliness (91%), ward comfortable and calmness (71.4%). Of (81.7%) participants were satisfied with providing medical requirements by the staff while only a small number were dissatisfied (18.3%). One–third of the respondents (84.6%) answered that hospital departments (laboratory, radiology, and pharmacy…) can be easily found by the patients. Over half (68.8%) of the patients were satisfied with ward water facilities and sanitary while (31.2%) have a negative aspect. The majority of the patients (89.4%) were satisfied with the patient’s system registration. More than half (59.5%) of the patients were satisfied with the availability of medical requirements (drugs and medical items). A large number of the participants (92.3%) were satisfied with the doctor’s performance for medical procedures at the time needed. A high percentage (90% vs 91.6%) of the respondents was satisfied with the time patients examine by the physician and the finding of laboratory results respectively.
Table 2: Response to Patient Satisfaction with hospital services and environment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Satisfied N=311</th>
<th>Dissatisfied N=311</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitals services</td>
<td>280 (90%)</td>
<td>31 (10%)</td>
</tr>
<tr>
<td>Number of the patient’s ward and bed</td>
<td>190 (61.1%)</td>
<td>121 (38.9%)</td>
</tr>
<tr>
<td>Patient’s ward cleanliness</td>
<td>283 (91%)</td>
<td>28 (9%)</td>
</tr>
<tr>
<td>Patient’s ward comfortable and calmness</td>
<td>222 (71.4%)</td>
<td>89 (28.6%)</td>
</tr>
<tr>
<td>Providing medical requirements by the staff</td>
<td>254 (81.7%)</td>
<td>57 (18.3%)</td>
</tr>
<tr>
<td>Easy to finding hospital departments (pharmacy, laboratory, radiology....)</td>
<td>263 (84.6%)</td>
<td>48 (15.4%)</td>
</tr>
<tr>
<td>Water facilities and sanitary</td>
<td>214 (68.8%)</td>
<td>97 (31.2%)</td>
</tr>
<tr>
<td>Patients registration system</td>
<td>278 (89.4%)</td>
<td>33 (10.6%)</td>
</tr>
<tr>
<td>Availability of medical requirements (drugs, medical items)</td>
<td>185 (59.5%)</td>
<td>126 (40.5%)</td>
</tr>
<tr>
<td>Implementing each procedure at time</td>
<td>287 (92.3%)</td>
<td>24 (7.7%)</td>
</tr>
<tr>
<td>Laboratory results</td>
<td>285 (91.6%)</td>
<td>26 (8.4%)</td>
</tr>
</tbody>
</table>

3.2- Patient satisfaction with nurse care

Regarding patients satisfied with nurse care was shown in Table 3. The majority (90.7%) of the respondents were satisfied with nurse behavior and patient respect. Of (89.7% vs 91.6%) were satisfied with nurse responsibility at any time. We noted a participant (92.3%) recorded that nurses have implemented each medical procedure adequately, (89.4%) of them providing a good explanation about the situation to the patients, and (87.5%) of respondents were answered that nurses were medically qualified.

Table 3: Response to Patient Satisfaction with nurse care

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Satisfied N=311</th>
<th>Dissatisfied N=311</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nurse behavior and respect</td>
<td>282 (90.7%)</td>
<td>29 (9.3%)</td>
</tr>
<tr>
<td>Nurse response to the patients</td>
<td>279 (89.7%)</td>
<td>32 (10.3%)</td>
</tr>
<tr>
<td>Nurse response at night</td>
<td>285 (91.6%)</td>
<td>26 (8.4%)</td>
</tr>
<tr>
<td>Implementing each procedure at time</td>
<td>287 (92.3%)</td>
<td>24 (7.7%)</td>
</tr>
<tr>
<td>Nurse providing good explanation to patients</td>
<td>278 (89.4%)</td>
<td>33 (10.6%)</td>
</tr>
<tr>
<td>Nurse are medically qualify</td>
<td>272 (87.5%)</td>
<td>39 (12.5%)</td>
</tr>
</tbody>
</table>
3.3- Patient satisfaction with physician care

The study findings showed that (91%) of the 282 patients were satisfied with the doctor’s behavior and dealing respectfully with patients during an examination. (87.8%) respondents during visiting hospitals were strongly satisfied with the doctor responded to the patient’s condition and hear them. Also (84.2%), were mostly satisfied with doctors providing a good explanation about the disease condition and the best medical care requirements. Of 90% of the patients were satisfied with the time of examination by the physician. The majority of the patients (91.6%) were satisfied with the easy access to the physician as needed at night shift. Of these (87.5%) were satisfied with physician clarity of drug use and dose. We noticed that (75.6%) of patients were satisfied with the physician’s exam, while (24.4%) of the patients were unsatisfied.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Satisfied</th>
<th>Dissatisfied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doctors’ behavior and respect</td>
<td>282</td>
<td>28</td>
</tr>
<tr>
<td>Doctors response and listen to the patient</td>
<td>273</td>
<td>38</td>
</tr>
<tr>
<td>Instructions and directives given by doctor</td>
<td>262</td>
<td>49</td>
</tr>
<tr>
<td>Does doctor spent adequate time for patient exam</td>
<td>280</td>
<td>31</td>
</tr>
<tr>
<td>Doctors’ response at night</td>
<td>285</td>
<td>26</td>
</tr>
<tr>
<td>Drug use and the dose was explained adequately</td>
<td>272</td>
<td>39</td>
</tr>
<tr>
<td>Physician investigation</td>
<td>235</td>
<td>76</td>
</tr>
</tbody>
</table>

The score level of patients’ satisfaction with the study subject was presented in Figure 2. The satisfaction score was ranged from 0-10 on a scale of 10. Two hundred ninety-six (86.5%) of the study participants were satisfied with hospital services and environment, while a small number 42 (13.5%) of them were showed dissatisfied with a mean score of 9.5, SD ±1.8. The majority of patients 275 (88.4%) were revealed excellent nurse care while a very small number 36 (11.6%) was dissatisfied, a mean score of 5.4, SD ± 1.3. Patients’ satisfaction with doctor care was 245 (79.1%) and a negative aspect was found among a small number of 65 (20.9%) with a mean score of 4.5, SD ± 1.7.
Chi-square analysis was performed to find the correlation between socio-demographic factors and patient satisfaction level with hospital services and health care (doctors and nurses) Table 5: We found no significant correlation between the participant’s age, gender, marital state, and occupation with the level of satisfaction. Except significant association was found between participant’s residence with hospital services (P-value= 0.04) and between education level with doctor care (P-value= 0.002).

Table 5: Comparison of socio-demographic factors of patients’ satisfaction with hospital services, nurses and doctors care

<table>
<thead>
<tr>
<th>Characters</th>
<th>Hospital services</th>
<th>Nurses care</th>
<th>Doctors care</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Satisfied</td>
<td>Dissatisfied</td>
<td>Chi, P-Value</td>
</tr>
<tr>
<td>N=311, %</td>
<td>N=311, %</td>
<td>N=311, %</td>
<td></td>
</tr>
<tr>
<td><strong>Residence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside</td>
<td>128 (47.9)</td>
<td>27 (64.3)</td>
<td>4.1, 0.04</td>
</tr>
<tr>
<td>Outside</td>
<td>141 (52.1)</td>
<td>15 (35.7)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>44 (16.4)</td>
<td>8 (19.1)</td>
<td>0.2, 0.7</td>
</tr>
<tr>
<td>&gt;25</td>
<td>225 (83.6)</td>
<td>34 (80.9)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>220 (81.8)</td>
<td>32 (76.2)</td>
<td>0.7, 0.4</td>
</tr>
<tr>
<td>Female</td>
<td>49 (18.2)</td>
<td>10 (23.8)</td>
<td></td>
</tr>
</tbody>
</table>
Multiple logistic regressions for the level of satisfaction with hospital services and health care providers with socio-demographic factors (residence, age, gender, marital state, occupation, and education) were presented in Table 6. A residence was found significantly associated with hospital services satisfaction the coefficient 0.68, P= 0.05, and level of education was found significantly associated with doctor care satisfaction the coefficient 0.88, P= 0.01, while no significant association was found with remained factors.

Table 6: Regression analysis of socio-demographic factors associated with the level of patient satisfaction

<table>
<thead>
<tr>
<th>Socio-demographic factors</th>
<th>Hospital services satisfaction</th>
<th>Nurse care satisfaction</th>
<th>Doctor care satisfaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>SE</td>
<td>P</td>
</tr>
<tr>
<td>Residence: Inside city</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence: Outside city</td>
<td>0.68</td>
<td>0.35</td>
<td>0.05</td>
</tr>
<tr>
<td>Age: &lt;25</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age: &gt;25</td>
<td>0.23</td>
<td>0.50</td>
<td>0.6</td>
</tr>
<tr>
<td>Gender: Male</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender: Female</td>
<td>0.09</td>
<td>0.47</td>
<td>0.8</td>
</tr>
<tr>
<td>Marital: Married</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital: Unmarried</td>
<td>-0.63</td>
<td>0.41</td>
<td>0.1</td>
</tr>
<tr>
<td>Occupation: Employee</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation: Unemployed</td>
<td>0.59</td>
<td>0.46</td>
<td>0.2</td>
</tr>
<tr>
<td>Education: Educated</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education: Uneducated</td>
<td>-0.50</td>
<td>0.39</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Discussion

This study examined the level of patient satisfaction with a health care provider (nurses and physicians) and services performed in the non-private hospitals in Sulaimani city. We evaluated 311 patients among whom were highly satisfied with the nurse’s care (88.4%), physician’s care (79.1%), and hospital services (86.5%). The findings of our study are corresponding with the Saudi experience study and the study of Lagos University Teaching Hospital reported that patients with high levels of satisfaction with nurse care (2, 14) and the study of Southern Saudi Arabia (15). A lower number of patient’s dissatisfaction with hospital services and health care was recorded in this study. Such a low perception of satisfaction with staff care and hospital services by patients may create problems in the health system by decreasing trust and less utilization of services as well as delayed health-seeking with subsequent poor health outcomes of the public. We found the study participants were more satisfied with nurse care compared to doctor care. The result of this study appeared consistent with the study done by the Kingdom of Saudi Arabia (16) while inconsistent with the study reported by Southern Saudi Arabia (15). The study discovered that there were no significant differences between patients’ satisfaction and age and gender. Indeed, older respondents did not affect satisfaction values. However, several studies indicated that older participants are generally more satisfied with the health care provider and hospital services compared to younger respondents (17, 18). More females were participated in this study (81%) but no relationships were found between gender and level of satisfaction. Our results are similar to the study that reported patient gender did not affect satisfaction values (18). The Ha’il City, Saudi Arabiastudy also reported no relationships were found between gender and patient satisfaction levels (19). The results of this present study indicated that there was a significant difference between the level of education and patients’ satisfaction. Patient with a lower level of education was more satisfied with doctor care services than the higher education the P=0.002. The finding of this study is similar to some studies that reported that lower education level was greater satisfaction with health care service (20, 21) and also is consistent with a study that reported a higher level of education was less satisfied with health care (22). This can occur because patients with high educational levels possess more information about treatment alternatives and expect higher care standards and therefore are more critical in this regard. In regression analysis, significant associations between place of residence and patient satisfaction were identified, respondents in the rural area remained significantly associated with respect to satisfaction with hospital services the coefficient 0.63, p=0.05. The result of this study is in line with the study (23). This may be due to that some of the rural places they don’t have large hospitals and if they found, the quality of the services may be at the lowest level compared to urban a place. Our study has much strength including the use of validated measures of patient satisfaction, nurses and physician’s care, hospital services, and environment across four large government hospitals. The study has limitations as well. Data from both health care providers and hospital services are cross-sectional, thus limiting causal inferences about the associations found. The open bedside interview we use to collect data might also influence the participants’ responses to some extent even if the data collectors were not part of the treating team. According to our knowledge, this is the first quantitative study to determine the level of patient satisfaction with health care providers (nurses and doctors) and hospital services in Sulaimaniyah city.

Conclusions

In general, the study revealed that patients were highly satisfied with hospital service and health care providers in Sulaimani city. The proportion of patients satisfied with nurse care was found to be higher than the proportion of patients satisfied with doctor care and those with a lower level of education was more satisfied with doctor care than a higher level of education because the second group have adequate treatment information and predicted adequate care they received. Besides, respondents with rural residents are more satisfied with hospitals’ quality and services because some of the
respondents living in rural areas have a lack of large hospitals and inadequate health system services. If we improve the quality of hospital services and health care, it is clear that evaluating patients’ satisfaction should be constant to reformulate the baseline and regulation and to be able to assess interventions and changes in the health system. Further study is suggested on patient satisfaction with doctor care to find factors that influence low patient satisfaction compared to nurse care if we must improve the quality of health.

Acknowledgments: We would like to extend our gratitude to the Sulaimani directorate of health and Sulaimani polytechnic university for their permission. Furthermore, our gratitude also goes to all participants who voluntarily agreed to participate in this study and helping to complete this study.

Conflict of Interest: The authors declare that there was no conflict of interest.

Financial Disclosure: Self

References


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Multimodal Strategy to Improve Hand Hygiene Compliance to Practices among Staff Nurses

Nilima Vasava1, Praful Damor2, Jayshree Vasava3
1Lecturer, Medical Surgical Nursing, 2Lecturer, Community Health Nursing, 3Lecturer, Obstetric and Gynecological Nursing, Parul Institute Of Nursing, Limda, Waghodia Gujarat

Abstract

Introduction and Background: Healthcare-acquired infections [HCAI] account for 10-30% of all hospital admissions, according to the World Health Organization [WHO]. An estimated 1.4 million people are infected with HCAI at any given time.1 As the burden of health-care-associated infections grows, so does the seriousness of disease and treatment difficulty, which is exacerbated by multi-drug-resistant pathogens.2 Health care providers are reverting to the fundamentals of infection control by employing common steps such as hand hygiene.3 The WHO’s global initiative on patient safety programmers has made “Clean Care is Safer Care” a top priority. It is time for developing countries to formulate the much-needed policies for implementation of basic infection prevention practices in health care set-up.4

Nurses, the “nucleus of the health-care system,” spend more time with patients than any other health-care worker, and their adherence to hand-washing protocols appears to be more important in preventing disease transmission among patients. Hand hygiene is thought to be the most important factor in preventing healthcare-associated infections (HAI). Hand hygiene programmes with a variety of components have been shown to increase compliance among healthcare workers and, as a result, minimise infections. One such initiative is the World Health Organization’s implementation of an evidence-based definition called “My five moments for hand hygiene.”5

The investigator seeks to study the compliance to hand hygiene practices among staff nurses, working in the obstetrical and gynaecological wards. The research is an endeavour to increase the awareness and educate nurses about the need for compliance to hand hygiene practices.

Aim: A. The aim of this study was to see how well health care workers in the intensive care unit kept their hands clean (ICU)

B. To determine the causes of noncompliance, and

C. To investigate the effectiveness of a multimodal intervention approach in enhancing compliance.

Methodology: This Quasi experimental one group pre-intervention and post intervention study compares the compliance to hand hygiene practices before and after a multimodal interventional strategy

Setting: The study was conducted in ICU ward in hospital

Result: The study needed a sample size of 64 nurses to achieve an 80 percent power and a 5% error rate. A t test was used to compare the mean pre- and post-test scores. ANOVA was used to examine the relationships between the baseline variables. Hand hygiene enforcement among staff nurses increased dramatically from 45.31 percent to 65.78 percent following multimodal intervention strategies. (P<0.0001) Hand hygiene techniques have improved significantly (hand wash technique mean score increased from 5.97 ± 1.284 to 8.16 ± 1.158 (p<0.05) and hand rub technique score increased from 4.52 ± 0.992 to 6.69 ± 1.489 (p<0.05)). The private wards had considerably better hand hygiene than the general wards.

XI. Conclusion: The results of this study show that when multimodal interventional interventions were used, hand hygiene compliance improved.

Keywords: Multimodal, Hygiene, Staff Nurses
Introduction

If there is an abundance of foreign matter in the air, it is referred to as air pollution. This can be harmful to both the person and the environment. The phenomenon known as “pollution” is an unavoidable result of man’s existence and behavior. The word “air pollution” refers to the presence of pollutants (e.g., chemicals, mixtures of gases, and particulate matter) in concentrations in the atmospheric (surrounding) environment that are harmful to human health. Chemicals join the food chain or are found in drinking water, constituting an additional source of human exposure, whether for safety or comfort, or because they are harmful to plants and animals. The direct impact of air pollution on plants, animals, and soil can have an impact on the structure and function of the eco system, including its ability to self-regulate, lowering quality of life.

Pollution is the introduction of pollutants into an atmosphere that cause the ecosystem, i.e. physical structures or living organisms, to become unstable, disordered, harmed, or uncomfortable. Pollution may take the form of chemical compounds or energy in the form of noise, heat, or light. Pollutants, or pollution elements, can be foreign substances or energies, or they can occur naturally; when they do, they are called pollutants when their quantities exceed natural levels. Pollution is often classified as either point source or nonpoint source. A variety of pollution-related diseases and health problems, such as respiratory infections, heart disease, COPD, stroke, and lung cancer, are all linked to air pollution.

Breathing difficulties, wheezing, coughing, asthma, and worsening of chronic respiratory and cardiac conditions are all possible health consequences of air pollution. Increased drug usage, increased doctor or emergency department visits, further hospital admissions, and premature death are all possible outcomes of these results. Bad air quality has a wide range of health consequences for humans, but it mostly affects the respiratory and cardiovascular systems. Individual responses to air pollutants are influenced by the type of pollutant, the degree of exposure, as well as the person’s health and genetics. Particulates, ozone, nitrogen dioxide, and sulphur dioxide are the most important sources of air pollution. In terms of overall deaths due to indoor and outdoor air pollution, children under the age of five years in developed countries are the most affected group.

Multi Modal Intervention Strategies:

XIII. The following multimodal intervention techniques were used in this study:

XIV. 1. Educational Initiatives: Demonstration and lecture focused on WHO’s Your Five Minutes of Hand Hygiene: Before patient touch, before the aseptic task, after the danger of body fluid exposure, after patient contact, and after contact with the patient’s surroundings

XV. 2. The HICC protocol is being reinforced.

XVI. • Signs throughout the nurses’ station and in the wards to serve as visual reminders.

XVII. • Verbal reminders to use a hand hygiene product

XVIII. • Hand hygiene solutions and services are readily available.

XIX. 3. One-on-one instruction and community discussion

Methods and Materials

Instrument: Three parts make up the data collection method.

Section A: Demographic data interview schedule includes age, years of experience, education, and field of employment.

Section B: Hand hygiene specific enforcement observation form Section B: Observation check list (WHO).

Tool for data collection
The researcher obtained written permission from the consent authority of prior of data collection. Data collection date waseldon. The purpose of the study was explained to the sample sand written informed consent was obtained. Their response was assessing.

Individual explanation was given regarding structured Knowledge questionnaires to assess the knowledge regarding effects of air pollution on health. Knowledge test was checked. The average time taken 60 minutes. All people gave good cooperation during data, investigation, analyzed the data and made findings.

**Study participants and size:** Sample size determine by power analysis calculated based on previous studies.

\[
N = \frac{4pq}{L^2}
\]

Where \( p = 0.087 \), \( q = 0.913 \)

\[
= \frac{4 \times 0.087 \times 0.913}{0.017 \times 0.017} = 0.317 = 1097
\]

Calculation sample size for this study was 1097, due to time constraint and global pandemic situation investigator consider 64 participants for the study.

**Result**

**SECTION 1: Distribution of Nurses according to the demographic variable**

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Basic Line variables</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age in Years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>21-24</td>
<td>34</td>
<td>53.1</td>
</tr>
<tr>
<td></td>
<td>25-29</td>
<td>24</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>&gt;=30</td>
<td>6</td>
<td>9.4</td>
</tr>
<tr>
<td>2</td>
<td>Educational Qualification</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diploma</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Degree</td>
<td>48</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>Area of Work</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Common Ward</td>
<td>37</td>
<td>57.8</td>
</tr>
<tr>
<td></td>
<td>Private Ward</td>
<td>27</td>
<td>42.2</td>
</tr>
<tr>
<td>4</td>
<td>Years of Work</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-2</td>
<td>44</td>
<td>68.8</td>
</tr>
<tr>
<td></td>
<td>2-4</td>
<td>12</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>&gt;4</td>
<td>8</td>
<td>12.5</td>
</tr>
</tbody>
</table>
Table 1 shows that the bulk of the staff nurses (53:1) are between the ages of 21 and 24. In terms of educational status, the majority (75%) had a bachelor’s degree. In terms of work location, 57.8% of staff nurses worked in a common ward. The majority of the participants (68.8%) had 0-2 years of experience.

SECTION II: Comparison of hand hygiene practice before and after interventional strategies.

Table II a: Comparison of hand hygiene compliance before and after multi modal interventional strategies.

<table>
<thead>
<tr>
<th>SL NO</th>
<th>Items Score (%)</th>
<th>Max (%)</th>
<th>Range (%)</th>
<th>Mean</th>
<th>SD</th>
<th>Test of significance</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Paired t test</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Pre test</td>
<td>100</td>
<td>30-70</td>
<td>45.31</td>
<td>10.23</td>
<td>22.014</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>2</td>
<td>Post test</td>
<td></td>
<td>40-80</td>
<td>65.78</td>
<td>9.225</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant

Table II: comparison of hand hygiene techniques (hand wash) before and after multimodal intervention strategy

<table>
<thead>
<tr>
<th>SL NO</th>
<th>Items Score (%)</th>
<th>Max (%)</th>
<th>Range (%)</th>
<th>Mean</th>
<th>SD</th>
<th>Test of significance</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Paired t test</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Pre test</td>
<td>10</td>
<td>4-8</td>
<td>5.97</td>
<td>1.284</td>
<td>17.13</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>2</td>
<td>Post test</td>
<td></td>
<td>5-10</td>
<td>8.16</td>
<td>1.158</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant

Before the intervention, the mean score for hand hygiene enforcement was 45.31, according to the data in the table above. After the intervention, the mean score improved to 65.78. At the 0.05 stage, the obtained t test value of 22.014 is important. As a result, before and after multimodal intervention techniques, there is a noticeable difference in hand hygiene compliance. Table II c: comparison of hand hygiene techniques (hand rub) before and after intervention.

<table>
<thead>
<tr>
<th>SL NO</th>
<th>Items Score (%)</th>
<th>Max (%)</th>
<th>Range (%)</th>
<th>Mean</th>
<th>SD</th>
<th>Test of significance</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Paired t test</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Pre test</td>
<td>10</td>
<td>3-6</td>
<td>4.52</td>
<td>0.992</td>
<td>17.36</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>2</td>
<td>Post test</td>
<td></td>
<td>4-10</td>
<td>6.69</td>
<td>1.489</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant
Table II shows that the pre-test and post-test scores for hand washing techniques vary significantly. Before intervention, the mean pre-test score was 5.97; after intervention, it was 8.16. At the 0.05 stage, the obtained t value is meaningful.

With a t value of 0.05, the data in Table II c shows that there is a substantial difference in hand hygiene technique (hand rub) before and after intervention. Thus, at the 0.05 stage, Hypothesis 1 is accepted: there is a substantial difference in hand hygiene practise enforcement before and after multimodal interventional strategies among staff nurses.

SECTION III: Association between compliance to the practice of hand hygiene with base invariables.

Table- III- Association between compliance to the practice of hand hygiene with baseline variables.

<table>
<thead>
<tr>
<th>SL No</th>
<th>Baseline variables</th>
<th>Frequency</th>
<th>HH Comp Pre score Mean</th>
<th>Standard Deviation</th>
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<td>45</td>
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<td>3</td>
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<tr>
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<td>Diploma</td>
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<td>42.5</td>
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<td></td>
<td>Degree</td>
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<td>46.25</td>
<td>10.027</td>
</tr>
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<td>4</td>
<td>Area of work</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Common ward</td>
<td>37</td>
<td>4.81</td>
<td>7.593</td>
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<tr>
<td></td>
<td>Private Room</td>
<td>27</td>
<td>51.48</td>
<td>10.267</td>
</tr>
</tbody>
</table>

A - ANOVA, t- independent t test:
Table III Demonstrates that there is a strong link between hand hygiene enforcement and the type of job (private room). At the 0.05 stage, the obtained t value is meaningful. Some baseline variables have no meaningful relationship (Age, Years of Experience and Education). At the 0.05 stage, Hypothesis 2 is agreed for the work field.

Discussion

The current research included 64 staff nurses between the ages of 21 and 24 years old. The majority of them (53.1 percent) have a nursing degree. Staff nurses were employed in obstetrics and gynaecology general wards by 48 (75%) of them, and 37 (57.8%) of them had clinical experience of up to two years. The age group of 21-24 was found to have the highest level of hand hygiene compliance in the sample. The baseline characteristics were close to those of a previous descriptive cross-sectional analysis on nurse compliance with hand hygiene procedure and expertise conducted in the intensive care units of a hospital in Kuala Lumpur. There were a total of 84 nurses on board. It revealed that the majority of people (53.6%) agreed with the statement the staff nurses ranged in age from 21 to 30 years old. The respondents had 1 to 5 years of work experience (34.5%), and the majority of them had a Diploma as their educational qualification.

The results of this study show that after multimodal interventional interventions, there is a substantial difference in pre-test and post-test scores in hand hygiene enforcement and practises. Compliance improved (p<0.001) from 45.31±10.23 to 65.78±9.22. Hand rub techniques improved from 4.52±0.99 to 6.99±1.48 (p<0.001), while hand wash techniques improved from 5.97±1.28 to 8.16±1.16 (p<0.001). In a tertiary care hospital in northern India, a similar prospective study was performed in the adult ICU. Hand hygiene compliance was initially measured over a 6-week span, with pre-intervention hand hygiene compliance of 21.48 percent and post-intervention hand hygiene compliance of 61.59 percent (p<0.0000).

Before and after the introduction of a multimodal interventional strategy, hand hygiene enforcement was 40 to 55 percent before patient contact and 42 to 62 percent after patient contact in the current report. Hand hygiene enforcement increased from 40 to 53 percent before patient contact in a neonatal ICU before the introduction of a multimodal implementation programme, and from 39 to 59 percent after patient contact, according to a report by Lam. They came to the conclusion that a good education programme could help people keep their hands clean.

Hand washing agents cause irritations and dryness, sinks are inconveniently located, shortages of sinks, always too busy, insufficient time, patient needs take priority, wearing of gloves, beliefs that glove use obviates the need for hand hygiene, low risk of infection from h. influenza Other studies have shown that non-compliance is due to a lack of understanding of guidelines/ procedures, a lack of rewards/ motivation, and a lack of a role model from a colleague.

Conclusion

The results of this study show that when multimodal interventional interventions were used, hand hygiene compliance improved. In order to improve hand hygiene practises among hospital staff nurses, formal communication and hand hygiene education, promotion, and leadership support are needed. As a result, the investigator is confident that this will be a reliable piece of knowledge for health-care professionals, allowing them to offer thorough patient care while also improving nursing practise efficiency.

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Conflict of interest: There is no conflict of interest

References


Antimicrobial Peptide Cathelicidin and Vitamin D Receptor Gene Polymorphism in Oral Health

Nireeksha 1, Mithra N Hegde 2, Suchetha Kumari N 3
1 Lecturer, 2 Vice Principal, Head of the Department, Department of Conservative Dentistry and Endodontics, A.B.Shetty Memorial Institute of Dental Sciences, Nitte University (deemed to be) Deralakatte, Mangalore, 3 Professor, Department of Biochemistry, K.S.Hegde Medical Academy, Nitte University (deemed to be) Deralakatte, Mangalore

Abstract

Background: Anti-microbial peptides and vitamin D levels in saliva, GCF play an important role in oral health and its maintenance. The peptide levels are regulated by the Vitamin D cathelicidin pathway and their association and influence on oral health is of utmost importance. The aim of the present review article is to establish the aim is to establish a relationship and possibly the role of Cathelicidins and Vitamin D receptor gene polymorphism in relation to oral health.

Methodology: The preferred reporting items for systematic review ad meta analysis (PRISMA) were used for reporting this systematic review. The study protocol has been approved by all co-authors. The study has been registered at the National institute for health research (NHR) under the Prospero ID: CRD42020200946.

Results: A total of 102 articles were retrieved through electronic database search. After evaluating the title, abstract and full text of these articles only 7 were selected for the present systematic review. However 3 articles were excluded because they were classified as high risk of bias according to PRISMA guidelines. Out of 7 studies selected, 4 were classified as low risk bias and 3 were classified as moderate risk bias.

Conclusion: The current evidence suggests anti-microbial peptide LL-37 and vitamin D receptor gene polymorphism plays an important role in caries prevalence and periodontal health.

Highlight: Understanding the role of antimicrobial peptides in saliva helps in enhancing focus on the effect of these components on oral health and its maintenance.

Keywords: Saliva, Anti-Microbial Peptide, Vitamin D, Dental Caries, Periodontitis.

Introduction

Organisms possess very strong defence system to combat the invading pathogens and micro-organisms. One of the principal defence system which plays a major role against microorganism is innate immunity system. The anti-microbial functionality of innate immunity modulates the disruption of gram negative, gram positive, fungi and virus principally by disruption of bacterial cell membrane. Anti-microbial peptides enhances the chemical and physical barrier in mucous membrane and skin. Various recognized anti-microbial peptides are highly conserved and expressed in organisms such as insects (drosophila) rabbits, mice, crabs. They are expressed in response to inflammatory conditions by phagocyte cells like monocytes, macrophages and granulocytes.

Oral cavity inspite of these numerous bacterial colonies and has high chances of invasion to the body through GIT, epithelium, gut, surgical procedure rarely leads to infection. Thus, indicates a strong and efficient host defensive mechanism that is in action. Production of antimicrobial peptides from neutrophils, salivary gland makes saliva rich in its anti-microbial, anti-fungal and anti-bacterial properties. They perform various functions...
like stimulation of epidermal growth factor thereby promotes wound healing, stimulates keratinocytes, chemotaxis and immune activation. The binding ability of LL-37 to lipopolysaccharides and neutralizing its activity facilitates a strong positive correlation between inhibition of lipopolysaccharides induced cytokine secretion. LL-37 influence epidermal growth factor levels, migration of keratinocytes and mediates angiogenesis. This quality of these AMP’s make them an area of interest and they are also extensively studied².

These anti-microbial peptides destroyed by attraction, attachment and insertion. Attraction is electrostatic attraction between cationic and anionic moieties on bacterial membrane, these antimicrobial peptides attach in parallel/perpendicular manner to the membrane thereby stretching the bacterial membrane further thinning it down leads to pore formation, further insertion of these antimicrobial peptides takes place by three different model systems barrel stave model, carpet model leading to bacterial dissociation. Several types of anti-microbial peptide adverse in their sequence and structure are identified. Among them cathelicidins are peptides which are crucial in inflammatory conditions.

Cathelicidins are transcribed from a single gene CAMP with a N-terminal [30 amino acid residues] a highly conserved pro-sequence and mature antimicrobial peptide name LL-37 at the C-terminal domain. It has been observed that LL-37 play a role in oral inflammatory conditions, few studies show their association of these peptides. The peptide has anti-microbial activity against gram positive and negative bacteria specifically on clinically relevant pathogens such as Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Salomonella typhimium at a mic of <10µg/ml also has an effect on lowering release of pro-inflammatory mediators. This peptide is located in azurophil granule, immature neutrophils, NK cells, monocytes, mast cells, squamous epithelium, airway, mouth, tongue, oesophagus, intestine, cervix and vagina. Widely produced in salivary glands, acinar cel of submandibular glands and minor glands⁵. This systemic review will focus on Cathelicidins [LL-37] and its role in oral inflammatory conditions.

### Materials and Methods

The preferred reporting items for systematic review ad meta analysis (PRISMA) were used for reporting this systematic review. The study protocol has been approved by all co-authors. The study has been registered at the National institute for health research (NHR) under the Prospero ID: CRD42020200946.

#### Systematic search strategy:

Electronic search strategy consisted of two stages: Stage 1 included studies that specifies antimicrobial peptide levels and vitamin D receptor gene polymorphism, Stage 2 included systemic search to identify various studies evaluating these levels in association with oral health and disease [Dental caries and Periodontitis].

Focused question: For identifying various studies the below mentioned focused questions were adapted using PICO criteria (Miller and forest 2001)

“What is the role of anti-microbial peptide LL-37 (Cathelicidins) in oral health and the association of vitamin D receptor gene polymorphism and dental caries”

**POPULATION:** Younger adolescence and middle aged with dental caries and periodontitis.

**INTERVENTION/EXPOSURE:** Salivary antimicrobial peptide levels and its protective anti-inflammatory effect on oral health.

**COMPARISON:** Control group with Good oral hygiene (Low caries index and good periodontal health) Case group with poor oral health status (High caries index and poor periodontal health).

**OUTCOME:** Effect of LL-37 levels and vitamin D receptor gene polymorphism on caries prevalence and periodontal health.
Scope:

Clinical studies which involved estimation of LL-37 and vitamin D receptor gene polymorphism conducted in human subjects. Comparison done between healthy individuals and study subjects with dental caries and periodontitis. Animal studies, abstracts, letter to editors, narrative reviews and case reports were excluded.

Sources:

Electronic search was performed in Pubmed medline, ISI web of science and medline database applying Mesh terms “dental caries”, “periodontal disease” “Cathelicidins” “LL-37” “Vitamin D” “VDR receptor gene polymorphism” “saliva”. The search system reviewed the article published between 2009 to 2019. Also a hand search of references and related articles were performed.

Study Selection:

Controlled observational studies comparing and evaluating dental caries and periodontitis with LL-37 and vitamin D levels were selected. Studies concerning permanent dentition individuals with any other systemic disease and under medication that effect the salivary composition were excluded. Case report, review articles were excluded. Studies which presented at least 8 of evaluating criteria were considered as low risk bias and 4 to 7 criteria were considered “moderate risk” less than 3 criteria it was considered as high risk bias and excluded from the study due to low evidence.

| 1. Definition of inclusion criteria |
| 2. Definition of exclusion criteria |
| 3. Dental caries diagnosis criteria |
| 4. Periodontal condition and evaluation criteria | 5. Saliva collection description |
| 6. Statistical analysis description | 7. Risk of Bias |
| 8. Level of evidence |

Chart 1: Quality assessment criteria

Result

Electronic search retrieved 102 no-duplicate records. However, after checking the titles, abstract and full texts, 9 studies were selected for this systematic review. Two of them [Davidipoulou et al and Putsep K et al] were excluded because they were classified as high risk bias leaving a total of 7 for systematic review, from the selected studies. 4 classified as low risk and 3 classified as moderate risk bias. Table 1 shows quality assessment, table 2 gives description of study group.

Simon Sara included 33 children’s grouped based on caries at baseline and developing caries were further evaluated. Turkaglu et al conducted a study including 4 participants with gingivitis and healthy periodontal tissue were included and presence/absence of caries was not of concern. Another study conducted
by Davidopoulu included 49 systemically healthy individuals evaluated for Decay index, periodontal index and gingival index and were divided into 3 groups based on caries experience7.

Takeuchi conducted a study among 69 patients, comparison between them was done based on probing depth, clinical attachment levels, Bleeding on probing, Plaque record. No comparison with healthy individuals was done9. Turkoglu in 2009 evaluated IL-18 and LL-37 in 18 healthy 41 individuals with chronic periodontitis and comparison of these levels was done10. BsmI and Folk gene role in VDR receptor gene polymorphism was analyzed by Yu M et al and Kong YY among 400 and 380 consequently. Both the studies included caries free group for comparison11,12.

Based on the results of selected studies 5 studies showed statistical difference between groups with/without caries and with/without periodontitis. Two of the studies showed association between BsmI and Folk gene polymorphism and dental caries.

### TABLE 1: QUALITY ASSESSMENT OF SELECTED ARTICLES

<table>
<thead>
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<tr>
<td>Exclusion criteria</td>
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<tr>
<td>Periodontal condition and evaluation criteria</td>
<td></td>
</tr>
<tr>
<td>Saliva/GCF/Buccal swab collection description</td>
<td>X</td>
</tr>
<tr>
<td>Statistical analysis description</td>
<td>X</td>
</tr>
<tr>
<td>Risk of Bias</td>
<td>Low</td>
</tr>
<tr>
<td>Level of evidence</td>
<td>High</td>
</tr>
</tbody>
</table>
Discussion

The selected studies were those which satisfied the minimum criteria that would be able to evaluate the role of LL-37, Vitamin D in caries and periodontal conditions. Rolf Kotsmann in 1956 first reported condition called morbus Kostmann syndrome (infantile genetic agranulocytosis) with a missing serum factor. These patients were highly susceptible to repetitive infections primarily gingivitis and periodontitis persisted even after antibiotic treatment. Later absence of LL-37 in saliva and load were reported. LL-37 is tested as an anti-biofilm compound, due to its broad spectrum effect, rare resistance development by bacteria’s, bacterial growth inhibition and bactericidal activity. These Anti-microbial peptides help in maintaining oral health by allowing more resistant non-pathogenic bacteria to dominate the colonization. The conducive factor for anti-microbial activity is the low ionic strength of saliva. Therefore evaluation of LL-37 plays a major role in caries risk assessment and development of treatment strategies.

Majority of studies showed relationship between salivary LL-37, periodontitis and dental caries. In a study microbial composition was compared with LL-37 levels in children significant increase in LL-37 levels in healthy individuals throughout the study. This longitudinal study concluded that both antimicrobial composition of saliva and bacterial composition predispose development of dental caries. In another study LL-37 levels were evaluated in different age group in patients with gingivitis showed that inflammation of gingiva as an increasing effect on LL-37 levels, independent of individual agegroup.

Caries experience and LL-37 were evaluated in relation to age, gender, type of dentition i.e. primary, mixed and permanent in a study which showed that there is low peptide at early childhood and 100 fold increase in adolescence in ng/ml. Variation in gender was served based on differences in immuno-competency. Thus suggested that LL-37 may serve as prognostic tool for disease and efficient therapeutic agent. A study compared LL-37 in chronic periodontitis shows increase in LL-37 which may be attributed to response of innate defense mechanism against peri-odontopathic bacteria.

In contrast LL-37 was elevated in chronic periodontitis compare to healthy individuals, suggesting an important role of LL-37 in pathogenesis of chronic periodontitis they concluded that LL-37 in gingival crevicular fluid was sufficient to decrease or prevent periodontal destruction. IL-18 was evaluated to identify whether both LL-37 and IL-18 contribute to innate immunity in periodontal destruction. IL-18 is a pro-inflammatory cytokine stimulates mediator’s of inflammation and activate neutrophil’s thus a part of host defense it was showed that LL-37 can stimulate production of IL-18. But in this study there was no correlation between the LL-37 levels and IL-18 in chronic periodontitis.

These peptides being one of the important effector molecules of innate immunity is produced by the circulating cells, bone marrow progenitor cells, various epithelial surfaces, inflamed gingival tissues. Vitamin D is critical for regulation of CAMP gene and DEFB4 gene which codes for cathelicidin and defensins in both normal and hematopoietic cells. Therefore, vitamin D boosts innate immunity system and helps in wound healing and infection control. Presence of pathogen stimulates toll-like receptor’s (TLR) of macrophages inducing expression of CYP27B1 (25- hydroxyvitamin D-1α-hydroxylase) which, in turn, leads to the production of bioactive 1,25(OH)2D from circulating inactive 25(OH)D. Locally increase in 1,25(OH)2D activates CAMP gene which codes for cathelicidin. The release of cytokines are also mediated through stimulation of toll-like receptorsthrough vitamin D. Thus, Vitamin D levels play a major role in modulating immunity.

Kong YY and Yu m et al studied the possible correlation between genetic polymorphism of vitamin D receptor and occurrence of dental caries. Vitamin D regulates the calcium metabolism and promotes calcium depositing on enamel, also in a meta-analysis they mentioned that supplementation with vitamin D decreases he occurrence of caries by 40%. Both these
studies showed correlation between vitamin D and caries pattern even though the specific mechanism is not understood. A single variable like LL-37 is difficult to establish as a marker because dental caries is multifactorial as a maker in nature. Understanding the complexity of peptide and their role in innate immunity makes it complex to understand the role of these LL-37 peptides in dental caries. They target a broad spectrum of microbial activity, the ease of synthesis they are ideal for formulation of peptide based oral health care products along with routine maintenance of oral hygiene methods. Since they help to kill bacteria selectively in mixed culture, they can be used as an adjuvant to standard hygiene care in immune-compromised patients, control biofilms, progression of caries, biofilms on implants also prevention of plaque mediated dental diseases. These Anti-microbial peptides can be idealized has biomarkers for dental caries assessment, the up regulation and down regulation of these peptides may help in monitoring treatment outcome pre and post treatment. They are highly conserved and show relatively lower potential to resistance against microbes.

**Conclusion**

The selected studies showed a significant variation in LL-37 levels in dental caries, periodontitis, gingivitis and vitamin D polymorphism with dental caries. Still the complexity in mechanism of action of LL-37 and its relationship with Vitamin D levels makes it difficult to conclude. Only with more evidence and analysis we will be able to establish vitamin D and L-37 have a major role to play in occurrence and progression of dental caries.

**Ethical Statement**: Not relevant.

**Source of Funding**: Self

**Conflict of Interest**: Nil

**References**


Primary Closure after Open Common Bile Duct Exploration in Comparison to T-tube Drainage. Our Experience in a New Medical College of Assam

Nirmal Kumar Agarwal¹, Jon Bordalai¹, SantanuSarma¹
¹Asso. Prof. of Surgery, Tezpur Medical College, Tezpur

Abstract

Background: Traditionally T-tube drainage of CBD is most commonly done. However T-tube has its own problems mainly longer hospitalisation, longer absence from work and many complications. This study done to assess outcome of primary closure of CBD after open CBD exploration.

Methods: This retrospective study was done in Tezpur Medical College which is a new medical college in Assam. All the patients who underwent CBD exploration in our unit were analysed to compare the results. All together 38 patients underwent CBD exploration of which in 18 primary closure of CBD was done.

Results and Conclusion: Our results indicate lesser complications for primary closure, much shorter hospital stay in comparison to T-tube drainage.

Primary closure of CBD after choledocholithotomy is safe, much simple and effective and has lesser complications in comparison to T-tube drainage.

Keywords: Common bile duct (CBD), primary closure, T-tube, choledocholithotomy

Introduction

Gallstone disease is very common prevalence in population and along with Gall bladder(cholelithiasis), stones are present in 5-15% of gallstone patients in common bile duct(choledocholithiasis). Surgical intervention is necessary for CBD stones. Management options are- either endoscopic sphincterotomy in case of smaller stone or surgery is required in case of ERCP failure and in case of larger CBD stones. After surgical exploration of CBD i.e. choledocholithotomy, traditionally common bile duct is closed over a T-tube. The purpose of using T-tube drainage after open CBD stone removal is to decompress the CBD and to allow for oedema at sphincter of Oddi to subside. It also allows radiological visualisation of CBD in post-operative period and a potential route for stone extraction of any retained stones. T-tube is kept for about 10-28 days and a T-tube cholangiogram is usually done at 10-14 days to look for any retained stones. Traditional CBD closure over T-tube carries potential complications. These include bacteremia, dislodgement of tube, obstruction and/or fracture of tube. Further leakage of bile may be encountered after removal. Patients may have to carry it for several weeks before removal. All of these lead to prolong length of hospital stay and increased cost. The role of T-tube has been challenged...
and primary closure of CBD after exploration has been described more than a century ago. Primary closure of CBD has been described in literature to overcome adverse consequences of T-tube\(^9\). Traditionally it is thought T-tube has definitive role after CBD clearance, but many authors found no significant difference in the complication rate or mortality between primary closure and T-tube drainage\(^10,15\). Others found higher morbidity in terms of more biliary infection, discomfort from tube, delayed discharge from hospital\(^11\)-\(^19\). However still there are apprehensions in the minds of surgeons and primary closure is not performed routinely.

This study was conducted to assess the outcome of primary repair of CBD in terms of patient comfort, duration of hospital stay and post-operative complications.

**Materials and Methods**

This retrospective study was conducted in department of surgery of Tezpur Medical College which is a new medical college in Assam. All together 38 patients of common bile duct stones were analysed from January 2017 to February 2020. Out of 38 patients 20 underwent T-tube closure (group I) while 18 patients (group II) underwent primary closure of CBD. The patients were investigated with required blood investigations for operative procedure like haemogram, blood sugar, renal function test, liver function test, PA view x-ray chest, E.C.G., prothrombin time, thyroid status and viral markers. Ultrasonography of abdomen was done to see dilatation of CBD, stones in CBD and MRCP done in selected cases. Contrast enhanced CT was done in selected cases to rule out malignancy, pancreatic diseases causing jaundice and other conditions like renal failure and these patients were not included in study. The studied patients underwent open cholecystectomy through moderate size subcostal incision followed by supraduodenal CBD exploration between stay sutures. Stones were removed from CBD with Desjardin forceps and CBD was irrigated with normal saline ensuring no stone is left. Also patency of CBD checked with 10F feeding tube and Bakes dilator passing readily into duodenum. Intraoperative choledochoscopy could not be done because of nonavailability. This is also a reason for not doing primary closure in many cases. T-tube was inserted in 20 cases (group I) and primary closure was done in 18 cases (group II). Primary closure of CBD was done with 3-0 vicryl interrupted sutures. 12F size T-tube was inserted in group I patients and opening in CBD around T-tube was made watertight with 3-0 vicryl sutures.

![Figure 1-Primary closure of CBD after choledocholithotomy](image)

In all patients a subhepatic tube drain was inserted which was removed after 3-6 days. T-tube was removed after 3 weeks after a satisfactory T-tube cholangiography after 10-14 days. The other options of CBD stone management like choledochoduodenostomy, transduodenal sphincterotomy/sphincteroplasty which are necessary in selected cases of CBD exploration for stone are not discussed in this study. All patients were given standard post-operative care with IV fluids. Antibiotics, analgesics and followed up at 3 weeks, 3 months and 1 year. Primary closure was done in those patients with few stones (up to 2 wholesome stones) not breaking easily while holding with Desjardins forceps. T-tube was given in those patients with large number of stones of different sizes, easily crushable stones, CBD much inflamed.
Results

Out of 38 patients who underwent CBD exploration 28 were females (73%) and 10 (27%) were males. Age group varied from 32 to 67 years. In group I average duration of hospital stay was 12 days (8-16) and in group II, hospital stay ranged from 4-7 days average 5 days. In group I, one case (5%) had tube dislodgement on 4th post-operative day which was again inserted operatively and another patient developed bile leak about 200ml which was managed conservatively by drain. In group II, one patient developed bile leak about 300ml on 2nd post-op day which subsided by 4th post-op day. In group I one patient had residual stone which was referred for ERCP removal. Other complication were superficial wound infection one in each group. No postoperative haemorrhage, biliary peritonitis, acute pancreatitis were found. However one patient with residual stone developed pain and jaundice which was persistent. Size of CBD ranged from 8-17mm. average operation time was 65-85 minutes more in T-tube group. Serum bilirubin level ranged from 1mg(normal) to 9.6mg/dl.

COMPARISON OF TWO GROUPS:

<table>
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<tr>
<th></th>
<th>Group I(20)</th>
<th>Group II(18)</th>
</tr>
</thead>
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<tr>
<td>Hospital stay</td>
<td>12(8-16) days</td>
<td>5(4-7) days</td>
</tr>
<tr>
<td>Bile leak</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tube dislodgement</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Residual stone</td>
<td>1</td>
<td>nil</td>
</tr>
<tr>
<td>Wound infection</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CBD size</td>
<td>10-17mm</td>
<td>8-13mm</td>
</tr>
<tr>
<td>Average operation time</td>
<td>75-85minutes</td>
<td>65-75minutes</td>
</tr>
</tbody>
</table>

Discussion

The current standard protocol for the treatment of CBD stones is to clear CBD by means of Endoscopic retrogradecholangio-pancreatography(ERCP), followed by laparoscopic cholecystectomy in today’s minimally invasive era. However, these minimally invasive approaches are not widely practised in many developing countries including ours due to the lack of equipment and trained endoscopists. Even in the developed world, in rural setting there is lack of equipment for these techniques. In many studies, ERCP was less successful compared with open surgery in CBD stone clearance and was associated with a higher mortality rate. There is also an increased incidence rate of CBD stones after endoscopic removal. Post ERCP pancreatitis, chance of ascending cholangitis, haemorrhage and very rare possibility of increased chance of malignancy cannot be ignored. Traditionally after exploration of CBD, t-tube is placed. The T-tube drainage is helpful to prevent bile stasis, decompress the biliary tree, allows spasm or oedema of sphincter of Oddi to settle after the trauma of exploration and acts as a stent in CBD and minimises the risk of biliary drainage. A T-tube has also provided an easy access for cholangiography and extraction of
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retained stones. Despite these potential advantages, morbidity rates related to T-tube have been reported to be at a rate of 4-16.4%. The T-tube related complication includes accidental T-tube displacement leading to CBD obstruction, bile leakage, persistent biliary fistulas and excoriation of the skin, cholangitis from exogenous sources through the T-tube, and dehydration and saline depletion. Additionally, CBD stenosis has been reported as a long term complication after T-tube removal. After discharge, indwelling T-tube becomes uncomfortable, requiring continuous management, thus restricting patient’s activity because of the risk of dislodgement and prolonged absence from joining work. As T-tube drainage is associated with these significant disadvantages, therefore, primary repair of CBD has been advocated in literature. The four requirements for a safe and successful primary closure are patent ampulla of Vater, complete removal of all intraductal calculi, absence of pancreatic pathology and meticulous suture of the duct. This study shows that hospital stay in the T-tube group (8-16) days versus 4-7 days in primary group was longer than primary closure group which is in agreement with studies conducted by Zhang et al, Ambreen et al, and KywinTah Noe et al. In our study in T-tube group there were complications like bile leak, infection and dislodgement of T-tube which required replacement operatively. The main drawback of T-tube was that it was uncomfortable, require continuous management and it restricts patient’s activity because of risk of dislodgement. It also affects the patient’s quality of life. Retained stones following CBD exploration remained a significant complication and it was reported in up to 10% of cases in relevant studies. In present study in T-tube group, one patient (5%) had retained stones and these were removed by ERCP. Post-operative hospital stay was much shorter in primary closure group and hospital expenses were also lower than in the T-tube group. After discharge from hospital there is no effect on patient’s quality of life and they can join their jobs early. Biliary complications are considered to be major consequences after primary repair of CBD. However their overall frequency are not higher than T-tube closure. In this study one patient in each group had bile leak which subsided with conservative management. The use of primary closure was limited in the treatment of patients with severe acute biliary pancreatitis, acute pyogenic cholangitis or ampullary stenosis because they required CBD decompression and biliary drainage or other preferable therapeutic options. A small diameter CBD (8mm) may be a contraindication for primary closure because this might increase the risk of bile duct stricture. To minimise post-operative complications, the indications for T-tube must be strictly followed, such as CBD stones secondary to intrahepatic duct stones or if there is undefined residual stone in the intrahepatic or extrahepatic ducts, vague patency of the Oddi’s sphincter or failure to pass bae’s dilator through Vater’s ampulla due to oedema or obstructed stones, acute suppurative cholangitis with severe oedema of the CBD wall.

Results of our early experience with primary closure did not increase the risk of bile leak after the operation. Post-operative hospital stay and operation time (75min) were shorter and lower hospital expenses. With primary closure, we could surely avoid T-tube related complications.

Therefore we can conclude that primary closure without T-tube drainage after cholecystolithotomy is safe, much simpler and cost effective. Post-operative primary closure should be preferred in most cases after CBD exploration wherever possible. However larger randomised trials with longer follow up are necessary to find whether there is stenosis or any other problems after primary closure.

Conclusion

From the study we can conclude that primary closure without external drainage (T-tube) after cholecystolithotomy is safe, feasible, much simple and effective. It reduces the hospital stay and cost, can be done in many cases of CBD exploration and is much comfortable for the patient. However randomised trials on a larger scale of patient and a longer follow up are necessary to address the issue.

Conflict of Interest: None
Funding: no source of funding

References


Detection of Mn – Dependent Chitinase for Wheat Root Rot Disease Control by Real time PCR

Noor Maath Ahmed¹, Akeel Hussian Ali Al-Assie¹, Abdullah Abdulkareem Hassan²

¹Researcher, Department of Biology, University of Tikrit, College of Science, ²Prof. Department of Plant Protection, University of Tikrit, College of Agriculture

Abstract

The use of some mineral salts with concentrations of 10, 30 and 50 mm Muller led to the inhibition of the activity of the enzyme chitinase, but to varying degrees. Manganese chloride MnCl₂ showed more effect in stimulating the activity of the enzyme, as the effectiveness reached 74.87% in addition to calcium chloride CaCl₂, which had a stimulating effect for the enzyme as well. The salts EDTA and NH₄Cl had an inhibitory effect on the enzyme activity. The field experiment was conducted for the purpose of inducing systemic resistance of wheat plants with the fungus T. longibrachiatum under conditions of infection with the pathogen Fusarium oxysporum and evaluating the efficacy of the chitinase enzyme in improving plant growth for three varieties of wheat, which are Iba 99, Sham 6 and July 2 for 45 days, after which the vegetative characteristics were taken. For cultivated plants and the efficacy of the chitinase enzyme for the shoot and root system, the induction treatment (pathogenic fungus + T. longibrachiatum + MnCl₂) proved its role in the highest reduction of the severity of infection with the pathogen fungus of wheat, reaching 18.05% and the high vegetative and root growth indicators under conditions of pathogen infection and for all the studied varieties. The results of induction were due to the increased expression of the chitinase enzyme gene. RT - PCR technique was used. It showed that all the studied cultivars with 99, Sham 6 and July 2 had higher expression than the control treatment.

Keyword: Wheat, chitinase, MnCl₂, Trichoderma, RT-qPCR

Introduction

Wheat Triticum L. belongs to the Poaceae family, and it is one of the oldest agricultural crops known to man, as it is grown on a large scale and produced in large quantities. Many of the economic losses in agricultural crops are due to its infection with fungi, and the fungi of roots rot the largest share, and at the forefront of these crops is wheat and barley. The symptoms of field infection are the absence of plants from some areas or the presence of scattered areas in the field of yellowish or wilted plants due to infection with the fungi of root rot, and the infection of different wheat plants with different types of fusarium, which affects the wheat yield. The severity of the disease was inversely related to the plant height. It also significantly contributed to reducing the number of heads and their infestation with mold[1].

There are many problems facing the process of growing the wheat plant, which cause a significant reduction in its production and quality, including the pathogens that afflict it in all stages of its growth and attack its various parts, causing the deterioration of its production in terms of quantity and quality and the occurrence of economic losses, especially when the appropriate conditions are available, including disease of seed rot, roots and death. Wheat seedlings caused by the fungus Pythium spp., Rhizoctonia solani, Fusarium spp. and other fungal species [2]. The fungus Fusarium sp. Fusarium head blight (FHB) for wheat is the most important problem in cereal cultivation, which leads to large quantitative losses and deterioration of the quality of grain fields and their products. Crop losses due to FHB can be excessive and in maximum cases up to 70% [3]. Fusarium head blight (FHB) mainly caused by F. graminearum is one
of the most destructive fungal pathogens of wheatgrass and very important economically\cite{4}[5].

Many studies have emphasized the importance of enzymes in biological control, including chitin degradation enzymes, as they received great attention in this regard and were developed as biopesticides or chemical defense proteins in genetically modified plants or microbial biological control agents. The biological control of the fungal diseases that carry the soil is interrelated with the production of chitinase, as the fungi and bacteria producing the enzyme chitinase are more virulent in inhibiting the growth and killing of pathogenic fungi \cite{6}. The chitinase enzyme is one of the hydrolysis enzymes secreted by the plant when stimulating its resistance, and its secretion to this enzyme gives it protection from pathogens and increases its resistance to pathogens at different levels as it works to release N-acetyl glucosamine upon infection \cite{7}[8].

Some enzymes show an absolute need for a specific inorganic ion for their catalytic activity, while other enzymes show increased activity when certain cations are added to the reaction medium. Some divalent cations can replace each other, but sometimes one of them competes with the other, while inhibitors reduce the activity Catalytic enzymes\cite{9}. Found Lee\cite{10} that the purified chitinase from Penicillium sp. LYG0704 was inhibited by (79 and 81)% in the presence of iron ions (Fe + 2) and mercury (Hg + 2). Respectively, while the magnesium ions (Mg + 2) and molybdenum (Mo + 2) had an inductive effect on the activity of the enzyme by (26 and 22)%, respectively, at a concentration of 1 mmol at a temperature of 37 C for an hour. Told Yi\cite{11} that zinc ions (Zn + 2), calcium (Ca + 2) and iron (Fe + 2) had an effect on the activity of the enzyme beta 1 - 3 clokinase purified from the fungus Trichoderma viride TP09. Due to the spread of wheat root rot disease and its increase in recent years And the importance of the chitinase enzyme in biological control and the role of T. RT-PCR cannula in detecting enzymatic activity. The study aimed:

1 - Use of RT - PCR technique to determine and quantify the expressivity of the chitinase gene.

2 - The effect of mineral salts in activating and inactivating the enzyme chitinase induced by wheatgrass, class Iba 99.

3 - The effect of the mineral inhibitor MnCl2 and the chitinase enzyme on pathogenicity of Fusarium oxysporum on the wheat plant.

Materials and Methods of Work

The Effect of Mineral Salts on the Effectiveness of chitinase

This experiment was conducted on pure chitinase induced from wheatgrass type Iba 99 bioavailable using the fungus Trichoderma longibrachiatum\cite{12}. The salts have prepared Na₂MoO₄, MgCl₂, K2SO₄, ZnSO₄, FeCl₃, LiCl, (NH₄)SO₄, MgSO₄, CoCl₂, MnCl, FeSO₄, Na₂SO₄, MnSO₄, NH₄Cl₂, CaCl₂, KCl, LiF, CuSO₄10, CaCl₂, KCl, LiF, CuSO₄, 3-D, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3, CaCl₂, KCl, LiF, Cu 50) Milli-Muller and add 1 ml of each concentration to 1 ml of enzyme chitinase. Then incubate in a water bath at a temperature of 35 C for an hour, then take 1 ml of it and add 1 ml of pure chitin solution to it, then put it in a water bath at a temperature of 35 For an hour, then 1 ml of DNS was added and then placed in a boiling water bath at a temperature of 100 ° C for 5 minutes. After that, the tubes were cooled with tap water. The readings were taken on a spectrophotometer with a wavelength of 540 nm and then the enzymatic activity of chitinase was estimated.

Estimate the Efficacy of the Enzyme Chitinase

The method has been folloled by Tweddell\cite{13} for the determination of the chitinase enzyme, as the reaction mixture consisted of adding 0.5 ml of a solution of chitin (1%) and 0.5 of the enzyme extract and incubated in a water bath at a temperature of 37 C for two hours. After which 1 ml of DNS was added and the mixture was placed in a water bath at a temperature of 100 M for 5 min, the tubes were cooled and the absorbance was measured in a Spectrophotometer at a wavelength of 540 nm. The standard curve for n-style glucose amine was adopted in estimating the enzymatic activity. The
enzymatic activity was defined as the amount of enzyme required to release 1 micromol of n-stylet glucose amine per minute and according to the reaction conditions.

**Effect of mineral inhibitor MnCl₂ and chitinase enzyme on pathogenicity of Fusarium oxysporum on wheat plant.**

The potting experiment was carried out in the 2019-2020 season. Mixed soil was cleaned and sifted well, then sterilized with 5% formalin, then covered with nylon for 10 days. Then the nylon was lifted to perform a 3-day aeration of the soil. Soil was distributed in the pots at a rate of 3 kg per pot and the experiment included 5 treatments of three varieties and three replications to produce 45 experimental units as follows:

**Field experiment parameters**

The experiment included three varieties of wheat (Sham 6, Tamuz 2, Iba 99). The experiment also included the following treatments:

- 1 Treatment of healthy plants only.
- 2 Treatment of the pathogen F. oxysporum only.
- 3 Treatment of the pathogen F. oxysporum + Trichoderma longibrachiatum (T1).
- 4 Treatment of the pathogen F. oxysporum + MnCl₂.
- 5 Treatment of the pathogen F. oxysporum + Trichoderma longibrachiatum (T1) + MnCl₂.

A suspension of F. oxysporum spores was added at 1 x 10¹⁰ colony forming units (CFU) / ml at 100 ml per pot. After 3 days, a suspension of Trichoderma longibrachiatum (T1) was added at 1 x 10¹⁰ CFU / ml at 100 ml. For each pot, 15 seeds of each variety of wheat were planted separately for each pot. As for the treatment of manganese chloride MnCl₂, 100 ml of it was added at a concentration of 10 mmol per pot after planting the seeds. The plant service was carried out from bush hoe (if any) and the irrigation was carried out according to the plant’s need.

**Studied traits**

The studied traits were taken after 45 days of germination, except for the efficacy of chitinase, which were tested after two weeks of germination

**Germination Rate**

It was calculated by dividing the number of plants growing in pots for each variety of wheat by 3 replications for each treatment by the total number of seeds planted.

**Chlorophyll Estimate**

The percentage of chlorophyll in wheat plant leaves was estimated in the morning using a Chlorophyll meter, as three plants were taken from each repeat randomly for all varieties and treatments.

**Weights Estimate for Dry and Wet Root and Vegetable Masses**

Samples were taken randomly from the pots from all the duplicates, then the plants were cleaned and washed with water to get rid of soil residues on the roots. Then the weights were measured for the vegetative and root system (wet), after that, the plants were dried on sunlight until the weight stabilized and according to the dry weight (in grams) using the sensitive balance.

**Estimate injury severity**

According to McKinney’s equation\(^{[14]}\), the percentage of injury severity was calculated in all treatments depending on the appearance of the injury and according to the pathological evidence adopted by it\(^{[15]}\).

**The degree appearance of the injury**

0 The plant is healthy, the root system is large, and the roots are white

1 slight brown discoloration on the roots and yellowing of a specified number of leaves

2 Full coloration of the roots, with a complete
yellowing of leaves

3 The coloration extends from the roots to the bases of the stems

4 general death

The severity of the injury was estimated according to the following equation:

\[ \text{Severity of injury} = \frac{\text{Number of plants in grade } 0 \times 0 + \text{number of plants in degree } 1 \times 1 + \text{number of plants in degree } 4 \times 4}{\text{Total examined plants}} \times 100\% \]

**The Effectiveness of Chitinase in the Root System**

The efficacy of the chitinase enzyme was estimated by the root group of the three varieties after two weeks of germination, as the plant was uprooted and washed well with sterile distilled water. After which the root group was separated from the vegetative and weighed 1 g per portion and placed in a ceramic mortar each part separately and 10 ml of acetate buffer solution was placed on top pH 5.6. The parts were crushed with the solution inside an ice bath until the plant part was well crushed and the plant cells were ruptured. After that, the solution was filtered and the solution was centrifuged at 5000 revolutions / minute for 10 minutes for the solution to get rid of the plant parts, the scent which represented the raw chitinase enzyme was collected and kept in the refrigerator at a temperature of 4°C until use, then the chitinase activity was estimated.

**Extraction of RNA**

The RNA was isolated from fungi and wheat plants of the three varieties using the GENEzol™ TriRNA Pure Kit provided by the Taiwanese company Geneaid, to extract the ribonucleic acid according to the instructions of the supplier.

**Measurement of RNA Concentration and Purity**

The concentration and purity of the extracted RNA was measured using a nucleic acid concentration and purity meter (NANO DROP). RNA purity was measured by dividing the RNA absorbance at length 230 by the absorbance product at 280, while the purity was calculated using the following equation: Concentration (µg / ml) = OD 260 x dilution factor x 40 µg / ml.

**Converting RNA into cDNA**

The conversion process was performed using the AccuPower RocketScript™ RT PreMix kit prepared from the Korean company BIONEER. The cDNA reaction mix includes 18 µl of template RNA (100pg), 2 µl of Oligo dt20 (50 pmoles), after that, the reaction mixture was transferred to transparent white tubes of 0.2 ml, and the mixture was mixed with the Vortex device for 3 minutes at a speed of 3000 rpm. The reaction conditions as follow: Primer annealing (Oligo dT 20) at 37°C for 10 min, cDNA synthesis at 42°C for 60 min and heat inactivation at 95°C for 3 min.

**Real Time PCR reaction**

**Prepare the qPCR mixture**

The reaction mixture was prepared using the AccuPower GreenStar™ qPCR PreMix prepared from the Korean company BIONEER and according to the company’s instructions, 5 ml of cDNA and 3 ml of the initiator (F + R) were added at a concentration of 10 pmole and 12 l DEPC - distilled water and then the mixture was applied in opaque white tubes of 0.2 ml for the kit and the Real Time PCR machine, then all the tubes were transferred to the Vortex machine to mix the mixture.

**Prefixes used in the Reaction**

The primers for the chitinase gene for wheatgrass were designed by Dr. Ahmed Abdul-Jabbar Sulaiman, prepared from Macrogen; the first primer was Wheat
Primer “F: 5’-CTACACGTACGCCTCCTA-3’” and “R: 5’-TGCCCTTGCTTATCTCTCC-3’” whose annealing temperature was 58 and it’s produced band was 194 bp. The second primer was ADP “F: 5’-CCTCATGGTCGGTCTCGATG-3’” and “R: 5’-GGATGGTGGTGACGATCTCT-3’”. the annealing temperature of ADP was 59 and it’s produced band was 80 bp.

The prefixes were dissolved with Nuclease Free Water to obtain a final concentration of 100 pmole as a storage solution and kept at a temperature of -20 ̊C. Upon reaction, 10 pmole of the storage solution was prepared by taking 10 ml of the storage initiator and adding to it 90 ml of Nuclease Free Water to obtain a final volume of 100 ml that was involved in the qPCR.

The program used to amplify the prefixes in a real-time polymerase chain reaction (qPCR) is shown below:

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predenaturation</td>
<td>95 ̊C</td>
<td>3 min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95 ̊C</td>
<td>20 Sec</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>55 ̊C</td>
<td>40 Sec</td>
<td>40</td>
</tr>
<tr>
<td>Detection (Scan)</td>
<td></td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>Melting</td>
<td>55 - 95 ̊C</td>
<td>1 min</td>
<td>1</td>
</tr>
<tr>
<td>Incubated</td>
<td>25 ̊C</td>
<td>1 min</td>
<td></td>
</tr>
</tbody>
</table>

Statistical Analysis

The laboratory experiments and the CRD were carried out and the data were analyzed using the SPSS program, and the averages were compared according to the Least Significant Differences LDS at a probability level of 0.05 [16].

Results and Discussion

Inhibition and Activation of Minerals on the Effectiveness of Chitinase

The results indicated that if Manganese Chloride recorded the highest activity of the enzyme, the percentage increase in activity was (74.82, 71.11, 66.33) %, followed by CaCl2 (43.76, 57.63, 73.98%). The lowest activity of the enzyme was recorded when using K2SO4, as the percentage increase in activity was (12.23, 7.03, 4.22%) for the concentrations used respectively.

The results also indicated that there is an inhibitory effect for all EDTA concentrations, as the percentage of chitinase reductase activity was (22.75, 34.12, 56.43), followed by the salt NH4Cl, as the percentage of enzyme reductase was (18.87, 33.51, 42.32)%. These results were in agreement with Haider [17], who mentioned there is an induced effect of manganese chloride on the activity of the chitinase enzyme, but for calcium chloride the result was opposite to what appeared to us as it had an inhibitory effect on the enzyme activity. The results are also in agreement with those found by Adrangi [18] showed that manganese ions had an effect on the two forms of the intracellular enzyme (chi-56 and chi-64) purified from Massilia timonae. As for the effect of EDTA on decreasing the enzyme’s effectiveness, it was identical to what Al-Fakihi [19] found in his study on the effectiveness of purified alpha amylase from malt type Iba 99, as it was also found a decrease in the
activity of the enzyme by increasing the concentration of EDTA, but its results were opposite to what we found with regard to manganese chloride. The activity of the enzyme decreased when incubated with this salt.

The higher activity in the presence of Mn ions may be attributed to the enzyme’s need for this ion in the form of co-factor. The evidence for the enzyme’s requirement for this ion is the lower activity when using the EDTA chelating agent. The decrease in activity when incubating the enzyme with the mineral salts under study may be attributed to the effect of these salts on the enzyme synthesis or at the enzyme’s active sites on the one hand or in the base material on the other hand through the formation of complexes that impede the enzyme’s attachment to the base material. The mechanism by which the positive and negative ions activating the enzyme works is also different, as the ion may change the interstitial direction of the protein in order to allow the correct association between the enzyme and its base material[20].

Effect of mineral inhibitor MnCl₂ and chitinase enzyme on pathogenicity of Fusarium oxysporum on wheat plant.

The Effect of Treatment with the Fungus T. longibrachiatum T1 and Manganese Chloride on Shoots Weight

The results listed in Table (1) show that the highest shoots weight at the level of treatments was when treating the fungus Tricho. + Mncl2 in the presence of the pathogen F. oxysporum, which reached 0.82 g, followed by the Trichoderma treatment in the presence of the pathogen, which reached 0.67 g compared to the weight of the shoot in the treatment of healthy plants (control), which amounted to 0.66 g. Whereas, the lowest weight of the shoot total was recorded in the MnCl₂ treatment with the presence of The pathogenic fungus was 0.25 g, which did not differ significantly compared to the treatment of pathogenic fungi only (0.21 g). As for the average of the varieties, cultivar Iba 99 showed the highest weight of the shoot total, which reached 0.56 g, while the lowest weight of the shoots was 0.49 g in the Sham 6 cultivar. As for the interaction, the cultivar showed a positive 99 with the highest shoot weight of 0.86 g in the treatment of the pathogen + Tricho. + MnCl₂ without significant differences with the control treatment compared to the minimum shoot weight of 0.17 g in the cultivar Cham 6 in the treatment of pathogenic fungi only.

Table (1) Effect of Treatment with the fungus T. longibrachiatum T1 and manganese chloride on shoots weight (gm) of three varieties of Iraqi wheat under conditions of infection with the pathogen F. oxysporum.

<table>
<thead>
<tr>
<th>Average of transactions</th>
<th>Ibba Class 99</th>
<th>Sham Class 6</th>
<th>July Class 2</th>
<th>Transactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (healthy plant)</td>
<td>0.66</td>
<td>0.69</td>
<td>0.62</td>
<td>0.67</td>
</tr>
<tr>
<td>Pathogen fungi only</td>
<td>0.21</td>
<td>0.25</td>
<td>0.17</td>
<td>0.21</td>
</tr>
<tr>
<td>Pathogen fungi + Tricho.</td>
<td>0.67</td>
<td>0.70</td>
<td>0.64</td>
<td>0.68</td>
</tr>
<tr>
<td>Pathogen fungi + MnCl₂</td>
<td>0.25</td>
<td>0.28</td>
<td>0.21</td>
<td>0.25</td>
</tr>
<tr>
<td>Pathogen fungi + Tricho. + MnCl₂</td>
<td>0.82</td>
<td>0.86</td>
<td>0.79</td>
<td>0.82</td>
</tr>
<tr>
<td>Average varieties</td>
<td>0.56</td>
<td>0.49</td>
<td>0.53</td>
<td>0.53</td>
</tr>
</tbody>
</table>

For Items = 0.071 Transactions = 0.084 Items x Transactions = 0.13   LSD 0.05
The Effect of Treatment with the Fungus T. longibrachiatum T1 and Manganese Chloride in Root Mass Weight

The results listed in table (2) show that the highest weight of the root group at the level of treatments was when treating the fungus Tricho. + MnCl2 in the presence of the pathogen F. oxysporum, which reached 0.53 g, followed by the treatment of Trichoderma in the presence of the pathogen, which reached 0.43 g compared to the weight of the root total in the treatment of healthy plants (control), which amounted to 0.4 g, while the lowest weight of the root group was recorded in the treatment of MnCl2 with the presence of the pathogenic mushrooms, which amounted to 0.15 gm, did not differ significantly compared to the treatment of pathogenic fungi only (0.13g).

As for average cultivars, cultivar Iba 99 showed the highest root total weight of 0.372 g, while the lowest root total weight was 0.29 g in Sham 6. With regard to the interaction, the cultivar showed a test of 99 with the highest root total weight of 0.59 g in the treatment of the pathogen + Tricho. + MnCl2, compared to the minimum root weight, was 0.11 g for the cultivar Cham 6, in the treatment of pathogenic fungi only.

Table (2) the effect of treatment with the fungus T. longibrachiatum and manganese chloride on the weight of the root system (gm) of three varieties of Iraqi wheat under conditions of infection with the pathogen F. oxysporum.

<table>
<thead>
<tr>
<th>Average Transaction</th>
<th>Ibaa Class 99</th>
<th>Sham Class 6</th>
<th>July Class 2</th>
<th>Transactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (healthy plant)</td>
<td>0.40</td>
<td>0.35</td>
<td>0.40</td>
<td>Control (healthy plant)</td>
</tr>
<tr>
<td>Pathogen fungi only</td>
<td>0.13</td>
<td>0.16</td>
<td>0.11</td>
<td>0.12</td>
</tr>
<tr>
<td>Pathogen fungi + Tricho</td>
<td>0.43</td>
<td>0.48</td>
<td>0.38</td>
<td>0.42</td>
</tr>
<tr>
<td>MnCl2+Pathogen fungi</td>
<td>0.15</td>
<td>0.18</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Pathogen fungi + Tricho. + MnCl2</td>
<td>0.53</td>
<td>0.59</td>
<td>0.48</td>
<td>0.51</td>
</tr>
<tr>
<td>Average varieties</td>
<td>0.37</td>
<td>0.29</td>
<td>0.316</td>
<td>Average varieties</td>
</tr>
</tbody>
</table>

LSD 0.05

For Items = 0.051 Transactions = 0.063 Items x Transactions = 0.086

The Effect of Treatment with the fungus T. longibrachiatum T1 and manganese chloride on the efficacy of the enzyme ketinase radical total.

The results listed in Table (3) show that the highest efficacy of the enzyme chitinase is in the area. Radical on the level of transactions was the treatment of Tricho mushrooms. + MnCl2 in the presence of the pathogen F. oxysporum, which reached 1.92 units / ml, followed by the treatment of Trichoderma in the presence of the pathogen, reaching 1.75 units / ml, compared to the effectiveness of the enzyme in the treatment of healthy plants (control), which amounted to 0.07 units / ml, while the lowest activity of the enzyme was recorded. Chitinase, MG. Radical treatment of MnCl2 in the presence of the pathogen, reaching 1.26 units / ml, which was not significantly different compared to the treatment of pathogenic fungi only (1.08 units / ml).

As for the average of the varieties, class Iba 99 showed the highest efficacy of the enzyme chitinase Mg. Radicals of 1.36 units / ml, while the lowest activity of
the enzyme chitinase was mg. Radical was 1.03 units / mL in the Sham 6 cultivar. As for the interaction, the cultivar showed 99 highest efficacy of the enzyme chitinase Mg. Radicals amounted to 2.07 units / ml in the treatment of pathogenic fungi + Tricho. + MnCl₂ compared to the lowest efficacy of the Mg chitinase enzyme. Radicals amounted to 0.07 units / mL in the Sham 6 cultivar in the control treatment.

Table (3) the effect of treatment with the fungus T. longibrachiatum T1 and manganese chloride on the efficacy of the enzyme chitinase. Root (unit / ml) for three varieties of Iraqi wheat under conditions of infection with the pathogen F. oxysporum

<table>
<thead>
<tr>
<th>Average of transactions</th>
<th>Class Ibba 99</th>
<th>Class Sham 6</th>
<th>July class 2</th>
<th>Transactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>1.08</td>
<td>1.31</td>
<td>0.82</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>1.75</td>
<td>1.87</td>
<td>1.55</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>1.26</td>
<td>1.50</td>
<td>0.96</td>
<td>1.31</td>
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<tr>
<td></td>
<td>1.92</td>
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<td>1.76</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td>1.36</td>
<td>1.03</td>
<td>1.25</td>
<td>Average of Transactions</td>
</tr>
</tbody>
</table>

For Items = 0.061 Transactions = 0.066 Items x Transactions = 0.096 | LSD 0.05

The Effect of Treatment with the Fungus T. longibrachiatum T1 and Manganese Chloride on Pathogen Severity

The results listed in Table (4) show that the highest severity of pathogen infection at the level of treatments was when treating the pathogen only. As it reached 75.44%, followed by MnCl₂ treatment in the presence of the pathogen, reaching 73.94%, compared to the severity of pathogen infection in the treatment of healthy plants (control), which amounted to 0 %. While the lowest pathogen severity was recorded in Tricho treatment. + MnCl₂ in the presence of the pathogen, reaching 18.05%, which does not differ significantly compared to the treatment of the fungus Trichoderma with the presence of the pathogen (23.71%). This is confirmed by the study of Hasan & Aldoury [21], that there is a significant superiority in the severity of infection within the treatment of pathogenic fungi only compared to the rest of the treatments.

As for the average of the varieties, the Sham 6 variety showed the highest pathogen severity, which was 40.0%, while the lowest pathogen severity was 36.22%, in the IBA class 99. As for the interaction, the cultivar Sham 6 showed the highest pathogen intensity, which was 78.40% in the treatment of pathogenic fungi only, compared to the lowest pathogen severity, which was 17.05%, in the cultivar, Ibb 99, in the treatment of the pathogen + Tricho. + MnCl₂.
Table (4) Effect of treatment with the fungus *T. longibrachiatum* T1 and manganese chloride on the severity of pathogen infection (%) for three varieties of Iraqi wheat under conditions of infection with the pathogen *F. oxysporum*

<table>
<thead>
<tr>
<th>Average of transactions</th>
<th>Class IBA 99</th>
<th>Class Sham 6</th>
<th>July class 2</th>
<th>Transactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>Control (healthy plant)</td>
</tr>
<tr>
<td>75.44</td>
<td>71.37</td>
<td>78.40</td>
<td>76.55</td>
<td>Pathogen fungi only</td>
</tr>
<tr>
<td>23.71</td>
<td>22.66</td>
<td>25.63</td>
<td>22.85</td>
<td>Mushrooms. pathogen + Tricho</td>
</tr>
<tr>
<td>73.94</td>
<td>70.04</td>
<td>76.77</td>
<td>75</td>
<td>Pathogen fungi + MnCl2</td>
</tr>
<tr>
<td>18.05</td>
<td>17.05</td>
<td>19.23</td>
<td>17.87</td>
<td>Pathogen fungi + Tricho. + MnCl2</td>
</tr>
<tr>
<td>36.22</td>
<td>40.00</td>
<td>38.45</td>
<td></td>
<td>Average varieties</td>
</tr>
</tbody>
</table>

For Items = 1.21 Transactions = 1.43 Items x Transactions = 3.05 LSD 0.05

Table (5) shows the results of gene expression and its values for the chitinase gene in the wheat plant

<table>
<thead>
<tr>
<th>Transactions</th>
<th>Class Ebaa 99</th>
<th>Class Sham 6</th>
<th>2July class 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ct</td>
<td>ΔCT</td>
<td>ΔΔCT</td>
</tr>
<tr>
<td>Pathogen fungi only</td>
<td>29.21</td>
<td>4.75</td>
<td>-1.86</td>
</tr>
<tr>
<td>Pathogen fungi + Tricho.</td>
<td>24.38</td>
<td>3.32</td>
<td>-3.29</td>
</tr>
<tr>
<td>Mushroom pathogen + MnCl2</td>
<td>28.50</td>
<td>13.29</td>
<td>6.68</td>
</tr>
<tr>
<td>Pathogen fungi + Tricho. + MnCl2</td>
<td>17.73</td>
<td>-3.6</td>
<td>-10.21</td>
</tr>
</tbody>
</table>

It was noticed from the results of the experiment that the treatment with the pathogenic fungus only gave the lowest values for the rate of germination, chlorophyll, plant height and dry weight for both vegetable and root parts; as well as, the experiment gave the highest rates for the dead cells, highest harshness and infection rates; this indicates that the virulence of the pathogenic fungus and the occurrence of damage in the plant, Pathogenic fungus enzymes such as cellulose, pectinase and protease cause degradation of the root and components of plant cells; as well as, the production of pathogenic fungus for fungal toxins that directly effect on plant growth, inversely the low vegetative growth traits and high indicators of injury[22]. The treatment
with MnCl2 alone did not positively affect on the characteristics of vegetative growth nor did it affect in the reduction of plant diseases and this was laboratory proven by Ahmed[23]as it may provide Mn element to the pathogen, while the treatment with *Trichoderma* improved the proportions of seed germination and all the characteristics of vegetative growth and reduced the incidence and severity of the infection due to the mechanisms’ action of *Trichoderma* and their significant role in vital resistance such as antibiosis, competition, Mycoparasitism, Enzymes, resistance induction, production of plant hormones, increased readiness of elements and tolerance of external stress. In case of treating *Trichoderma* with MnCl2 treatment, it is observed that there is a significant superiority statistically to all the characteristics of vegetative plant growth and a decrease in infection indicators, which due to the role of *Trichoderma* and its direct and indirect mechanisms against the pathogen. It should be mentioned that the important factor is activation of Chitinase, which is well known as Mn dependent enzyme.

**Real Time PCR Gene Expression Results**

RT-qPCR was used to detect and estimate the level of gene expression for the gene (Primer Chitinase Wheat) responsible for producing the enzyme chitinase in wheat plant. The ADP gene was used as a reference gene, and the relative quantitative expression method was used where data are presented for the reference gene where the expression is expressed. For reference genes in all cells of the organism under normal and pathological conditions, and although some reference genes are expressed at constant levels in most cases. There are genes whose expression may change depending on the situation, the interval (Ct) where Ct is the number of cycles required for the fluorescence to be emitted from the dye to reach the threshold level to detect the reaction.

The results listed in Table (5) show the gene expression values represented by the value of Cycle threshold (Ct), which indicates the degree of gene expression inversely (as the lower the value of Ct, the more the gene expression process increases), as well as the Fold Fold, where the results showed the high gene expression of the chitinase gene. In wheat plants, class Ibaa 99 for treatment No. 4 (pathogenic mushrooms + Tricho. + MnCl2), which amounted to 1184.449 compared to the control treatment, in which the value of the volt was 1, followed by treatment No. 2 for the same variety (pathogenic mushrooms + Tricho), reaching 9.78, i.e., it increased by approximately 9 times for the Control treatment, which equals 1.

As for the variety Sham 6, the results showed that the highest gene expression for treatment No. 4 (pathogen + Tricho) was 3956.475 compared to the Fold value for Control (1), followed by treatment No. 2 (pathogenic fungus + Tricho), reaching 92,411 compared to A control transaction.

As for the July 2 variety, the results of its gene expression showed that the highest value was recorded for treatment No. 4 (pathogenic fungus + Tricho), reaching 19.027, followed by treatment No. 2 (pathogenic fungus + Tricho), which reached 7, which increased by 7 times over the control treatment (1).

It is evident from the results shown above that the gene expression increased for the three cultivars by the last treatment No. 4 (pathogenic fungus + Tricho), but the highest gene expression was for type Sham 6. The reason for the high gene expression of the chitinase gene in Treatment No. (4) and for the three varieties is due to the treatment of plants with the fungus *Trichodrema*, which stimulates biological resistance and thus stimulates induced systemic resistance (ISR) in addition to the presence of salt (MnCl2), which acts synergistically with the fungus and leads to increased stimulation. The production of the enzyme chitinase and thus leads to the stimulation of acquired systemic resistance (SAR) in the plant, which lies in the stimulation of genes encoding some pathogen-related proteins such as the enzyme chitinase, which has a high activity in analyzing the cell wall of pathogenic fungi and improving plant growth[24][25]. This has been demonstrated in our previous experiments in induction of chitinase enzyme biosynthesis in 12 varieties of wheat plants, as the mushroom-induced
varieties showed the highest efficacy of the chitinase enzyme compared to the enzyme activity of the varieties without the inducement factor. As well as what was demonstrated in the experiment of steroids and enzyme inhibitors of a group A wide range of salts and three concentrations, as the MnCl2 salt recorded the highest activity of the enzyme and of the three concentrations. In another experiment on the effect of Mineralogenerator MnCl2 and the enzyme chitinase extracted from wheat induced by Trichoderma fungus on some pathogenic fungi. The results proved that the presence of the salt and enzyme mixture in the middle of the development of pathogenic fungi inhibits the growth of these fungi compared to the control plate. Thus, the salt and enzyme mixture inhibited the growth of pathogenic fungi due to the maximum effectiveness of the enzyme Manganese ions. The results of the gene expression were consistent with the findings of Al-Jassani [26] in his study of three varieties of date palms treated with salicylic acid and hydrogen peroxide. Which led to an increase in the gene expression of the gene responsible for the enzyme Superoxide SOD dismutase (which is considered one of the defense enzymes to reduce the damage caused by exposure to The plant under saline stress conditions). As the variety Burhi gave the highest gene expression when treated with salicylic acid 500 mg, when the value of Folding (3.36) was reached. As for the crescent variety, the highest value of gene expression in the treatment of salicylic acid was 250 mg, as the value of Folding (5.09) and the unknown variety gave the highest an expression value in the treatment of hydrogen peroxide at a concentration of 6%, as it reached (2.82).

**References**


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On Health Care Sector, What External Environment that Important? : A Review Literature

Noor SyamSidiq Himawan¹, Nur Wening²

¹Doctoral Student, ²Associate Professor, Post Graduate Program of Management, University of Technology Yogyakarta

Abstract

This Paper is a literature review related to various studies that discuss macro environmental analysis (political, economic, socio-cultural and technological) in the field of health care. The purpose of this study is to explore and connect theories from various studies related to macro business environment factors that are important in the field of health care, how much research is related and what are the opportunities for future research. This paper identifies 21 articles that discuss the analysis of the business environment in the health care sector. In this study, the criteria for selecting literature were applied, namely the selected literature must be in the form of articles not handbooks, in English, discussing business environmental analysis, especially in the field of health services and published for a maximum of the last 10 years. The research related to political factors was 31.25%, economic factors 18.75%, socio-cultural factors 18.75% and technological factors 31.25%. Research related to environmental analysis (political, economic, socio-cultural and technological factors) in the health sector is still very minimal, especially in the economic and socio-cultural sectors so that this can actually become an opportunity in developing research on the business environment in the health sector.

Keywords: PEST analysis, health sector, political factors, economic factors, socio-cultural factors, technological factors, external environment, strategic management.

Introduction

Analysis of external environmental factors is an important thing in the formulation of strategies for improving the performance of health organizations ¹². Based on research, business environment analysis can have a significant impact on a company p value 0.025 ³. Macro environmental conditions are conditions outside a company and cannot be controlled by a company but must be analyzed to adjust the company’s strategy to changes in the business environment. The main strengths of the macro environment are political, economic, social, technological, legal, ecological, demographic, ethical and regulatory aspects. The macro environment can be analyzed by various methods such as PEST, PESTEL, PESTLE, STEEPLE, STEEPLED ⁴. The challenge faced by health services today is how to be able to control patient care costs efficiently and adapt to policy changes that occur quickly ⁵. Economic and political instability are also factors that affect the health service system ⁶. In addition, the socio-cultural condition of the organization also plays an important role in health services ⁷. The term PESTLE has been used regularly in the last 10+ years and its true history is difficult to establish. The earliest know reference to tools and techniques for ‘Scanning the Business Environment’ appears to be by Francis J. Aguilar (1967) who discusses ‘ETPS’ – a mnemonic for the four sectors of his taxonomy of the environment: Economic, Technical, Political, and Social. Shortly after its publication, Arnold Brown for the Institute of Life

Corresponding author:
Noor SyamSidiq Himawan.
Post Graduate Program of Management, University of Technology Yogyakarta,
Email: noorsyamsh@gmail.com+623815249295
Insurance (in the US) reorganized it as ‘STEP’ (Strategic Trend Evaluation Process) as a way to organise the results of his environmental scanning. Thereafter, this ‘macro external environment analysis’, or ‘environmental scanning for change’, was modified yet again to become a so-called STEPE analysis (the Social, Technical, Economic, Political, and Ecological taxonomies). In the 1980s, several other authors including Fahey, Narayanan, Morrison, Renfro, Boucher, Mecca and Porter included variations of the taxonomy classifications in a variety of orders: PEST, PESTLE, STEEPLE etc. Why the slightly negative connotations of PEST have proven to be more popular than STEP is not known. There is no implied order or priority in any of the formats. Quite who and when added what elements to the mnemonic is a mystery, but what is known is that the actual order and words contained are common to certain parts of the world and streams of academic study.

**Literature Review**

Macro environmental conditions are conditions outside a company and cannot be controlled by a company but must be analyzed to adjust the company’s strategy to changes in the business environment. The main strengths of the macro environment are political, economic, social, technological, legal, ecological, demographic, ethical and regulatory aspects. The macro environment can be analyzed using various methods such as PEST, PESTEL, STEEPLE, STEEPLED. The explanation of these methods is as follows:

- **PEST**

  PEST is an external environmental analysis using approaches political, economic, social, and technological.

- **PESTEL**

  PESTEL is an external environmental analysis using approaches political, economic, social, technological, environmental and legal.

- **STEEPLE**

  STEEPLE is an external environmental analysis using social, technological, economic, environmental, political, legal and ethical.

- **STEEPLED**

  STEEPLED is an external environmental analysis using the social, technological, economic, environmental, political, legal, ethical, and demographics dimension.

The explanation of the above components is as follows:

**Political**

This factor looks at the extent to which the government can influence a particular economy or industry. Political factors are generally in the form of various national policies taken by the government that affect an industry. Such as: various policies (tax, fiscal, trade, evil that support certain industries), licensing, processes and services, laws and regulations. These factors determine the extent to which a government may influence the economy or a certain industry.

**Economic**

Factors are factors of economic performance that directly impact the company and have a long term impact. Such as: local economy, international economy, economic trends, inflation, corporate taxes, market and trade cycles, GDP, consumer purchasing power, interest and exchange rates. Economic factors include inflation rate, interest rates, foreign exchange rates, economic growth patterns etc. It also accounts for the FDI (foreign direct investment) depending on certain specific industries who are undergoing this analysis.

**Social Factor**

Are conditions that affect market and consumers. These factors include: Demographics, lifestyle psychology, consumer perceptions of brands, consumer purchasing behavior, influence of advertising and public relations, influencers /role models, influence (race, ethnicity and religion). The sociological factor
takes into consideration all events that affect the market and community socially. Thus, the advantages and disadvantages to the people of the area in which the project is taking place also need to be considered. These factors scrutinize the social environment of the market, and gauge determinants like cultural trends, demographics, population analytics etc. An example for this can be buying trends for Western countries like the US where there is high demand during the Holiday season.

**Technological Factors**

This is a technological innovation that can affect industry and the market. Such as: technology in product design, production, distribution, price and consumption, machines or software that support business processes. These factors pertain to innovations in technology that may affect the operations of the industry and the market favorably or unfavorably. This refers to automation, research and development and the amount of technological awareness that a market possesses. This factor takes into consideration all events that affect technology. Since technology often becomes outdated within a few months after it is launched, it is important to consider this. This factor could also take into consideration all barriers to entry in certain markets and changes to financial decisions.

**Legal**

This factor takes into consideration all legal aspects like employment, quotas, taxation, resources, imports and exports, etc. These factors have both external and internal sides. There are certain laws that affect the business environment in a certain country while there are certain policies that companies maintain for themselves. Legal analysis takes into account both of these angles and then charts out the strategies in light of these legislations. For example, consumer laws, safety standards, labor laws etc.

**Environmental**

These factors include all those that influence or are determined by the surrounding environment. Factors of a business environmental analysis include but are not limited to climate, weather, geographical location, global changes in climate, environmental offsets, ground conditions, ground contamination, nearby water sources, etc. 8,9

Based on previous research conducted on health facilitation research in the Lubin area, the environmental analysis obtained is as follows:

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Assessment</th>
<th>Sub Specifications</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Political and legal factors</td>
<td>2.0</td>
<td>Health service reform</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Changes in health care policies</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Volatility of regulations and legal norms</td>
<td>2.3</td>
</tr>
<tr>
<td>Economic factors</td>
<td>1.7</td>
<td>Economic situation</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inflation</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Population income equity</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Availability of loans</td>
<td>1.8</td>
</tr>
<tr>
<td>Socio-cultural factors</td>
<td>0.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Technological factors</td>
<td>2.0</td>
<td>Procedures techniques and diagnostics</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Changes in health regulations</td>
<td>2.0</td>
</tr>
<tr>
<td>Demographic factors</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Epidemiological factors</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Other research related to PEST analysis in the health service sector suggests that important political, economic, social and technological factors in the health sector are as follows:

Table 2. Overview of PEST analysis in the health service sector

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Sub-specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Political factors</td>
<td>Role of the government in relation to the promotion of the quality of health services</td>
</tr>
<tr>
<td></td>
<td>Government initiatives in the health sector that can change</td>
</tr>
<tr>
<td></td>
<td>Government strategies in the health sector</td>
</tr>
<tr>
<td>Economic factors</td>
<td>Investment targets in government in the health sector</td>
</tr>
<tr>
<td></td>
<td>Priority setting</td>
</tr>
<tr>
<td>Social factors</td>
<td>Age population</td>
</tr>
<tr>
<td></td>
<td>Changes in lifestyle</td>
</tr>
<tr>
<td></td>
<td>Community expectations regarding health care</td>
</tr>
<tr>
<td></td>
<td>Changes in work practices</td>
</tr>
<tr>
<td>Technological factors</td>
<td>Latest health intervention technologies</td>
</tr>
<tr>
<td></td>
<td>Telemedicine</td>
</tr>
<tr>
<td></td>
<td>Scientific evidence in the health sector</td>
</tr>
</tbody>
</table>

Method

This research is a review of literature related to various studies that discuss the analysis of the business environment, especially the macro environment (political, economic, cultural-cultural and technological) in the field of health services. The collection of articles is done using search engines such as mendeley, google and google scholar. In this study, there were 21 articles discussing the analysis of the business environment in the field of health services. In this study, criteria were applied in the selection of literature. These criteria are that the selected literature must be in the form of articles not handbooks, in English, discussing business environmental analysis, especially in the field of health services and published for a maximum of the last 10 years. Furthermore, the literature obtained was analyzed related to components in important political, economic, socio-cultural and technological factors in the health sector, research that had been carried out for each of these factors, conclusions and future research opportunities.

Results and Discussion

Based on important factors in the above PEST analysis. Several studies have been conducted to
examine each factor in the analysis of the business environment as follows:

**Table 3. Research related to political, economic, social, and technological factors in the field of health care**

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Percentage</th>
<th>Research topics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Political factors</td>
<td>31.25%</td>
<td>Budget allocation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Policies Care cost policies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Health service reform, policy changes, volatility of regulations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Political and policies in health facilities</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Politics and public health Political</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Structure and its influence on health</td>
</tr>
<tr>
<td>Economic factors</td>
<td>18.75%</td>
<td>Situation, inflation, population equity and availability of loans</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Macroeconomics in the health sector</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Impact of economic factors on the quality of health services</td>
</tr>
<tr>
<td>Socio-cultural factors</td>
<td>18.75%</td>
<td>Health organizational culture</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Factor context social in influencing the health sector</td>
</tr>
<tr>
<td>Technological factors</td>
<td>31.25%</td>
<td>Information on health services</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Digital technology in health services</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High technology in health care</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Technology and the future of health services</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Technological factors in health information systems</td>
</tr>
</tbody>
</table>

Sources\(^{2,5,18–21,7,10,12–17}\)

Research related to environmental analysis (political, economic, socio-cultural and technological factors) in the health sector is still very minimal, especially in socio-culture, demography, epidemiology so that this can actually be an opportunity development of research on the business environment in the health sector, even though careful analysis of environmental factors important for improving the performance\(^2,10\)

**Conclusion**

Analysis of external environmental factors is an important thing in the formulation of strategies for improving performance health organization. The main strengths of the macro environment are political,
economic, social, technological aspects. Political factors that are important to note in the health sector include (the role of the government in terms of promoting the quality of health services, government initiatives in the health sector that can change, government strategies in the health sector, health care reform, changes in health care policies, volatility in regulations and legal norms). Economic factors include (government investment targets in the health sector, priority setting, economic situation, inflation, equality of population income, availability of loans). Socio-cultural factors include (age of the population, changes in lifestyle, community expectations regarding health services, changes in work practices). Technological factors (latest health intervention technology, telemedicine, scientific evidence in health, changing health regulations). For each of these facilitators, very little research has been carried out so that it can become an opportunity for future research, especially related to the economic and socio-cultural sectors.

**Ethical Clearance:** No Need Ethical Clearance on this Research

**Source of Funding:** This research was funded privately and did not receive funding from any party.

**Conflict of Interest:** The author confirm that there are no conflicts of interest to disclose.

**References**


The Effectiveness of Dayak Song Art as a Media for Increasing Mother’s Knowledge about the Health and Development of Toddler

Noordiati
Lecturer of Midwifery Department, Polytechnic of Ministry of Health, Palangka Raya, Center of Kalimantan, Indonesia

Abstract

The Dayak song serves as a medium or medium for conveying various development messages, physical development, health, and mental and spiritual development. The Dayak song is very appropriate if used as an innovative medium in conveying the information given to the Mother Toddler class’s implementation. Every mother in Palangka Raya City can easily accept and remember the material given. This type of research is a quasi-experimental study with a two-group comparison pretest-posttest design. Samples were selected based on inclusion and exclusion criteria and were taken by a consecutive sampling of mothers who have children aged 0-60 months. The results of the statistical test p-value of 0.000 (p <0.05), it can be concluded that there is a significant difference in increasing the knowledge of mothers with children under five years about health and growth between the group using the Dayak song media and the flipchart group on the first pre-test and post-test. The results showed a significant difference in the pre and post-test knowledge scores in the Dayak Song and flipchart groups. The knowledge scores between the Dayak song groups and flipcharts shows that Dayak songs are more effective as a medium for delivering information with an increase of 77.41 compared to the increase in knowledge using flipcharts 39.59.

Keywords: Dayak song, knowledge, mother, health and development, toddler

Introduction

Most causes of infant mortality are due to neonatal problems such as low birth weight 11.1%; respiratory disorders 35.9%; prematurity 32.4%, sepsis 12%, hypothermia 6.3%; blood / jaundice disorders 5.6%; post matur 2.8% and 1.4% congenital abnormalities. Infectious diseases are the most common causes of under-five mortality with diarrhea 12.3%, ARI 12.8%, pneumonia 4.8%. Toddlers who experience malnutrition 3.8% and malnutrition are still high, namely as much as 11.4%. Early initiation of breastfeeding (EIB) and exclusive breastfeeding, which plays a major role in reducing neonatal mortality, infants and toddlers can reduce morbidity as diarrhoea and pneumonia. Although the coverage shows an increase, which based on The Indonesian Basic Health Research 2018, the EIB coverage has become 58.2% and the coverage of breastfeeding alone in the last 24 hours is 74.5%, efforts still need to be optimally increased. Immunization can also reduce infant and child mortality due to preventable diseases by immunization. Complete immunization coverage for children aged 12-23 months has decreased from The Indonesian Basic Health Research 2013 of 59.2% to 57.9% in 2018.

The variables that can affect the incidence of infant mortality in Indonesia during the period 1984 to 2012 are Gross Regional Domestic Product (GRDP) per capita, total fertility rate (TFR), female labor force participation rate, and female literacy numbers. Of these variables,
TFR is the determinant that has the greatest influence on infant mortality. One of the strategic efforts to increase the independence of families and communities in maintaining and caring for maternal and child health is using the MCH Handbook. In order for the MCH book to be used properly and its benefits will be felt by the community, it is necessary to provide an understanding of the MCH book through a communication platform that exists in the community which is then referred to as the mother class. The mother class is one of the important activities in increasing the use of the MCH Handbook in the community and as a learning effort for mothers, husbands, and families to understand the MCH Handbook’s contents through the method of collective learning activities facilitated by competent health workers. The mother class was developed for two targets, namely pregnant women through the pregnant mother class and mothers who have toddlers through the mother toddler class. Both are implemented to increase the coverage and use of the MCH Handbook in maternal and child health services. The pregnant mother class activities aim to prepare pregnant women for safe, comfortable and safe delivery, as well as for babies to be born healthy and smart. While the toddler mother class activities aim to realize optimal toddler growth and development.

In several community health center in Palangka Raya City, almost every community health research already has a mother class facilitator, the implementation of the pregnant women class at the community health center has been implemented, but it is different from the maternal toddler class, almost all community health center have not implemented it due to the unavailability of operational funds, ignorance health workers that the implementation of the mother under five class can be integrated with integrated healthcare center services and planning at the community health center level does not yet know technically that this activity is an indicator and priority activity of the ministry of health.

Considering the importance of the mother’s class for toddlers as an effort to realize optimal health and development of toddlers, the researcher intends to contribute in the form of cultural-based learning media innovation to complement existing media, namely in the form of song lyrics containing health material for toddlers in Dayak language songs. One of the functions of the Dayak Song is as a means or medium for conveying various messages of development, both physical development, health and mental and spiritual development, so the Dayak song is very appropriate if it is used as an innovative medium in conveying information given to the implementation of the Mother Toddler class, so that every mother is Palangka Raya City easily accepts and remembers the material given. In addition, families and communities can easily find out children’s health messages under five through Dayak Songs.

**Materials and Methods**

This type of research is a quasi-experimental study with a two-group comparison pretest-posttest design. The research was conducted in two sub-districts of the working area of Community Health Center Kereng Bangkirai, where the mothers with children under-fives used Dayak language daily. This study’s population was all mothers who have children under five aged 0-60 months in the family. Samples were selected based on criteria of ink warp and exclusion and taken with consecutive sampling as follows.

The inclusion criteria in this study were:

a. Have children under the age of 0-60 months.

b. Willing to take part in mother-to-child classes which are held 3 times in a row every month.

c. Residing in the village area which is the place for the mother’s toddler class to be held.

The exclusion criteria are:

a. Not attending all mother-to-child class meetings

b. Have taken a mother class before

c. Not following the post-test.

The difference in the average change in the expected
knowledge score from mothers of children under five is 1.70, the sample obtained for each group is 52 people, in anticipation of missing the sample during the study added 10% so that each group to 58 people. The variables in this research include independent variables, namely the knowledge of mothers under five about the health and development of children under five. The dependent variable, namely the Dayak language song media and flipcharts, and confounding variables are the mothers’ education age who have toddlers. Data analysis was descriptive and analytic (univariate and bivariate).

Findings and Discussion

Table 1. Differences in the level of knowledge of mothers before and after receiving intervention using the Dayak song media and the flipchart.

<table>
<thead>
<tr>
<th>Variabel</th>
<th>Mean (SD)</th>
<th>Beda Mean</th>
<th>P value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre and post-test I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flipchart</td>
<td>1.50 (0.502)</td>
<td>43.66</td>
<td>0.000</td>
<td>58</td>
</tr>
<tr>
<td>Dayak song</td>
<td>8.34 (4.828)</td>
<td>73.34</td>
<td></td>
<td>58</td>
</tr>
<tr>
<td>Pre and post-test II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flipchart</td>
<td>1.50 (0.502)</td>
<td>39.59</td>
<td>0.000</td>
<td>58</td>
</tr>
<tr>
<td>Dayak song</td>
<td>21.53 (11.255)</td>
<td>77.41</td>
<td></td>
<td>58</td>
</tr>
</tbody>
</table>

Based on table 1, the results of the statistical test with the p-value of 0.000 (p <0.05), it can be concluded that there is a significant difference in increasing the knowledge of mothers under five about health and growth and development between the groups using the Dayak song media and the flipchart group on the first pre-test and post-test. The increase in the group’s knowledge score that received the Dayak song intervention by 73.34 was higher than the increase in the knowledge score of the group that received the flipchart intervention by 43.66. In the pre and post-test II, increased knowledge score intervention group that received Dayak songs of 77.41 is higher than the increase in knowledge scores intervention group receiving a flip chart at 39.59. The increase in knowledge of the group that received the Dayak song media intervention was higher than the group that received the flipchart intervention, so it can be concluded that the intervention using the Dayak song media was effective in increasing maternal knowledge compared to the flipchart media intervention, and the results of the increase in knowledge were greater in the second pre-test compared to the first pre-test.

The results showed a significant difference in the pre and post-test knowledge scores in the Dayak Song and Flipchart groups. The difference in knowledge from the pre and post-test of each group shows that both the Dayak song median and the turning sheet used as a medium for conveying information in the toddler mother class implementation can improve knowledge.

The mother toddler class is held in a participatory manner, it means that mothers are not positioned only to receive information because the passive position tends to be ineffective in changing behaviour. Therefore, the
mother toddler class is designed with a participatory learning method where mothers are not seen as students, but as learning citizens. In practice, mothers are encouraged to learn from others’ experiences, while the facilitator acts as a guide to correct knowledge. Facilitators are not teachers or lecturers who teach, but they can be a source of learning in a limited scope.5

In the implementation of the Toddler Mother Class program based on the Indonesian Ministry of Health, flipcharts are used as a medium for delivering information that aims to increase the MCH books’ use. The flipchart used contains pictures related to children’s health and development under five, then the facilitator explains what is on the flipchart. For the Mother Class Toddler group using the Dayak song media, the participants and the facilitator sang Dayak songs whose lyrics were adjusted to the material to be delivered according to the age group, after which the facilitator explained the material contained in the song lyrics using the lecture or question and answer method. The knowledge obtained from the resource persons is inseparable from the experience and knowledge they already have, the facilitator helps the learning community bring back this knowledge to what is happening around them.

Behavioural change in the adult education process (andragogy) is generally more difficult than behavior change in children’s education (pedagogy), because adults already have prior knowledge, attitudes and skills, so that media and learning methods are wrong. A component in the learning process plays a very important role, because these two aspects are interrelated. The choice of a particular teaching method will affect the appropriate type of learning media, although other components must still be considered in selecting instructional media.6

The results of statistical tests show an increase in the knowledge of mothers under five who follow the mother toddler class using Dayak Songs and Flipchart. The increase in knowledge of mothers under five was higher in the group that used Dayak songs than in the Flipchart group so that Dayak songs were considered effective in increasing maternal knowledge compared to Flipchart.

This study’s results are in line with research in West Africa, which shows that music has an important role in improving the health and well-being of people in Sierra Leone. Health messages attract listeners when the existing rhythm is used to be heard by the community.7 The effectiveness of Cirebon tarling culture (regional music) on increasing the knowledge of pregnant women in Cirebon Regency, West Java showed that the change in the knowledge score of pregnant women was higher in the group using Cirebon art media than using flipcharts. The use of local culture in increasing knowledge is one form of creative media that has contextual meaning.5

In Uganda states that by combining scientific knowledge about health and distinctive music as cultural heritage, messages/information will be more able to enter into life to become a culture that is commonly practised. Health messages will attract listeners if they are packaged in the form of a song, especially if the song is well known and has important meaning for the listener. Messages/information can be conveyed through songs to the wider community.7

In this study, the Dayak song lyrics were taken from the material contained in the Facilitator’s Handbook module and then translated into Dayak to easily remember the lyrics sung because they used the language they used daily as a means of communication. The culture-based learning approach can provide opportunities for students to create meaning and achieve an integrated understanding of the scientific information they obtain, as well as the application of scientific information in the context of the problems of their cultural community.8

Health promotion efforts must be carried out most effectively and efficiently to achieve the goals. Various educational strategies, including audiovisual entertainment such as storytelling and videos, are valuable tools in increasing people’s knowledge and attitudes in health promotion. Many health promotion programs use music to increase health promotion, increasing knowledge, social, and building behaviour. In yet another example, traditional song in Laos has proven
effective in imparting knowledge about HIV/AIDS, teaching prevention skills and motivating listeners to change their behavior regarding disease prevention.9

Conclusion

1. There are differences in the level of knowledge of mothers with children under five before and after receiving intervention using the Dayak song media in the implementation of the toddler mother class from a knowledge score of 46.97 to 74.47.

2. There are differences in the level of knowledge of mothers with children under five before and after receiving intervention using flipchart media in the implementation of the toddler mother class from a knowledge score of 48.19 to 63.76.

3. Comparison of the increase in knowledge scores between the Dayak song groups and flipcharts shows that Dayak songs are more effective as a medium for delivering information with an increase of 77.41 compared to the increase in knowledge using flipcharts, namely 39.59.

Ethical Clearance: This research has gone ethical feasibility testing by the Ethical Committee of the Polytechnic of Ministry of Health, Palangka Raya.

Source Funding: This study was done by self-funding from the authors.

Conflict of Interest: The authors declare that they have no conflict interests.

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Qari (Male Voice) Increases Higher Expression of *Synapsin 1* than Qariah (Female Voice) in the *Cerebrum* Newborn *Rattus Norvegicus*

Nur Laila Faizah¹, Hermanto Tri Joewono², Widjiati³

¹Posgraduate Student of Reproductive Health Science, Faculty of Medicine, Universitas Airlangga, Surabaya 60131, Indonesia, ²Lecturer and Consultant in Department of Obstetrics and Gynecology, Universitas Airlangga-Dr. Seotomo Teaching Hospital, Surabaya 60131, Indonesia, ³Lecturer in Department of Embryology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, 60115, Indonesia

**Abstract**

**Introduction:** Pregnancy is the golden period to prepare children for the future. Stimulation is needed during pregnancy, one of which is brain stimulation. This can be done with Al Qur’an Murrotal Surah Ar-Rahman stimulation. This study aims to analyze expression of *synapsin 1* in the *cerebrum* of newborn *Rattus norvegicus* when given murrotal Surah Ar-Rahman stimulation by qari, qariah, and not given any stimulation during pregnancy.

**Methods:** Experimental with post-test only control group design. Sampling in this study was considered homogeneous because it met the inclusion criteria. The samples obtained were distributed randomly to each experimental group and randomized into 3 groups, including control group, group stimulated by qari, and group stimulated by qariah. The treatment was given on the 6th day of pregnancy for 1 hour in a soundproof room with an intensity of 65 dB.

**Results:** The highest mean expression of *synapsin 1* in the *cerebrum* is qari group (6.96 ± 2.59), then qariah group (5.84 ± 2.90), and the lowest is control group (5.74 ± 2.32). There is no significant difference in expression of *synapsin 1* in the *cerebrum* between control and qari groups (p = 0.306), control and qariah groups (p = 0.932), qari and qariah groups (p = 0.347).

**Conclusion:** The highest expression of *Synapsin 1* in the *cerebrum* of newborn *Rattus norvegicus* is the group stimulated by qari.

**Keywords:** qari, qariah, synapsin 1, cerebrum, Rattus norvegicus

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**Introduction**

Pregnancy is the golden period to prepare children for the future. In that pregnancy, most women focus on filling their pregnancy with activities that make the fetus healthy and smart, to prepare quality human resources. Report from the World Economic Forum Indonesia’s competitiveness position in 2019 was at level 50, down compared to 2018 which was in 45th position. The lowering of this competitiveness ranking is due to the low quality of human resources(1). Intelligence is related to the quality of the brain which states that stimulation will increase the potential for intelligence if it is done during pregnancy and early in life. Lack of brain stimulation during pregnancy can lead to the child’s
inability to learn and adapt to the demands of society\textsuperscript{(2)}.

Efforts to improve the quality of human resources through the First 1000 Days of Life program continue to be voiced in Indonesia. At 1000 HPK, there is a rapid rate of proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis\textsuperscript{(3)}. Good brain development will affect cognitive and motor skills to prepare for the development of future generations. The brain consists of the cerebrum which plays a role in cognitive function. This process is supported by synapsin 1 as a marker for synaptogenesis. Synapsin-I (Syn-I) is the main isoform in neurons which is a phosphoprotein that binds to synaptic vesicles\textsuperscript{(4)}.

Dr. Al Qadhi, the main director of the Islamic Medicine Institute for Education and research in Florida, United States, said that 97\% of Al-Qur’an murattal therapy reduces reflective nerve tension and these results are recorded and measured quantitatively and qualitatively by computer-based tools. The most frequently used Murattal of the Koran is the Surah Al-Rahman\textsuperscript{(5)}. Research on the spectrum analysis of the human voice based on gender has been carried out. The results showed that the frequency ranged from 120 - 150 Hz for men and 200 - 280 Hz for women\textsuperscript{(6)}. Murottal sounds certainly have their respective sound characteristics based on the frequency and intensity values of the sound source. From the description above, a study was carried out on qari (male voice) increase higher expression of synapsin 1 than qariah (female voice) in the cerebrum newborn Rattus norvegicus.

**Materials and Methods**

This researched was a laboratory experimental study with a post-test-only control group design. The samples of this studied were white adult female rats (Rattus norvegicus) aged 2-3 months of 6 days of gestation with an initial body weight of 120-160 grams with three groups were randomized, including control group (K1), qari (K2) and qariah (K3). On the 18th day of pregnancy, Rattus norvegicus was sacrificed, then expression of synapsin 1 was calculated using immunohistochemical methods and analyzed with the appropriate statistics. In the expression of synapsin 1 variable, each subject was assessed according to the Remmele method. The data normality test was performed using the Shapiro Wilk test. Normally distributed data will be analyzed using one-way ANOVA followed by the Least Significant Differences test between groups.

**Results**

Expression of synapsin 1 in the cerebrum Rattus norvegicus which is the variable of this study. The table 1 shows the mean and standard deviation of expression of synapsin 1 in the cerebrum of Rattus norvegicus which was highest in the Ar-Rahman voice treatment group by Qari (K2).

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mean ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>5.74 ± 2.32</td>
</tr>
<tr>
<td>K2</td>
<td>6.96 ± 2.59</td>
</tr>
<tr>
<td>K3</td>
<td>5.84 ± 2.90</td>
</tr>
</tbody>
</table>

Note: K1: Control group  
K2: Qari group  
K3: Qariah group

The figure 1 shows the expression of synapsin 1 in the cerebrum uses immunohistochemical methods. Red arrows indicate the expression of Synapsin 1 in the brain which indicates the presence of a chromogen brown color. The tissue was observed under a light microscope at 5x the field of view at 400X magnification.
The results of the normality test using the Shapiro-Wilk test showed that the distribution expression of synapsin 1 data in the cerebrum in each group was normally distributed. The ANOVA test results with a significance value of 0.519. Then the Analysis Least Significant Difference further test.

Table 2. Test Results LSD Against Expression Of Synapsin 1 In Cerebrum Rattus norvegicus

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>LSD Test Significance Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K1</td>
</tr>
<tr>
<td>K1</td>
<td>-</td>
</tr>
<tr>
<td>K2</td>
<td>0.306</td>
</tr>
<tr>
<td>K3</td>
<td>0.932</td>
</tr>
</tbody>
</table>

The results of the Table 2 LSD advanced test showed no significant difference between the control group (K1) compared to the Ar-Rahman voice treatment group by Qari (K2), there was no significant difference between the control group (K1) compared to the Ar-Rahman voice treatment group by Qariah (K3), and there is no significant difference between the Ar-Rahman voice treatment group by Qari (K2) compared to the Ar-Rahman voice treatment group by Qariah (K3) with a significance value of more than 0.05.

Discussion

The cerebrum of the newborn Rattus norvegicus showed that the mean expression of Synapsin 1 which was given muotton surah Ar-Rahman stimulation by the qari during pregnancy was higher than that qariah and control group, but there were no significant differences in expression of synapsin 1 in the cerebrum. During this period the fetus can deliver information in the form of sound waves to the brain. The sound waves activate a specific area of the bacillary membrane according to the frequency of the vibrations\(^7\). The qari used in this
research is the voice of Muzammil Hasbalah. A qari who reads every word of the surah in a good and easy way can make a person concentrate on listening and can provide a more pleasant stimulus. listening to soothing sounds can increase, reduce pain perception, and provide sound stimulation that will affect physiological functions (8).

The advantages of learning Al Quran with audio visual media by Hj wafiq azizah. Audio visual media that are more varied can increase the activeness and learning outcomes of students. Murottal surah Ar-Rahman stimulation by qariah which is given during the prenatal period from an early age is expected to improve the intellectual function and emotional function of children as they grow and develop in the future (9).

Surah Ar-Rahman has a duration of 13 minutes 55 seconds with a tempo of 79.8 beats per minute, a tempo of 79.8 bpm is a slow tempo. The slow tempo has a range between 60-120 bpm. The slow tempo itself is a tempo that is in line with the human heartbeat, so the heart will synchronize the beat according to the tempo of the sound (10). Music that is rich in high frequency and has a high rhythm (similar to the rhythm of the fetal heartbeat), which can be associated with simulation and energizing functions. Mozart’s music has the best combination with mellow sound colors dominated by violin instruments which help mothers to relax, there are no minor tones, the number of beats in the rhythm is around 60-80 times/minute which corresponds to the mother’s heart rate (11).

Likewise with the murottal Surah Ar-Rahman by the qari has a frequency that can be associated with fetal stimulation.

At 26-28 weeks’ gestation, the fetal hearing function begins to respond and process to auditory stimulation through stimulation of the auditory cortex located in the cerebral cortex and brainstem. 30-35 weeks of gestation, the fetus can hear and respond to the mother’s voice and begins to distinguish between sounds around it. At 42 weeks, the lines of the auditory system with the central nervous system undergo a process of maturity and will then develop after the baby is born (12). In this study, the murottal element that can be adjusted is the intensity where all types of murottal that are presented have been adjusted to an intensity of 65 dB using a sound level meter and analyzed using Cubase 5, it was found that it was murottal qari around 15,400 Hz and the murottal qariah around 13,000 Hz. This difference in frequency will be captured differently by the tonopy of the cochlea, so the response to the brain will be different (13).

From the recorded sound spectrum, the basic frequency is determined. The results showed that there were fewer males than females. The mean formant value of boys was slightly higher than that of girls, including 525 Hz for girls and 512 Hz for boys, respectively (6). This is to support that the mother’s voice, which is higher in frequency, is received by the fetus. Auditory stimulation of the mother’s voice has been applied in several studies. This is in line which states that the ability to receive auditory stimulation and memorization has begun since the fetus in the womb has a permanent impact on brain development (14).

The initial male frequency was smaller than the female initial frequency, the male final frequency was smaller than the female final frequency, the highest male frequency was smaller than the female high frequency, the lowest male frequency was smaller than the lowest frequency girls. Broadly speaking, it can be said that the male voice frequency is smaller than the female voice frequency (15). From this study, it is related to the current research that the results of the analysis show that the voice frequency of male voice is higher than that of female voice. Therefore, it is obtained a higher expression in the voice of the qari than the voice of the qariah.

**Conclusion**

The highest expression of Synapsin 1 in the cerebrum of newborn Rattus norvegicus is the group stimulated by qari, but there is no significant difference among control, qari, and qariah groups.

**Conflict of Interest:** The authors state that there is no conflict of interest associated with this research.

**Source of Funding:** The authors have not received specific grants from any funding agency in the public, or
not-for-profit sector.

**Ethical Clearance:** This study was approved by the Ethical Committee Faculty of Veterinary Medicine Universitas Airlangga with Number: 1.KE.009.01.2021.

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Evaluation of Root Canal Morphology of Maxillary 2nd Premolars Using Cone Beam Computed Tomography in Chennai Population

Nur Qistina Binti Ahmad Fauzi¹, Mahesh R²

¹Undergraduate Student, ²Reader, Department of Pedodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai

Abstract

Background: Using the CBCT, this technique has the ability to identify the root canal morphology as detailed as using the root canal staining and other clearing techniques that in the past have been of higher value to the other conventional techniques that can be implemented to give a three-dimensional perspective of the complete morphological details. With this, analysing the variation in root canal morphology of human maxillary second premolar between different gender in Chennai population can be done.

Methods: One hundred Cone Beam Computed Tomography (CBCT) images from different patients are selected randomly from Department of Oral Radiology of Saveetha Dental College and Hospital, India. All the CBCT images of patients are examined for the inclusion criteria in maxillary second premolars between different genders based on Vertucci classification. All root canal configurations of maxillary second premolars between different genders are investigated and compared with Vertucci Classification.

Conclusion: Of all the 100 maxillary second premolars, the most common type of canal configuration for both the genders is Type I (41%), Type III (22%), and Type V (22%). In females, the most common type of canal configuration discovered is Type I (35%), Type III (63.6%) and Type V (83.3%). In males, the most common type of canal configuration determined is Type I (65%) and Type III (36.4%).

Keyword: Root Canal Morphology, maxillary second premolar, Cone Beam Computed Tomography, Vertucci’s classification.

Introduction

An endodontic procedure can be deemed successful depending on the cleaning and shaping, the disinfection process and the filling of the root canal system. Each tooth has their own anatomical feature and if variations are present, it could pose as an important issue during a root canal treatment.¹ In cases when there are missing root canals, there could be a possibility of the presence of necrotic tissues and microorganisms. As time goes by, these microorganisms can proliferate and thus resulting in a periapical periodontitis. With that reason, clinicians should be aware of the complexity surrounding different root canal structures in order to provide proper treatment techniques and protocols allowing for an increase in the rate of success of the treatment.²

Premolars are generally considered as a challenging tooth when a root canal treatment is involved. For a
maxillary first premolar, there could be either be two roots and two canals (56%) or one root with two canals (40%). In some studies, it was found that both maxillary and mandibular premolars more than often could have additional roots and canals.3-7 Based on a study made by Vertucci and Gegauff, it was concluded that there were three root canals seen on approximately 5-6% of maxillary premolars. 6 On the other hand, a study made by Caliskan et al found that there was no three-seperate-rooted first maxillary premolars as a result. Second premolars will more than often have one roots with a single oval-shaped canal. 8 Based on a study made by Ok et al. it was established that one canal second maxillary premolars were seen in 59.7% of their cases while there were two canals in 40% of the cases and three canals in 0.30% of their cases. 9 Constant to this finding, Vertucci et al. even concluded that there was an incidence where there was 1% of three-rooted-plus-three-canal in second maxillary premolars. 10

When the root canals are classified, various types of classification can be used which comprises of Weine, Vertucci and Gulabivala classifications. In describing the root canal morphology, Vertucci’s classification is known to be the most commonly used classification and it comprises of eight categories: Type I (1), Type II (2-1), Type III (1-2-1), Type IV(2), Type V (1-2), Type VI (2-1-2), Type VII(1-2-1-2) and Type VIII(3).

In investigating the root canal anatomy of the tooth, various methods have been proposed which includes that of in vivo and in vitro methods. In vivo methods comprise of the basic clinical evaluation of the root canal treatment, the retrospective assessment of the patient records, any conventional radiographic evaluation, and advanced radiographic techniques like cone-beam computed tomography (CBCT).11-13 In vitro methods comprise of the root canal staining and tooth clearing,14,15 root sectioning, microscopic examination, examination of conventional radiographs and using three-dimensional modalities such as microcomputed tomography.16,17,18 Using the CBCT, this technique has the ability to identify the root canal morphology as detailed as using the root canal staining and other clearing techniques that in the past have been of higher value to the other conventional techniques that can be implemented to give a three-dimensional perspective of the complete morphological details.2

With these in mind, the purpose of this study is to analyse the variation in root canal morphology of human maxillary second premolar between different gender in Chennai population.

**Materials and Methods**

One hundred CBCT images of Saveetha Dental College patients (53 females and 47 males) which are aged between 16-60 years old, with an average of 45 years, whom were seeking the routine dental diagnosis for further dental treatment. These patients were referred to the Radiology Department of Saveetha Dental College between the years 2017-2018.

The samples that were collected and analysed were purposive where at the very least there was either one maxillary second premolar with fully developed roots; which was taken as the inclusion criteria. When determining which samples to examine, unclear or distorted CBCT images, beforehand endodontically initiated or treated teeth, teeth with posts or crowns, periapical lesions, and any physiological or pathological process such as immature apex was excluded. The final sample that was collected was 100 including both right and left second maxillary premolars, was further screened for the number of roots and root canal configuration. The total data observed and collected were then evaluated and classified under Vertucci’s classification. Other additional data that was collected also included the number of canals, the distance between the occlusal pit to the pulp chamber, the distance between pulp floor to the furcation as well as the distance between the CEJ to the pulp chamber. The genders of each patients were also recorded in order to be analysed.
The collected CBCT samples were retrieved and assessed at the Radiology Department of Saveetha Dental College, Chennai using the Galileo software used for viewing the CBCT that are taken.

**Results and Discussion**

The results of the study are presented in Tables 1-5 and the overall data has been graphically represented in Graph 1. Based on Table 1, of all the 100 maxillary second premolars, the most common type of canal configuration for both the genders is Type I (41%), Type III (22%), and Type V (22%). In females, the most common type of canal configuration discovered is Type I (35%), Type III (63.6%) and Type V (83.3%). In males, the most common type of canal configuration determined is Type I (65%) and Type III (36.4%).

### Table 1: Root Canal Configuration

<table>
<thead>
<tr>
<th>Gender</th>
<th>Type I (1)</th>
<th>Type II (2-1)</th>
<th>Type III (1-2-1)</th>
<th>Type IV (2)</th>
<th>Type V (1-2)</th>
<th>Type VI (2-1-2)</th>
<th>Type VII (1-2-1-2)</th>
<th>Type VIII (3)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>14 (35%)</td>
<td>4 (50%)</td>
<td>14 (63.6%)</td>
<td>2 (66.7%)</td>
<td>18 (83.3%)</td>
<td>0 (0%)</td>
<td>1 (50%)</td>
<td>0 (0%)</td>
<td>53 (100%)</td>
</tr>
<tr>
<td>Men</td>
<td>27 (65%)</td>
<td>5 (50%)</td>
<td>8 (36.4%)</td>
<td>1 (33.3%)</td>
<td>4 (16.7%)</td>
<td>0 (0%)</td>
<td>1 (50%)</td>
<td>1 (100%)</td>
<td>47 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>41 (41%)</td>
<td>9 (9%)</td>
<td>22 (22%)</td>
<td>3 (3%)</td>
<td>22 (22%)</td>
<td>0 (0%)</td>
<td>2 (2%)</td>
<td>1 (1%)</td>
<td>100 (100%)</td>
</tr>
</tbody>
</table>

Based on Table 2, when the number of canals for each tooth were evaluated, female patients showed a mean value of 1.708 with a standard deviation of 0.470 while in male patients, the mean value is 1.888 with the standard deviation of 0.471. When both the genders were compared in terms of the number of canals, there was no significant difference (p-value: 0.9835).

### Table 2: Number of Canals

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Test of Significance</th>
<th>Degree of freedom</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>53</td>
<td>1.708</td>
<td>0.470</td>
<td>0.05912</td>
<td>95</td>
<td>0.9835</td>
</tr>
<tr>
<td>Male</td>
<td>47</td>
<td>1.888</td>
<td>0.471</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on table 3, when the distance between the occlusal pit and the pulp chamber was analysed and recorded, female patient showed a mean value of 3.706 with a standard deviation of 0.744 while in male patients, the mean value is 3.963 with a standard deviation is 0.840. With the p-value calculated, it was found that there was no significant difference between the genders.
in terms of the distance between the occlusal pit to the pulp chamber (p-value: 0.3950).

### Table 3: Distance between Pulp Floor to the Furcation

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Test of Significance</th>
<th>Degree of freedom</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DISTANCE BETWEEN OCCLUSAL PIT &amp; PULP CHAMBER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>53</td>
<td>3.706</td>
<td>0.744</td>
<td>0.1079</td>
<td>95</td>
<td>0.3950</td>
</tr>
<tr>
<td>Male</td>
<td>47</td>
<td>3.963</td>
<td>0.840</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on the table 4, out of all 100 samples, there was in total 42 (15 females and 27 males) second maxillary premolars that has two roots. In female patients, the mean value that was gathered was 4.835 with a standard deviation of 2.114 while in male patients, the mean value is 3.243 with a standard deviation of 0.518. Overall, there is a significant difference between the two genders (p-value: <0.0000001).

### Table 4: Distance between the Pulp Floor to the Furcation

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Test of Significance</th>
<th>Degree of freedom</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DISTANCE BETWEEN PULP FLOOR TO FURCATION</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>4.835</td>
<td>2.114</td>
<td>0.0005611</td>
<td>95</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
<td>3.243</td>
<td>0.518</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on the table 5, in regard to the distance between the CEJ to the pulp chamber, the female patients have a mean value of 2.484 with a standard deviation of 0.396 while in male patients, the mean value is 2.519 with a standard deviation of 0.547. Overall, when the two genders were compared, the distance between the CEJ to the pulp chamber showed a significant difference (p-value: 0.02465).

### Table 5: Distance between the CEJ to the Pulp Chamber

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Test of Significance</th>
<th>Degree of freedom</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DISTANCE BETWEEN CEJ TO PULP CHAMBER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>53</td>
<td>2.484</td>
<td>0.396</td>
<td>0.7126</td>
<td>95</td>
<td>0.02465</td>
</tr>
<tr>
<td>Male</td>
<td>47</td>
<td>2.519</td>
<td>0.547</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
To achieve a successful treatment in endodontics, a proper comprehension of both the root canal anatomy and the morphology is of utmost importance. A proper endodontic treatment involves procedures where all the canals are properly debrided, disinfected, shaped and obturated completely. When an endodontist come across failures in an endodontic treatment, the main cause to this is either because of an untreated canal, incomplete debridement or incomplete obturation. Because of these possible scenarios, a cautious clinical and radiographical examination should be of importance and essential criteria for a successful prognosis.

Another study that was done presented an intricate investigation on the root canal morphology of maxillary second premolars in Chennai population using Cone Beam Computed Tomography (CBCT). With various techniques being introduced and used till this day for the purpose of assessing the root canal morphology and configuration like macroscopic sections, transparent samples and polyester resin impressions, CBCT is considered as the most excellent technique for a proper three-dimensional assessment of the root canal morphology.

Overall, there are various studies that have attempted to fully investigate the root canal morphology and the root numbers of the maxillary second premolar. In terms of the number of canals present in the maxillary second premolar, a study that was made by Pineda and Kuttler\(^2\) showed that there was one canal in 55% of the obtained sample while there were two canals in 45% of the total samples. Vertucci et al, on the other hand discovered that 48 of the maxillary second premolars have one canal while there were two canals in 51 of the premolars and three canals in 1 premolar. Similar to the latter study, a study made by Bellizi and Hartwell reported that there were 40.3% of premolars that have one canal while 58.6% have been discovered to have two canals and 1.1% have three canals.\(^2\)

In regard to the root canal configurations based upon Vertucci’s classification, in the Turkish population, it was reported that the most common type of canal configuration is Type IV (60%) and Type I (38%). On the other hand, in females, the most common type of canal configuration is Type IV (63%) and Type V (34%). Based upon a study made by Ok et al. on the
Turkish population, for the maxillary second premolar, the frequency of canals is that of one canal (59.7%) and Type I (54.5%) of Vertucci’s root canal configuration.\(^9\) (PM 1). Comparing these studies to a study made by Abella et al. the most frequently seen canal configuration if of Type I (47.2%); which is seen in the Spanish population.\(^{23}\) Additionally, in the Pakistani population, commonly there were 84% of premolars that were single rooted with a Type I (53.4%) canal configuration.\(^{24}\)

In the current study that was made, out of all the 100 maxillary second premolars, the most common type of canal configuration for both the genders is Type I (41%), Type III (22%), and Type V (22%). In females, the most common type of canal configuration discovered is Type I (35%), Type III (63.6%) and Type V (83.3%). In males, the most common type of canal configuration determined is Type I (65%) and Type III (36.4%).

In terms of the number of canals, female patients showed a mean value of 1.708 with a standard deviation of 0.470 while in male patients, the mean value is 1.888 with the standard deviation of 0.471. When both the genders were compared in terms of the number of canals, there was no significant difference (p-value: 0.9835). These results are consistent with our study.

**Conclusion**

Within the limited number of samples available, it is proven that the Chennai population have maxillary second premolars with a root canal morphology of one canal and a Type I Vertucci’s classification. In addition to that, there some instances when there is more than one root with various canal configurations that have been identified. In order for a proper generalization of results to be produced, future studies should include even larger sample sizes.

**Conflict of Interest:** Nil.

**Source of Funding:** Funded by Saveetha Dental College and Hospitals.

**Ethical Clearance:** Ethical clearance taken from the Institutional Scientific Review Board, Saveetha Dental College and Hospitals.

**References**


Type of Manuscript: Research Article

Application of Berry’s Index in the Analysis of Bite Mark

Nur Qistina Binti Ahmad Fauzi¹, Gheena S²
¹Undergraduate Student, ²Reader, Department of Oral Pathology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai

Abstract

Background: This study was done to evaluate the importance of applying Berry’s index in bite mark analysis. With that, it is able to highlight the usefulness of Berry’s index in aiding the analysis of bite mark of an individual.

Methods: The study was conducted among 60 students between the ages of 18-30 from Saveetha Dental College, Chennai. The data that was obtained was then tabulated and statistically analyzed.

Conclusion: The results of the study revealed that the widths of both maxillary central incisors and the bizygomatic width were higher in males when compared to females. A positive correlation can be found in males compared to females in regard to both of the widths obtained. Berry’s index is a useful tool of high forensic value and can be used to help in the identification of an individual’s facial proportions based on the calculated width of the central incisors. Thus, it can be of correlation to that of forensic facial reconstruction.

Keywords: Berry’s formula, bitemark analysis, incisal width, bizygomatic width

Introduction

Dentistry is a form of science that can be applied in various legal applications. In regard to legal matters, if the detailed aspects of dentistry including the facts, experience and knowledge is used, it is then terms as Forensic Odontology. In today’s Forensic Dentistry, it has flourished and developed into applying evidence-based methods and procedures allowing for a more accurate result.¹

The identification of an individual through human remains is considered to be one of the most difficult tasks whenever an evidence is presented. Several methods have been used; one of the many being the analysis of the human bitemark.²

The significance of using bite marks was initially brought to light in the year 1937 where its application helped in securing an ongoing murder conviction.³ The bitemark evidence that was put forward gave proof of the victim’s struggles during the assault.⁴ Ever since that moment, bite mark analysis has progressed in terms of the techniques that are used making it more reliable and allowed a more reproducible result to be achieved.⁴,⁵

The human teeth can be considered a weapon whether it being a form of assault or a self-defense act against an assailant. For a bitemark to be of good forensic value, there should be specific characteristics that it should possess. Structurally, incisors have rectangular outlines, canines have triangular outlines and premolars have double triangles. Distinctive characteristics like missing teeth, fractures, etc. has even higher forensic
values. More than often, bite marks are seen as an elliptical or round shape of contusion or abrasion, which is significantly linked with indentations. When a person’s dentition needs confirmation to that of a set of bite marks, the procedure will include that of measuring the size, shape and the position of a specific individual’s teeth.

As a result of the human skin’s distortional property, the stiffness of tissues serves as a problematic thing during bite mark analysis. In instances where the teeth indent the skin especially loose tissues like the breast and the thighs, the teeth mark distance would be increased mesiodistally, the angle of rotation would be flattened, and there would be an increase of intercanine distance. On the opposite note, when the indents are made on areas like the forearm and the shoulders, the exact opposite characteristics would be produced.

In a typical bitemark analysis, it begins with photographic records of the injury including a universal measurement scale in order to record the measurements. Following that, it also requires the impressions for both the model of the injury as well as to represent the perpetrator’s dentition. In present days, advancement in technology has allowed a more accurate way of tracing the outlines of the bitemark for a more proper comparison.

In prosthodontics, the application of the width of the anterior teeth and the form selection is considered to be essential in order to provide the patient with a natural form of aesthetics. In relation to this, the maxillary central incisors are necessary to maintain a satisfying appearance. One of the methods that can be used is known as Berry’s Biometric Index. This index provides an overall compatible aesthetics during tooth selection in the instance that the pre-extraction of the patient is absent.

**Berry’s formula**

The formula used for this index includes the calculation of the width of the maxillary incisor in relation to the bizygomatic width:

\[
\text{Width of the maxillary central incisor} = \frac{\text{Bizygomatic width}}{16}
\]

When various available literatures where observed through PubMed, there has been a number of articles about Berry’s Index in regard to forensic odontology. With that in mind, this study was made to explore the correlation between these two. The obtained results would help provide great inference to the significance between that of the mesiodistal width of the central incisors and the bizygomatic width of the participants both males and females. This finding can further prove that Berry’s index could serve as a useful tool in the identification of damaged remains in forensics.

**Materials and Method**

This study was conducted among 60 students between the ages of 18 -27 from Saveetha Dental College, Chennai. Out of 60 subjects, 30 of the subjects were males and the other 30 were females. The objective as well as the procedure involved in conducting this study was explained beforehand to all of the subjects. An informal consent was also agreed upon by the participants before any steps where taken.

When this study was conducted, the inclusion criteria that was followed included:

- No missing maxillary and mandibular teeth
- Absence of any gingival and periodontal pathology
- Absence of anterior restoration
- Absence of interdental spacing and crowding

In order to measure the width of each participant’s incisor, bite registration was taken on a modeling wax.
For each participant, they were asked to sit on the dental chair in a position where their heads where upright and rested on the headrest, allowing them to face forwards into the horizon. The participant was then instructed to bite onto the modelling wax sheet with the occlusal plane of the maxillary teeth parallel to that of the floor. The distance between the disto-proximal surface indentation to the mesio-distal surface on the indentation of the maxillary right central incisor was measured and tabulated.

In order to measure the bizygomatic width of the participants, a face bow was used to determine the extent and a millimeter ruler was then used to measure the extent.

The data received was tabulated and analyzed using Statistical Package of Social Sciences (SPSS). Based on these values, the mean and standard deviation (SD) were calculated. The $P$ value of 0.05 or less was considered as statistically significant.

**Results and Discussion**

Once the data was calculated, Table 1 and Graph 1 reveals both the mean values and the standard deviation of the width of upper central incisors and bizygomatic width of both genders.

When the width of the upper central incisors was calculated, the mean value obtained was 0.7613cm and 0.7433cm respectively. On the other hand, the mean value of the bizygomatic width for both males and females were 12.32cm and 12.31cm respectively. After analyzing the obtained data, the t-test showed that there was a statistically significant difference in terms of the width of the upper central incisors between both genders. However, there was no statistically significant difference between males and females for the bizygomatic width.

<p>| Table 1: The mean values and standard deviation for the width of upper central incisors and bizygomatic width for both male and female patients |
|---------------------------------|-----|----------------|-------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Test of Significance</th>
<th>Degree of freedom</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WIDTH OF UPPER CENTRAL INCISOR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30</td>
<td>0.7613</td>
<td>0.01676</td>
<td>0.009022</td>
<td>95</td>
<td>0.0006605</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>0.7433</td>
<td>0.03241</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIZYGOMATIC WIDTH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30</td>
<td>12.32</td>
<td>0.39163</td>
<td>0.9210</td>
<td>95</td>
<td>0.9354</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>12.31</td>
<td>0.38572</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Graph 1: Graphical representation of the width of the upper central incisor and the bizygomatic width of the study population.

Table 2 conveys the statistical calculations done for the Pearson’s Correlation Coefficient (r) between the upper incisal width and the bizygomatic width for all the patients without regards to the gender of the participants.

Pearson’s correlation is a coefficient that calculates and evaluates the strength of relationship between two values. Based on Table 2, the correlation between the upper central incisor width and the bizygomatic width is concluded to be a weak positive correlation with a value of 0.1518.

Table 2: Observations and statistical calculations done for Pearson’s correlation coefficient (r) between the upper incisal width and the bizygomatic width in all patients

<table>
<thead>
<tr>
<th>CORRELATIONS</th>
<th>Bizygomatic Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width of upper central incisor</td>
<td></td>
</tr>
<tr>
<td>Pearson correlation</td>
<td>0.1518</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>N</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 3 exhibits the Pearson’s correlation between that of the upper central incisor width and the bizygomatic width of both males and females individually. The data obtained revealed that there was a negative correlation between the two values in terms of female participants (r =0.0267). On the other hand, there was a moderate positive correlation between the two mentioned values when compared to in males (r = 0.4318).
Table 3: Correlation Coefficient (r) between the upper incisal width and the bizygomatic width in males and females separately

<table>
<thead>
<tr>
<th>SEX</th>
<th>Bizygomatic Width</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
<td></td>
</tr>
<tr>
<td>Width of upper central incisor</td>
<td></td>
</tr>
<tr>
<td>Pearson correlation</td>
<td>0.0267</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>N</td>
<td>30</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
</tr>
<tr>
<td>Width of upper central incisor</td>
<td></td>
</tr>
<tr>
<td>Pearson correlation</td>
<td>0.4318</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>N</td>
<td>30</td>
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</tbody>
</table>

Discussion

As a forensic odontologist, the identification of human carcasses is helped with the ability to identify the dental records at a particular crime scene. Through the analyzation of various dental records, the possible cause (physical abuses or injuries) can be identified. It should be noted that the offender’s gender and age can also be determined thus allowing more evidence to be used during the court of law. Various evidences collected for example like tooth prints and radiographs are often used for forensic odontology. 14

Compared to other bones in the body, teeth are a structure that can be conserved for a longer period of time when compared to the latter. The earliest usage of teeth for a method of identification was utilized for the case of Lollia Paulina during the Roman times, which was aided by the distinctive arrangement of her teeth. 4When having to deal with an extremely decomposed body and other methods of identification is not an option, the dental records can be of good use even if additional information is needed. 15, 16

Bite mark analysis to this day has been an indispensable tool during a forensic investigation. 17By definition, it can be described as a representative pattern which is left in tissue or an object by the dentition of a human or an animal. 18On the contrary, even though it is said that bite marks of each individual are unique, the human skin is not an ideal material to which a proper bite registration can be obtained. 19When a bite mark is left on the skin, the pressure is either made against a cloth or wire grates or by the presence of cloth between the culprit’s teeth and the victim’s skin. In some cases, partial bite marks can also be seen in instances where when the bite was made, the victims was moving, resulting in a bitemark that is either partial or incomplete. In certain individuals, when there is a missing tooth, it
can be presented as a result of uneven pressure on the area. However, it is proven that whether it is the dead or living, bite marks can alter as time goes by.  

Berry’s biometric ratio was presented for the first time in 1906. He determined that the upper central incisor tooth is proportional to that of an individual’s face proportions. The maxillary central incisor is a definite item to produce an esthetically pleasing frontal profile for each person. Therefore, a proper selection of the teeth is definitely important to restore the anterior portion of completely or partially edentulous patients during a prosthodontic treatment. Even though this index is of very much use in prosthodontics, its application has yet to be fully examined in as a tool of identification. Thus, this study was made for the sole purpose of demonstrating the usefulness of Berry’s Index for bite mark analysis.

Based on this study, it was found that the bizygomatic width and the widths of the maxillary central incisors were more in males. The results of the bizygomatic width are in correlation with the study done by Anthony et al. in 2015 but is in contrast to that of the value of the width of the incisors. According to a previous study made by Hasanreisoglu et al, it was noted that the correlation between the width of the maxillary incisors and the bizygomatic width is more relevant in females than in males. However, in this study males are of more relevant in correlation compared to females. Compared to the same study, the results show that there is a positive correlation between the bizygomatic width and the width of the central incisors in males rather than in females while based on the later study both of the genders exhibited the positive correlations. The importance of this correlation can be used to aid in the determination of the facial characteristics of the victim as well as the culprit. At the same time, it allows a proper formula to be constructed in the determination of the facial width of destructed human bodies.

**Conclusion**

Even though the significance of bite mark analysis is impossible to be dismissed, it definitely cannot fully achieve a certain evidence. The reason behind this is because there are still drawbacks to this method. Using Berry’s Index enables the measurements that is collected during bite analysis to be utilized and aid in receiving details about a specific individual. Overall, based on the study that was conducted, utilizing the incisal width that is calculated from a bite mark has a possibility to achieve a proper impression in regard to the facial width of an individual. With the results of studies, it is of good use to apply Berry’s index as a subsequent method in bite mark analysis and thus making it a valuable forensic tool.

**Ethical Clearance:** Taken from the Department of Oral Pathology of Saveetha Dental College.

**Source of Funding:** This research was self-funded.

**Conflict of Interest:** Nil.

**References**


Mesiodistal Width of Mandibular Central Incisors between Different Genders in Chennai Population Using Cone Beam Computed Tomography

Nur Qistina Binti Ahmad Fauzi¹, Mahesh R²
¹Undergraduate Student, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India, ²Reader, Department of Pedodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai

Abstract

Background: Using the CBCT, this technique has the ability to identify the proper dimensions of the mesiodistal width of the tooth and other clearing techniques that in the past have been of higher value to the other conventional techniques that can be implemented to give a three-dimensional perspective of the complete morphological details. With this, analysing the mesiodistal width of mandibular central incisors between male and female based on CBCT can be accomplished.

Methods: One hundred Cone Beam Computed Tomography (CBCT) images from different patients are selected randomly from Department of Oral Radiology of Saveetha Dental College and Hospital, India. All the CBCT images of patients are examined for the inclusion criterias in the mesiodistal width of the mandibular incisors between different genders. All mesiodistal width of mandibular central incisors between different genders are investigated and compared.

Conclusion: When both the genders were compared in terms of the number of canals, there was a significant difference seen (p-value: 0.000002208). With the p-value calculated, it was found that there was a significant difference between the genders in terms of the distance between the occlusal pit to the pulp chamber (p-value: <0.0000001). Overall, there is no significant difference between the two genders at the level of the cervical third (p-value: 0.6554).

Keyword: Mesiodistal width, mandibular central incisor, Cone Beam Computed Tomography.

Introduction

The ratio of an individual’s tooth size is a reasonable diagnostic method to enable a proper treatment prognosis and outcome to be anticipated. In instances where there are complex cases, this will allow the limitation of requirements of any diagnostic setups. The relationship of the mesiodistal width of mandibular central incisors and other dentitions especially the opposing maxillary dentition will produce a proper treatment outcome preferably in correcting occlusion. In cases where an excellent orthodontic treatment wants to be achieved, the mesiodistal tooth size of both maxillary and mandibular arches should be of an ideal relationship.

Corresponding author
Dr. Mahesh
Senior Lecturer, Department of Pedodontics
Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, 162, Poonamalle High Road Chennai 600077, Tamil Nadu, India.
Email: maheshpedo@gmail.com
plan, it should consist of the identification of tooth size, both local and general, overbite, overjet as well as the occlusion. 4

Various studies have been done to display the differences of the tooth size in relation to a specific group. In a study made by Bolton, the relationship between the tooth size disharmonies and the treatment of malocclusions became the main focus in 55 patients; to which have excellent occlusions. With this, the ratios for the mesiodistal sizes of the maxillary and mandibular teeth were obtained. 5 A number of clinical cases were included in order to obtain the viability of the diagnostic aid and with thorough analysis, found that the need for a diagnostic aid was not necessary. 6 Using only the rough measurement of the labial portion of the teeth and the calculations made with Bolton’s ratio were identified to be enough to deliver a better and exact option of occlusal goals for a specific patient. 5,6

On the other hand, Arya et al. made a study in 1974 on the relationship of the tooth size with different genders. In addition to that, the study also included the presentation of how tooth size affects different types of malocclusions; which was not a success. Based on the study that was made, the calculated and recorded tooth size of each dentition for each specific group was differentiated. However, the differences for each of the individuals were not experimented on. 2 Lavelle in the year 1972 in contrast to the previous studies, determined that in different tooth dimensions there are various sexual dimorphisms related to it. 7 Similar to that of the study made by Arya, the ratio of both maxillary and mandibular arch tooth size was measured in relation to the different form of malocclusion. To oppose of the result obtained from Lavelle’s study, Nie and Lin in 1999 concluded that there an absence of any significant sexual dimorphism for both anterior and posterior tooth size ratio in relation to various types of occlusion. 8

Materials and Method

The data for this study were obtained from the records of Saveetha Dental College, Department of Oral Radiology, Chennai, India. The sample selection was collected based on the dental age of the patient with a permanent dentition specifically the presence of lower anteriors.

The total collected sample was 100 Cone Beam Computed Tomography (CBCT) which was sorted based on the gender of each individual. The following criteria were used:

1. No apparent loss of tooth substance due to attrition, caries or fillings.
2. Presence of at least one lower central incisor in each CBCT sample.

Once the sample CBCTs were collected, the mean, range and standard deviation were calculated in terms of the mesiodistal width of the teeth. The calculation is further divided into the incisal, middle and cervical third of the crown. In each division, the p-value was calculated and the significance in terms of gender of each of the divisions are determined.

Results and Discussion

The results of the study are presented in Tables 1-3 and the overall data has been graphically represented in Graph 1. Based on Table 1, when the mesiodistal width in the level of the incisal third were evaluated, female patients showed a mean value of 5.676 with a standard deviation of 0.304 while in male patients, the mean value is 5.167 with the standard deviation of 0.626. When both the genders were compared in terms of the number of canals, there was a significant difference seen (p-value: 0.000002208).
Table 1: Mesiodistal Width of Central Incisor (Incisal Third)

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Test of Significance</th>
<th>Degree of freedom</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MESIODISTAL WIDTH (INCISAL THIRD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>56</td>
<td>5.676</td>
<td>1.304</td>
<td>0.01921</td>
<td>95</td>
<td>0.000002208</td>
</tr>
<tr>
<td>Male</td>
<td>44</td>
<td>5.167</td>
<td>0.626</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on table 2, when the mesiodistal width of the incisor in the level of the middle third was analysed and recorded, female patient showed a mean value of 5.405 with a standard deviation of 1.501 while in male patients, the mean value is 4.726 with a standard deviation is 0.470. With the p-value calculated, it was found that there was a significant difference between the genders in terms of the distance between the occlusal pit to the pulp chamber (p-value: <0.0000001).

Table 2: Mesiodistal Width of Central Incisor (Middle Third)

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Test of Significance</th>
<th>Degree of freedom</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MESIODISTAL WIDTH (MIDDLE THIRD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>56</td>
<td>5.405</td>
<td>1.501</td>
<td>0.004762</td>
<td>95</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>Male</td>
<td>44</td>
<td>4.726</td>
<td>0.470</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on the table 3, in female patients in terms of the mesiodistal width of the incisor in the level of the cervical third, the mean value that was gathered was 3.943 with a standard deviation of 0.622 while in male patients, the mean value is 4.092 with a standard deviation of 0.582. Overall, there is no significant difference between the two genders at the level of the cervical third (p-value: 0.6554).

Table 3: Mesiodistal Width of Central Incisor (Cervical Third)

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Test of Significance</th>
<th>Degree of freedom</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MESIODISTAL WIDTH (CERVICAL THIRD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>56</td>
<td>3.943</td>
<td>0.622</td>
<td>0.2243</td>
<td>95</td>
<td>0.6554</td>
</tr>
<tr>
<td>Male</td>
<td>44</td>
<td>4.092</td>
<td>0.582</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A number of literatures regarding orthodontic studies have compared tooth size proportions in relation to that of various ethnics and malocclusion groups. With that in mind, as a result of the lack of knowledge in regard to gender, Angle’s classification as well as the mesiodistal width of the tooth serves a setback for these studies. In order to fully comprehend this topic, more added information should be obtained.  

When the tooth size discrepancy ratio is implemented as a diagnostic method, the main motivation to do so is to enable a proper view into how the both the functional and aesthetic outcome can be produced in instances when no diagnostic set-up is available. In relation to this, the most common use of this diagnostic method is in the evaluation of tooth size discrepancy in regard to different dental arches. 

The mesiodistal width of the crown is a criteria that is known to be a determining variable when we are in search of factors that can be related to the development of occlusal irregularities, the probable effects of the discrepancies in interdigititation, as well as the isolation of discrepant teeth with minor malocclusion that can be attended to through selective mesiodistal grinding and minor tooth movement. When this specific topic needs to be determined, the clinician has the ability to analyse various probable number of crown size relationships; which in this case is the relative size differences between a single tooth and a group of teeth, despite not knowing their location in the dental arch. 

In certain clinical settings, the Bolton’s analysis is known to be a useful method in order to guide the orthodontist whenever they come across cases where the tooth size discrepancies are extreme. However, this analysis has some setbacks and limitations. The reason behind this is because, Bolton’s estimates of variation at first was underestimated as a result of being obtained from samples of perfect Class 1 occlusion. On top of that, the population and gender composition using Bolton’s sample was not specific, which in results in it being a probable selection bias. With this finding, this index is considered not to be a universal acceptable index to be used in all population groups. Moreover, this analysis only identifies with either an anterior or overall ratio, meaning that it cannot identify each mesiodistal individual tooth size like smallness or excessiveness.
If there is a presence of tooth size discrepancy, this results in the prevention to achieve a proper occlusion. When there is a case of mismatched size between that of maxillary and mandibular dentition, this can result in a generalised spacing or crowding or even deviations from a Class 1 occlusion particularly in the posterior region. In order to fix the malocclusion, it is commonly specified only to that particular affected arch. 12

In recent studies, in regard to the gender difference, there have been reported to be a clinical relevance to the mesiodistal width. In a study done by Seipel, when the primary and the permanent dentition was compared, there was found to be less differences in terms of gender. When each individual tooth was evaluated, the maxillary canine and maxillary central incisors exhibit the greatest gender differences, while in another study, the upper lateral incisor and the lower central incisor showed to be the most homogenous.13 Based on this current study, there showed to be a significant difference between genders when an individual mandibular incisor was evaluated.

Conclusion

Within the limited number of samples available, it is proven that the Chennai population there is a significant difference between males and females when the mesiodistal width is compared. Although at the cervical region, both males and females exhibit the similar measurements, at the middle and the incisal edge, there is much significant difference between the two genders. In order for a proper generalization of results to be produced, future studies should include even larger sample sizes.

Conflict of Interest: Nil.

Source of Funding: Funded by Saveetha Dental College and Hospitals.

Ethical Clearance: Ethical clearance taken from the Institutional Scientific Review Board, Saveetha Dental College and Hospitals.

References


Predispositions Factors Affecting Quality of Life in Children with T1DM During the Coronavirus Disease of 2019 Pandemic

Nur Rochmah1,2, Muhammad Faizi1,2, Yuni Hisbiyah1,2, Ike Wahyu Triastuti2, Garindra Wicaksono2, Anang Endaryanto1,2, Soetjipto1,3
1Post Graduate Program, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia; 2Faculty of Medicine, Department of Child Health, Dr. Soetomo General Hospital, Universitas Airlangga, Surabaya, East Java, Indonesia; 3Department of Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia

Abstract

Background: Coronavirus disease 2019 (COVID-19) pandemic affects many aspects of society, including the quality of life (QoL) of children with type 1 diabetes mellitus (T1DM). This study analyzed predisposing factors that affect QoL of children with T1DM during the COVID-19 pandemic, mainly the socioeconomic factors.

Methods: Cross-sectional study was conducted in the Pediatric Endocrine Outpatient Clinic of Dr. Soetomo General Hospital, Indonesia, from March to October 2020. Children with T1DM were assessed using Pediatric Quality of Life Inventory 3.2 diabetes module. Socioeconomic characteristics were presented; correlation between variables were analyzed using Pearson and Spearman tests and \( p < 0.05 \) was statistically significant.

Conclusion: This study included 33 subjects (18 boys and 15 girls; mean age, 11.97 ± 2.91 years). Worry and treatment barrier for the child report correlated to the parent’s educational background (\( r=0.35; p=0.022 \)) and income (\( r=0.29; p=0.049 \)). Worry, treatment barrier, and diabetes aspects for parents’ report correlated to age group (\( r=−0.38; p=0.014 \)), parents’ educational background (\( r=0.37; p=0.015 \)) and income (\( r=0.29; p=0.049 \)). There’s correlation between diabetes aspect of parent’s report and age group (\( r=−0.38; p=0.014 \)).

Keywords: children, COVID-19, HRQOL, type 1 diabetes mellitus

Introduction

The World Health Organization Emergency Committee declared the coronavirus disease 2019 (COVID-19) pandemic as a global health emergency1. Its existence affects not only the health aspect but also the social and global economic aspects. In just 4 weeks, the lockdown conducted in New York cost an estimated $1.1 billion, which is 1% of the country’s gross domestic product2. In Indonesia, the losses incurred during the first quarter of 2020 amounted to 320,000,000,000 rupiahs3. Separately, the occurrence of social restrictions, independent isolation, restrictions on long-distance travel, dismissal, and closed schools led to multidimensional crisis2,4.

Type 1 diabetes mellitus (T1DM) is the most common chronic disease, with approximately 70,000 children diagnosed each year worldwide. The incidence was reported to be lower in Asia5. This century, the
prevalence of diabetes mellitus worldwide is increasing. In 2002–2014, there were 70 children with T1DM with 40% increment lately in 2020 in Surabaya, Indonesia. As of August 2020, the Chinese Center for Disease Control and Prevention reported 2143 infections in children aged below 18 years. Moreover, a report from the COVID-19 task force in Indonesia indicated that the infection rate in children is 11.4%, with 3.2% of deaths. This mortality rate is relatively the highest compared with other countries.

A multidisciplinary approach was very important in children with T1DM. Previous studies have reported that individuals with low socioeconomic status (SES) tend to have inadequate treatment; thus, they are at risk to develop complications. Alfian et al. stated that patient’s low compliance may result in a lower QoL. In addition, the International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines said that depression and anxiety affect patients with T1DM, resulting in bad glycemic control.

Parent education, parent income, and parent employment may influence the QoL of patients with T1DM, mainly because of the COVID-19 pandemic. The reduced capacity for contact with physicians owing to fear of going to the hospital may lead to a further decrease in QoL of patients with T1DM in pandemic conditions. This study aimed to analyze factors that affect the quality of life (QoL) of children with T1DM during the COVID-19 pandemic; the components assessed were diabetes, treatment barrier, treatment adherence, worry, and communication.

Material and Methods

Sample

This study involved children and adolescents diagnosed as having T1DM according to the ISPAD guidelines registered at the Pediatric Endocrinology Outpatient Clinic of Dr. Soetomo General Hospital, Surabaya, Indonesia, and their parents. The inclusion criteria were patients aged 5–18 years, who were diagnosed as having T1DM, and who already started their treatment. A total of 33 patients aged between 5 and 18 years (18 boys and 15 girls) were included in this study.

Socioeconomic factor

Socioeconomic factors such as parents’ education, parents’ income, child’s age, and children’s education were obtained. The educational background was classified based on the government program of 9-year compulsory education; hence, we divided it into 3 groups: <9 years, 9–12 years, and >12 years. Based on the regional minimum wage, we classified SES into 3 groups: low, medium, and high. The ages of children were then grouped into 5–7 years, 8–12 years, and 13–18 years.

Nutritional status

Nutritional status was classified according to the World Health Organization (body mass index for age) values defined for children and adolescents (5–18 years old): severe malnutrition (z < −3), moderate malnutrition (−3 < z < −2), normal (−2 < z ≤ 1), overweight (1 < z < 2), and obesity (z > 2). We grouped the subjects into severe malnutrition, moderate malnutrition, normal, and overweight or obese.

Pediatric Quality of Life Inventory (PedsQL)

The patient’s QoL was assessed using the PedsQL 3.2 diabetes module by Varni et al. for children based on the patient’s age. The PedsQL questionnaire that was used to assess the QoL of the children with T1DM has been translated into Bahasa Indonesia by a credible sworn translator. It assessed the aspects of diabetes, which assessed the diabetes symptoms (15 items); treatment barriers, which evaluated the obstacles or problems during treatment (treatment I) (5 items); treatment adherence (treatment II) (6 items); worry (3 items); and communication (4 items) with the items reverse scored. A 5-point scale (0 [never], 1 [almost never], 2 [sometimes], 3 [often], and 4 [almost always]) and a 3-point scale (0 [not at all], 2 [sometimes], and 4 [a lot]) were used for the Child Report for Young Children (ages 5–7 years).
These scores were transformed to a 0–100 scale: 0 = 100, 1 = 75, 2 = 50, and 4 = 0. Lower scores indicate more diabetes symptoms and management problems. In a study by Varni et al., the validity and reliability were tested in this PedsQL, showing good results15. Validity is assessed based on predicting the morbidity and disease burden of the patient and his parents. Reliability indicates consistent results from several repetitions of the test14. Moreover, the validity and reliability of the PedsQL questionnaire have been tested by the Statistics of Universitas Airlangga. The questionnaire was shared online to the participants by the same team during the study.

**Evaluation of data**

A comparison test was used to analyze our data using the SPSS 17.0 software (IBM SPSS). For the baseline and clinical characteristics, the mean and standard deviation of each element were used. Normality test was done for each data using the Shapiro-Wilk test because the study sample was >50, the correlation test was done using the Pearson test for parametric data and the Spearman test for nonparametric data, and the chi-square contingency was used for the nominal data to test their correlation. We considered \( p < 0.05 \) as statistically significant.

**Ethical permission**

This study’s ethical approval was granted by the ethics committee overseeing health research team at the Dr. Soetomo General Hospital, Surabaya, Indonesia (ref. no:0123/LOE/301.4.2/IX/2020).

**Results and Discussion**

This cross-sectional study involved 33 children with T1DM (18 boys and 15 girls; age, 11.97 ± 2.91 years). The socioeconomic characteristics of the patients with T1DM are presented in Table 1. Most of them have a good nutritional status (90.9%) and are active in teaching and learning activities. Most of the parents’ income was in the group of middle income (54.5%). The parent’s educational background was mostly 9–12 years (63.6%).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
</tr>
<tr>
<td>5–7 years old</td>
<td>3 (9)</td>
</tr>
<tr>
<td>8–12 years old</td>
<td>17 (51.5)</td>
</tr>
<tr>
<td>13–18 years old</td>
<td>13 (39.9)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>18 (54.5)</td>
</tr>
<tr>
<td>Girls</td>
<td>15 (45.5)</td>
</tr>
<tr>
<td><strong>Nutritional status</strong></td>
<td></td>
</tr>
<tr>
<td>Severe malnutrition</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Moderate Malnutrition</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Normal</td>
<td>30 (90.9)</td>
</tr>
<tr>
<td>Overweight or obese</td>
<td>1 (3)</td>
</tr>
<tr>
<td><strong>Socioeconomic status</strong></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>26 (78.8)</td>
</tr>
<tr>
<td>Medium</td>
<td>5 (15.2)</td>
</tr>
<tr>
<td>High</td>
<td>2 (6.1)</td>
</tr>
<tr>
<td><strong>Parents’s Educational Background</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;9 years</td>
<td>3 (9.1)</td>
</tr>
<tr>
<td>9–12 years</td>
<td>21 (63.6)</td>
</tr>
<tr>
<td>&gt;12 years</td>
<td>9 (27.3)</td>
</tr>
</tbody>
</table>
The average data of each aspect on the QoL assessment results of children and parents are presented in Fig. 1. The best value was obtained in the adherence treatment, whereas the worst was the worry score.

The results of the correlation between socioeconomic factors and QoL are presented in Tables 2 and 3. The correlation test revealed significant results in parent’s education with child’s worry ($r = 0.35; p = 0.022$) and parent’s worry ($r = 0.37; p = 0.015$). Moreover, the age group had a significant value on the diabetes score of the parents ($r = -0.38; p = 0.014$). Parent’s income significantly correlated to the treatment barrier ($r = 0.29; p = 0.049$).

Table 2. Correlation study between children QoL and socioeconomic factors

<table>
<thead>
<tr>
<th>Socioeconomic factors</th>
<th>Correlation coefficient (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetes</td>
</tr>
<tr>
<td>Child’s education</td>
<td>($r = -0.16; p = 0.183$)</td>
</tr>
<tr>
<td>Age group</td>
<td>($r = -0.22; p = 0.108$)</td>
</tr>
<tr>
<td>Parent’s education</td>
<td>($r = 0.11; p = 0.256$)</td>
</tr>
<tr>
<td>Parent’s income</td>
<td>($r = 0.18; p = 0.152$)</td>
</tr>
</tbody>
</table>

*p< 0.05
Table 3. Correlation study between parents’ QoL and socioeconomic factors

<table>
<thead>
<tr>
<th>Socioeconomic</th>
<th>Correlation coefficient (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetes</td>
</tr>
<tr>
<td></td>
<td>barrier</td>
</tr>
<tr>
<td>Child education</td>
<td>(r = −0.13; p = 0.235)</td>
</tr>
<tr>
<td>Age group</td>
<td>(r = −0.38; p = 0.014*)</td>
</tr>
<tr>
<td>Parent education</td>
<td>(r = 0.06; p = 0.360)</td>
</tr>
<tr>
<td>Parent income</td>
<td>(r = 0.28; p = 0.054)</td>
</tr>
</tbody>
</table>

*p < 0.05

Our study findings from the constituent components of SES indicated that parent education significantly correlates to the worry aspect of QoL in both parent and child. This finding is consistent with the findings of Costa and Vieira, who reported that parental education correlated with QoL, especially in the worry’s domain. In particular, it was reported that patients who have parents with higher education backgrounds will have better QoL and lower levels of anxiety and depression than those who have parents with lower educational backgrounds.

This study also found that parental income significantly correlates to treatment barriers. This finding consistent with the study of Costa and Vieira, who reported that parent’s income has a role in better QoL results in the satisfaction component and worries, which was also linked to happiness, which can then play a role in better compliance and self-management. T1DM is a disease that is closely related to the environment and lifestyle, in which lifestyle and environment are related to the SES of a family, which consists of parents’ education, parents’ income, and parents’ occupation. Other studies also reported that QoL was better in males than females.

Another finding indicated that older children with T1DM had a lower QoL, especially in the diabetes aspect. This can happen because when children are getting older, they have more personal opinions, and it becomes more difficult to control their treatment. This finding is also consistent with that of Abdul-Rasoul’s study, which showed that the 13–18 age group had the lowest QoL compared with the younger age group.

In this study, most of the subjects aged 8–12 years (51.5%), were male (54.5%), and had normal nutritional status (90.9%). This study is consistent with that of Abdul-Rasoul et al., who reported that most of the age group is 8–12 years.

Nevertheless, there is a limitation in this study. It was conducted at a referral hospital in East Indonesia, where the number of T1DM cases was lower than in Caucasians. Moreover, because of limited resources, the
management of T1DM is quite complicated in Indonesia. Besides maintaining routine control in the Pediatric Endocrine Outpatient Clinic, some factors, such as the socioeconomic and educational background of parents, can affect QoL.

We could not conclude whether there is any different T1DM QoL before and during the pandemic. There are no studies that evaluated factors affecting QoL during the COVID-19 pandemic. To the best of our knowledge, this is the first study on children with T1DM that evaluated the determining factors that affect QoL during the COVID-19 pandemic.

**Conclusion**

This study included 33 subjects (18 boys and 15 girls; mean age, 11.97 ± 2.91 years). Worry and treatment barrier for the child report correlated to the parent’s educational background (r = 0.35; p = 0.022) and income (r = 0.29; p = 0.049). Worry, treatment barrier, and diabetes aspects for parents’ report correlated to age group (r = −0.38; p = 0.014), parents’ educational background (r = 0.37; p = 0.015) and income (r = 0.29; p = 0.049). There’s correlation between diabetes aspect of parent’s report and age group (r = −0.38; p = 0.014).

**Acknowledgement:** The author reports no conflicts of interest in this work

**Source of Funding:** This research has no funding sources

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15. Varni JW, Seid M, Kurtin PS. PedsQL™ 4.0:


The Effect of Collagen-Chitosan-Sodium Hyaluronates Intrastromal Implantation on Corneal Clarity and Transforming Growth Factor (TGF-β) (Experimental Study On New Zealand Rabbit)

Nurul Fitri Shabrina¹, Reni Prasetyani², Prihatini Widiyanti³

¹Researcher, ²Researcher/Tutor, Department of Ophthalmology, Faculty of Medicine Universitas Airlangga, Dr Soetomo General Hospital Surabaya, ³Researcher/Tutor, Department of Biomedicine, Faculty of Science and Technology Universitas Airlangga

Abstract

Background: Stromal haziness due to various diseases could results in permanent blindness. Currently, many biomaterials are developed to help aid the healing, so, haziness does not occur. This study aims to examine the effect of the collagen-chitosan-sodium hyaluronic (Col-Chi-NaHa) as an alternative to healing the corneal stroma so that corneal haziness does not occur.

Method: A total of 30 New Zealand male rabbit eyes were divided into three groups. The first group was not treated at all. The second group performed intrastromal injuries by making a stromal pocket with the aid of a crescent knife. The third group carried out implantation of the Col-Chi-NaHa biomaterial which was inserted into the stromal pocket. On day 14, the corneal haziness level was examined using an eye microscope and handheld slit lamp. Immunohistochemical staining of the cornea was carried out using anti-TGF-β antibodies.

Result: After 14 days post-treatment showed that there was more significant haziness between the Col-Chi-NaHa composite implantation group and the control group (p = 0.00, \( \alpha > 0.05 \)). There were also lower TGF-β levels between the Col-Chi-NaHa implantation group and the control group (p = 0.00, \( \alpha > 0.05 \)).

Conclusion: Corneal clarity in the implanted group was lower than that of the control group but this was due to the short observation period which causing biomaterial not completely degraded. The TGF-β level in the implanted group was lower than control.

Keyword: Corneal clarity, stroma, TGF-β, biomaterial, Collagen, Chitosan, Sodium hyaluronic

Introduction

Corneal damage is the second leading cause of blindness worldwide. According to WHO, there are 36 million people who suffer from blindness worldwide, and 217 suffer from vision damage, 2.4% of the total blindness is caused by corneal disorders¹. The cornea is a transparent avascular tissue that functions protect the eye from infection and trauma. Due to its transparent
nature, the cornea has a refractive role in absorbing light and accounts for 2/3 of the eye’s total refractive function\(^2\). Corneal damage to the stromal layer due to corneal surgery, refractive surgery, trauma, or infection will cause corneal haziness due to extracellular matrix and imperfect collagen fibers arrangement. Haziness in the stroma will reduce the cornea’s clarity so that its refractive function will decrease, resulting in blindness\(^3\).

Until now, corneal transplantation is the only way to correct blindness due to corneal loss of transparency, but there is an imbalance between the donor and the need for a corneal transplant. There are currently only 40,000 people who receive corneal donor annually in the United States, with conditions that may worsen in developing countries. Due to this imbalance, many artificial corneas and biomaterials are developed to help heal the cornea properly so that corneal scar that reduces the clarity does not occur. Biomaterials are currently widely studied to treat corneal defects by reducing mechanical stress and reducing pro-inflammatory cytokines. Biomaterials that are widely studied for use in aiding corneal healing include chitosan, hyaluronic acid, silk fibers, and polyarginins\(^4\). Biomaterials to be implanted on the cornea must be non-toxic, non-immunogenic, transparent, flexible to adjust during implant insertion surgery, and must be able to support the growth of corneal cells. One of the biomaterials being developed is a combination of collagen-chitosan-sodium hyaluronate (Col-Chi-NaHa) discovered by Chen in 2005 fulfils the biomaterial criteria for the cornea because these three materials are similar to the extracellular matrix of the cornea\(^5\).

Collagen is the largest component of the extracellular matrix of the cornea. The extracellular matrix allows organogenesis and reconstruction during the wound healing process, so collagen is good for corneal repair and regeneration. Collagen is biodegradable, has low antigenicity, and has good biocompatibility due to its low toxicity and immune reactions. Collagen is widely used in ophthalmology for thread materials, bandage contact lenses, punctual plugs, and viscoelastic fluids during surgery. Collagen is also used for corneal reconstruction with good results because it has been shown to facilitate corneal cell and nerve regeneration in vitro. When implanted into the cornea, collagen has a brittle consistency, so it should be mixed with other ingredients to improve its strengths\(^6\). Chitosan is a linear polysaccharide consisting of glucosamine and N-acetylg glucosamine and is a deacetylated derivative of chitin. Chitosan is biocompatible, biodegradable, inert and non-toxic\(^7\). Chitosan also have anti-fungal, anti-bacterial, and hemostatic properties so it is suitable for use as a material for tissue engineering\(^29\). Sodium hyaluronate is a glycosaminoglycan in the extracellular matrix, which has a vital role in wound healing and inflammation. In the eye plane, sodium hyaluronate has viscoelastic properties, so it is often used to protect the corneal endothelium and maintain the anterior chamber’s depth during cataract surgery. Animal studies have also shown that sodium hyaluronate supports corneal epithelial wound healing by stimulating corneal epithelial migration, adhesion, and proliferation\(^8\). The addition of chitosan to collagen can improve its stability and structural integrity so that it can be implanted in the eye\(^9\) and the addition of sodium hyaluronate improves light transmission\(^28,29\). This study attempted to evaluate the differences in Collagen-Chitosan- sodium hyaluronates (Col-Chi-NaHa) intrastromal implantation to the corneal haziness level TGF-\(\beta\) expression in stromal injury on New Zealand rabbits eye.

**Methods**

**Animal**

Thirty New Zealand male albino rabbits weighing 2–3 kg were used. Ethical approval was given by the animal care and Use Committee of the Faculty of Veterinary Airlangga University (Surabaya, Indonesia). Animals were given at least one week for acclimatization. The rabbits then divided into three groups, with each group consists of 10 rabbits. The first group are the treatment group which was given a circular complex of Col-Chi-NaHa biomaterial intrastromal implantation. The second group is the positive control group who was given injuries by making the stromal pocket in the cornea. The
third group is a negative control group where rabbit eyes were not treated at all.

**Collagen-chitosan-sodium hyaluronates (Col-Chi-NaHa) membrane preparation**

Collagen-Chitosan-Sodium Hyaluronic (Col-Chi-NaHa) membrane was made at the biomaterial laboratory at the Institute of Tropical Disease (ITD) Airlangga university. Collagen 20% solution was mixed with 0.1 M acetic acid using a magnetic stirrer for 60 minutes. Then the solution was cross-linked with hydroxypropyl methylcellulose (HPMC). 10% w/v DD 95% chitosan is then dissolved in 13.4 M acetic acid. Collagen and chitosan, which had been wholly dissolved were mixed using a magnetic stirrer for 1 hour. Sodium hyaluronates with a concentration variation of 0.6% were then added to Col + Chi solution and stirred for 30 minutes. The homogeneous Col + Chi + NaHa solution was cast on a Perspex plate and heated using an incubator for 24 hours at 35°C until the membrane dries. After that, the membrane was immersed in PBS until the pH became neutral. The biomaterial membrane was then cut with a diameter of 3 mm and a thickness of 0.2 mm. Sterilization is carried out by rinsing thoroughly in distilled water, then rinsing again with 75% ethanol. The final stage was sterilization with UV for 30 minutes and immersed in sterile PBS buffer liquid, and the biomaterial was ready to be applied intrastromal to the rabbit’s cornea.

**Intrastromal implantation**

The corneal stromal pocket was created at 2 mm from the superior limbus with a diameter of 4 mm and a thickness of 0.2-0.3 mm using crescent knife. The first group was given a circular complex of Col-Chi-NaHa biomaterial intrastromal implantation of 3 mm and a thickness of 0.2 mm, and the stromal pocket was closed without suturing. The second group is the positive control group who was given injuries by making the stromal pocket in the same way as stated above. The third group is a negative control group where rabbit eyes were not treated at all. Each group consists of 10 rabbits with a total population of 30 rabbits. Levofloxacin 0.5% and fluorometholone 0.1% eyedrops were applied six times daily for 2 weeks. The rabbits were examined 2 weeks post op using eye surgery microscope and handheld slit lamp for corneal clarity grading. All the animals were euthanized, the eye were enucleated, and the samples were fixed in 10% NBF (BBC chemicals, Mount vernon, USA) and processed for light microscopy.

**Histopathology**

Immunohistochemical staining was performed on the corneal surface of the enucleated eye tissue in the second week after corneal stromal injury treatment was given. Paraffin-blocked tissue was cut with a thickness of 3µm and placed on a glass polylysine object, then incubated at 45°C for one night. The preparations were paraffinized and washed with flowing distilled water, then incubated with H₂O₂ for 3 minutes and washed with flowing distilled water. The preparations were incubated with citrate buffer pH 6 at 95°C for 45 minutes, then cooled for 30 minutes and washed with PBS 2x for 3-5 minutes. The preparations were then incubated with a blocking serum for 15 minutes and drained. Subsequently, immersion was carried out in alcohol with levels of 70%, 96%, 100%, and xylol. The tissue is then cooled for 15 minutes at room temperature and rinsed in phosphate-buffered saline (PBS) for at least 5 minutes. Tissues to be incubated in anti-TGF-β were given 0.5% casein in PBS for 10 min to block non-specific binding sites. The primary antibody incubation with rabbit polyclonal antibody TGF-β (1:50 Serotec MCA797, Oxford, UK). was carried out for 30 minutes. The tissue was rinsed in water, and 0.5% cobber sulfate in PBS was added to increase the staining intensity. The stained tissue was observed using a light microscope with a magnification of 400x.

**Corneal haze grading**

Corneal haze was graded using Sonoda and Streiler grading at two weeks post-op using ophtalmic surgery microscope and handheld slit lamp. The opacification observed and scored according to an established grading system as follows: 0, completely transparent cornea; 1, minimal corneal opacity, iris vessels easily visible; 2, moderate corneal opacity, iris vessels still visible; 3,
moderate corneal opacity, only pupil margin visible; 4, complete corneal opacity, pupil not visible.

**TGF-β immunoreactivity grading**

The level of TGF-β expression to assess the healing process of the corneal stroma was analyzed by semiquantitative histopathological examination using the Allred scoring method. Immunoreactive-score (IRS) can be assessed quantitatively by adding the results between proportion score (PS) and Intensity score (IS). Proportion score is the proportion of positive cells that are immunoreactive (scored on a scale of 0-5). Intensity score is staining intensity (scored on a scale of 0-3) from light brown to silver brown. The IRS scores range from 0-8, with the scores attached to table 1. Each sample’s data is the average IRS scores observed at 5 Fields of View at 400x magnification.

<table>
<thead>
<tr>
<th>Proportion Score (PS)</th>
<th>Intensity Score (IS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>score 0: no positive cell</td>
<td>score 0: no colour reaction</td>
</tr>
<tr>
<td>score 1: Positive cells 0-1%</td>
<td>score 1: weak intensity</td>
</tr>
<tr>
<td>score 2: Positive cells &gt;1-10%</td>
<td>score 2: intermediate intensity</td>
</tr>
<tr>
<td>score 3: Positive cells &gt;10-33.3%</td>
<td>score 3: strong intensity</td>
</tr>
<tr>
<td>score 4: Positive cells &gt;33.3-66.6%</td>
<td></td>
</tr>
<tr>
<td>score 5: Positive cells &gt;66.6-100%</td>
<td></td>
</tr>
<tr>
<td>Total PS+IS score 0-8</td>
<td></td>
</tr>
</tbody>
</table>

**Statistics**

The groups’ distribution was analyzed using the Shapiro-Wilk normality test. The comparison of variables between groups was analyzed using Anova if the variables were normally distributed or Kruskal-Wallis if the variables were not normally distributed. All the statistical tests are carried out with the help of SPSS 23 software.

**Results**

**The Effect Of Col-Chi-NaHa Intrastromal Implantation On Corneal Haze**

In the negative control group, all samples had clear corneas on the 14th day after surgery. In the positive control group that was given intrastromal injury, the mean corneal haze level was 0.30 ± 0.949, with the lowest corneal haze on a scale of 0 and the highest corneal haze was on scale 3 which was found on one rabbit. In the treatment group given the Col-Chi-NaHa implant, the mean corneal haze level was 1.80 ± 1.033 with the with the lowest corneal haze scale of 0 in two rabbits, a scale of 3 in two rabbits and a scale of 2 in the remaining six rabbits. The Kruskal-Wallis statistical test showed that there were significant differences between groups (P=0.00, P<0.01).
Table 2. Corneal haziness grading

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of samples</th>
<th>Corneal haziness grading, number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control group (normal)</td>
<td>10 rabbits</td>
<td>none</td>
</tr>
<tr>
<td>Positive control group (Stromal pocket)</td>
<td>9 rabbits</td>
<td>none</td>
</tr>
<tr>
<td>Treatment group (Stromal pocket + intrastromal implantation)</td>
<td>2 rabbits</td>
<td>1 rabbit</td>
</tr>
</tbody>
</table>

Table 3. Differences of corneal haziness grading between groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Corneal haziness grading (mean ± Standard deviation)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control group (normal)</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Positive control group (Stromal pocket)</td>
<td>10</td>
<td>0.30 ± 0.949</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment group (Stromal pocket + intrastromal implantation)</td>
<td>10</td>
<td>1.80 ± 1.033</td>
<td></td>
</tr>
</tbody>
</table>

Fig 1. chart comparison of corneal haziness grading between group
The Effect Of Collagen-Chitosan-Sodium Hyaluronates Intrastromal Implantation On TGF-β expression

Distribution statistical test using saphiro-Wilk showed a normal distribution in the TGF-β expression data (p = 0.093, p> 0.05). Test with the one way ANOVA method showed a significant difference between the positive control group and the treatment group (6.00 ± 0.66 with 4.10 ± 0.73, P = 0.00; P <0.05) and the positive control group with the negative control group (6.00 ± 0.66 with 2.10 ± 0.73), P = 0.00; P <0.05).

Table 4. Differences of TGF- β immunoreactivity score between groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean ± Standard deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control group (normal)</td>
<td>10</td>
<td>2.10 ± 0.73</td>
<td>1</td>
<td>3</td>
<td>0.00</td>
</tr>
<tr>
<td>Positive control group (Stromal pocket)</td>
<td>10</td>
<td>6.00 ± 0.66</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Treatment group (Stromal pocket + intrastromal implantation)</td>
<td>10</td>
<td>4.10 ± 0.73</td>
<td>3</td>
<td>5</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Fig 2. Mean TGF- β immunoreactivity score between groups

Fig 3. Immunohistochemistry staining under a light microscope at 400x magnification. (A) Negative control group (normal), (B) Positive control group (Stromal pocket), (C) Treatment group (Stromal pocket + intrastromal implantation). Green arrows show macrophage cells expressing TGF β, which is marked with a silvery brown color around the macrophages.
Discussion

The cornea is a transparent avascular tissue that functions as a protective and refractive structure for the eye and responsible for transmitting light. The corneal stroma is a structure that makes up almost 90% of the total thickness of the cornea, so it is imperative to maintain the transparency of the stroma in order to be maintained. Changes in the corneal stroma structure due to disease, trauma, or scarring can cause the loss of corneal transparency. Corneal stromal abnormalities cause millions of cases of blindness worldwide and currently there is an imbalance between corneal donors and the needs of corneal donors that are needed. A synthetic stromal substitute that can replace the endogenous regeneration ability of the stroma, however, until now there has not been found a synthetic material that can replace the corneal stroma because of the complex structure of the stroma consisting of the extracellular matrix, stromal cells and proteoglycans which are difficult to replicate in vitro. Therefore, research on synthetic materials is currently directed at helping stromal regeneration so that scar tissue that causes corneal haziness does not occur. Synthesis materials currently being researched are corneal tissue donors or other tissues such as lip mucosa, acellular corneal scaffold with bioengineering, adhesive tissue, 3-dimensional bioprinting and stromal stem cell therapy. Currently, many biomaterials are developed to help heal the cornea. Biomaterials have the advantage of inducing less immune reactions and can be fabricated in the laboratory, so the availability is relatively fast compared to waiting for donors’ availability. Good biomaterial can also function as a scaffold for additional cells and medication which are expected to accelerate healing even further.

Implantation of intrastromal biomaterials in rabbit eyes with fish scales as the main ingredient showed no biomaterial degradation until 54 weeks after surgery, and there were minimal corneal opacities around the implant site. In vitro biomaterials with fish scales can support the growth of stromal and epithelial cells. So that biomaterials that are difficult to degrade are more suitable for use to help stromal healing rather than replace the corneal stroma as a whole. Implantation of rabbit intrastromal biomaterials with collagen and Hydroxypropyl methylcellulose (HPMC) shows a mild inflammatory reaction two weeks post-op and neovascularization one-month post-op, which disappeared one week after. Furthermore, observations for seven months showed that the implanted biomaterial was well received without implant extrusion and the corneal condition was clear.

Thus, higher corneal haziness result in the treatment group is due to the short time of observation that causing implanted biomaterial to not fully absorbed and causes corneal haziness.
In this study, we also found 1/5 of the population in the treatment group had completely absorbed biomaterials resulting in transparent corneal condition after 14 days of implantation. The rate of degradation of the Col-Chi-NaHa biomaterial has been previously investigated in vitro by immersing the Col-Chi-NaHa biomaterial in 5 ml PBS and 0.05-gram collagenase for one week. With the results of biomaterial degradation of 0.68% per week\textsuperscript{16}. There has been no research on the degradation rate of the Col-Chi-NaHa biomaterial implanted intrastromally, further research is still needed on the timeline of Col-Chi-NaHa biomaterial degradation in vivo.

The lower corneal clarity in the treatment group could also occur because, in the second week postoperatively, the corneal wound healing phase was still in the remodelling phase. In general, the timeline for wound healing in the corneal stroma is that apoptosis of cells around the wound and neutrophil infiltration begins at six hours post-injury and reaches a peak at 24 hours after injury. Activation of keratocytes started at two days after injury and fibroblast and myofibroblast differentiation occurred 3-5 days after the injury occurred. Myofibroblasts also produce an extracellular matrix whose fibre direction is irregular, causing cloudiness in the cornea. Myofibroblasts have the contractile ability to go to the corneal wound and close the wound. The fibrosis expression gene will be detected within two weeks after injury, and remodelling and scar tissue deposition will occur 14-28 days after the first wound occurs\textsuperscript{17,18}. If the healing goes well and the wound is completely closed, the number of myofibroblasts in the stroma will decrease. In clinical conditions, this is characterized by the disappearance of corneal opacities and this occurs an average of 30-40 days after the injury occurs\textsuperscript{11}. We suggest more extended observation to assess the effect of Col-Chi-NaHa implantation on corneal healing.

Transforming growth factor (TGF-\(\beta\)) is a cytokine that has a significant role in healing corneal stromal wounds. TGF-\(\beta\) will activate the cornea’s wound healing pathway, including differentiate keratocytes into fibroblasts and myofibroblasts, induce changes in the extracellular matrix, and support the proliferation and migration of corneal epithelial cells. However, overexpression of TGF-\(\beta\) also negatively affects the cornea by causing the accumulation of myofibroblasts in the stroma and producing an irregular extracellular matrix leading to post-injury corneal opacification. Inhibition of TGF-\(\beta\) results in more transparent cornea after corneal alkali burn\textsuperscript{19}. Mice wounded by corneal alkali burn and given TGF-\(\beta\) inhibitor also had less inflammatory cell infiltration and myofibroblasts than the control group and had more transparent corneas and less neovascular at day 20 observation\textsuperscript{20}.

The primary ingredient of this biomaterial is collagen, a natural protein found throughout the body. Collagen is brittle and needs to be cross-linked with various ingredients. Mixing collagen with other ingredients can increase mechanical strength and defend collagen from enzyme degradation\textsuperscript{21}. Collagen-chitosan composites are reported to have good mechanical strength and clarity and can regenerate epithelial cells, stroma, and eye nerves after being implanted for 12 months in pig eyes\textsuperscript{21,22}. Chitosan is mixed with collagen to improve mechanical strength so that the biomaterial does not degrade quickly before the wound closes. Clinically, chitosan and its derivatives have been shown to aid in eye healing and are cytologically compatible with various cells in the eye. Administration of chitosan to rabbits with keratoconjunctivitis due to alkaline trauma can decrease symblepharon formation and increase collagen formation in the conjunctiva\textsuperscript{23}. Chitosan also accelerates the proliferation of epithelial cells and inhibits the proliferation of stromal cells in rabbits whose corneal epithelium is damaged due to chemical trauma. The cornea that is given chitosan treatment is more transparent due to rapid wound closure, and the stroma is still regular in structure\textsuperscript{24}. Chitosan also aids in corneal healing by inducing extracellular signal-regulated kinases (ERK) pathway\textsuperscript{27}. Another ingredient in this biomaterial is sodium hyaluronic that...
is present naturally in the extracellular matrix in various organs so that it has non-toxic and non-immunogenic properties. Hyaluronic acid can accelerate corneal healing by reducing pro-inflammatory cytokines, including CD44, IL-1β and MMP9\(^{26}\). Combining these three materials in the form of a biomaterial composite in this study was shown to reduce TGF-beta levels on day 14 compared to the control group, which is thought to occur because it accelerates healing and reduces pro-inflammatory cytokines.

**Limitation**

This study limitation is the short observation period and was not conducted serially to compare the level of corneal opacities at each phase of wound healing. This study also requires many other variables to be investigated to evaluate the chol-Chi-NaHa biomaterial composite’s effectiveness and investigate the inflammatory pathways this composite can inhibit.

**Conclusion**

In this article we reported on our study of Col–Chi–NaHA composite and its effect on corneal clarity and TGF-β level. After 14 days after stromal wounding, the results of the study showed that corneal clarity in the implanted group was lower than that of the control group, but this was due to the short observation period which causing biomaterial not completely degraded. The long-time effects of this biomaterials in cornea still need to be studied. TGF-β level in the implanted group was lower than control. Examination of other parameters, especially other pro-inflammatory cytokines and pro-inflammatory cells count can evaluate the effectiveness and further understand how the Col-Chi-NaHa composites work.

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**Ethical Clearance:** Given by the animal care and Use Committee of the Faculty of Veterinary Airlangga University (Surabaya, Indonesia).

**Source of Funding:** Self-funding.

**Conflict of Interest:** Nil.

**References**


Review on Mobile Apps for Toxicovigilance

Nurul Syafika Nadiah Mohd Zabarudin¹, Sharifah Mashita Syed-Mohamad², Yulita Hanum P Iskandar³

¹Student, ²Lecturer, School of Computer Sciences, ³Lecturer, Graduate School of Business, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia

Abstract

Toxicovigilance is the active process to detect, validate and follow up with poisoning occurrence within the community. The collection of data on poisoning occurrences over the years has been compiled into a poison information database that is accessible by users initially through a computer software and later as a web application. With mobile technology evolving rapidly, mobile apps have transformed and benefitted users in many areas including toxicology. This study conducted a review of existing mobile apps in the marketplace to find out available mobile apps for toxicovigilance and to evaluate its features and functionality to gain insight into the current trends in that area. Secondly, the objective of this study is to find out whether those apps are embedded with learning functionalities for health care professionals and examine its learning features. This study adopts a systematic searching method for apps selection in the marketplace through pre-determined keywords. A total of 22 apps were selected with a majority from the reference and information gathering category. The learning features in the apps focus on self-assessment and examination. The findings show that there are limited number of mobile apps for toxicovigilance and more features can be introduced such as patient monitoring and management or learning features for continuous learning, in addition to the assessment and evaluation features.

Key words: mobile applications, toxicovigilance, health care professionals.

Introduction

Toxicovigilance is an approach in medical toxicology concerned with the active process of detection, validation and follow-up for toxic exposure within the community (¹). It involves the identification and evaluation of toxic risk in the community and the evaluation of measures taken in eliminating and reducing the risks. This process is done by monitoring the occurrence of poisoning cases and identifying the cause that contributes to the occurrence with the intention to reduce the toxicological risk by analysing the inquiries reported to the poison centre (²) (³).

The poison centre received inquiries mostly from health care professionals (HCPs) and the public about toxic exposure. Experts or toxicologist in the poison centre will help to assess if the exposure is harmful and needs further actions. Especially in cases reported by the health care professionals, the experts will consult and give detailed information on the diagnosis and treatment management. From these inquiries, poison centres are able to create databases and repositories that can provide useful information on poisoning occurrence trends and risks which is a part of the toxicovigilance process.

Over time, the poison centre is able to create their own information databases and information management system from their analysis. The compiled information is organized into more structured information that better facilitates information retrieval and information sharing. For example, TOXBASE is a first generation poison information database developed by the National Poisons Information Service (NPIS) in the UK (⁴). The registered users in TOXBASE can easily access the information on poisoning management or products and substances. This database started as computer software, then moved to a more compatible system that utilized internet
access. This conversion enabled users to get access to information easily at anytime and anywhere.

The use of mobile device and apps is becoming increasingly common in the health-related area. On various devices and operating system platforms, there are various free and paid apps offered to aid and provide easy access to specific users or the public. The databases or information systems that previously can be accessed only through the web or stand-alone devices, can now be acquired through the mobile devices. Specifically, in the case of the HCPs, these databases and information systems give them quick access to information in assisting their jobs and also for their learning and education especially when using the mobile devices (5).

There are many existing mobile apps developed for various health purposes. The apps are typically more general-purpose like for diabetes, pregnancies, or smoking. Other apps are developed for more specific health areas such as emergency medicine, pharmacology or pediatrics. These apps could be used by general users, healthcare providers, students or healthcare professionals (6). Mobile apps benefit HCPs in terms of information management, time management, health record maintenance and access, communications and consulting, reference and information gathering, clinical decision making, patient monitoring and medical education and training (5).

This study aims to review available mobile apps related to toxicovigilance or toxicology and evaluate its features and functionality to gain insight into current trends in that area. Secondly, the objective of this study is to find out whether those apps are embedded with learning functionalities for HCPs and examine the learning features of the apps if it is present. Hence, this study conducted a review of available toxicovigilance mobile apps in the market.

**Methods**

**Systematic Review Design**

This review adapts a systematic search method by Kim (7) as this method is mainly used for studies that focuses on a specific domain and is applicable to this study as the review is focused on the context of toxicology. Two research questions are formulated for this review; (1) What are the available apps in the market that are for toxicovigilance, and (2) What are the features offered by those apps. These research questions set the baseline for designing app searching strategy.

**App Search Strategy**

The search was been conducted in June 2020 in two marketplaces, Google Apps Store for Android and Apple iTunes Store for iOS. Before the search, the researchers developed a set of related and relevant keywords to optimize the search process and identify related apps. The keywords are 1) Toxicovigilance, 2) Poison, 3) Toxicology, 4) Toxic, 5) Drug. Any apps related to toxicology or human poisoning will be included while excluding any games, entertainment, non-toxicology, and animal poisoning apps. The apps that are described in English or which the description could be translated to English are also reviewed. The apps that comply with all the pre-determined criteria will be reviewed. In Google Apps Store, the search was conducted in the Apps category by using the pre-determined keywords. Similar to the Apple iTunes Store, the search was conducted in the search box by using the same set of keywords. The researchers also viewed the recommendation of similar/related apps and reviewing the apps under the medical category that contain any of the keywords.

**App Selection**

The apps that are selected will be reviewed based on the description and screenshot provided. Relevant information such as general information, app description, and features were extracted and compiled into a spreadsheet file for analysis.

**Data Analysis**

The information that was compiled is analysed. The apps will be further classified based on the features and usage. The usage of mobile device and apps could be classified into eight (8) categories (5); 1) information management, 2) time management, 3) health record
maintenance and access, 4) communications and consulting, 5) reference and information gathering, 6) clinical decision-making, 7) patient monitoring, 8) medical education and training.

Following this classification, in order to answer the second research question, the selected apps are classified into five (5) categories that are relevant to our scope and the features for each category in the bracket: Information Management [write notes, dictate notes, record audio, take photographs, organize information and images, use e-book reader, access cloud service], Communication and Consulting [voice calling, video calling, texting, e-mail, multimedia messaging, video conferencing, social networking], Reference and Information Gathering [medical textbooks, medical journals, medical literature, literature search portals, drug reference guides, medical news], Patient Management and Monitoring[monitor patient health, monitor patient location, monitor patient rehabilitation, collect clinical data, monitor heart function], and Medical Education and Training[continuing medical education, knowledge assessment tests, board exam preparation, case studies, e-learning and teaching, surgical simulation, skill assessment tests].

**Results and Discussion**

The first aim for the study is to review the existing mobile apps for toxicovigilance to gain insight on the current trends. There are a lot of applications developed to assist HCPs for their tasks, learning and have become essential tools (8) for them. However, the findings shows that there are still limited number of mobile apps for toxicovigilance compared to the existing number of applications for health. From the review, there are a total of 30 apps that fit the criteria, with 19 apps (67%) on Android and the rest on iOS with 11 apps-37% (Refer Table 1, and Figure 1).

### Table 1: General Information of Apps (Android and iOS)

<table>
<thead>
<tr>
<th>Num</th>
<th>Apps Name</th>
<th>Platform</th>
<th>Category</th>
<th>Language</th>
<th>Cost</th>
<th>In-Apps Purchase</th>
<th>Rating</th>
<th>Developer</th>
<th>Hpc Targetted Apps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Poison Rx</td>
<td>Android</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>X</td>
<td>4.6</td>
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<tr>
<td>2</td>
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<td>Android</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>X</td>
<td>4.4</td>
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<td>3</td>
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<td>Android</td>
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<td>Free</td>
<td>X</td>
<td>4.7</td>
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<td>4</td>
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<td>Education</td>
<td>English</td>
<td>Free</td>
<td>X</td>
<td>5.0</td>
<td>JSS Academy of Higher Education &amp; Research</td>
<td>√</td>
</tr>
<tr>
<td>6</td>
<td>Poisoning - First Aid for Children</td>
<td>Android</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>X</td>
<td>4.4</td>
<td>kigorosa UG</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Poisoning &amp; Drug Overdose Info</td>
<td>Android</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>Yes (RM239.99 per item)</td>
<td>3.8</td>
<td>Skyscape Medpresso Inc</td>
<td>√</td>
</tr>
<tr>
<td>8</td>
<td>TOXBASE</td>
<td>Android</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>YES (RM 38.21 per item)</td>
<td>3.7</td>
<td>D4t4 Solution Plc</td>
<td>√</td>
</tr>
<tr>
<td>9</td>
<td>Austin Health Clinical Toxicology Guideline</td>
<td>Android</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>X</td>
<td></td>
<td>Austin Health</td>
<td>√</td>
</tr>
</tbody>
</table>
## Cont... Table 1: General Information of Apps (Android and iOS)

<table>
<thead>
<tr>
<th>No.</th>
<th>App Name</th>
<th>Platform</th>
<th>Category</th>
<th>Language</th>
<th>Version</th>
<th>Price</th>
<th>Rating</th>
<th>Developer</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Toxicology</td>
<td>Android</td>
<td>Book &amp; Reference</td>
<td>English</td>
<td>Free</td>
<td>X</td>
<td>3.7</td>
<td>SyuaLikesApple</td>
</tr>
<tr>
<td>11</td>
<td>Toxicology</td>
<td>Android</td>
<td>Book &amp; Reference</td>
<td>English</td>
<td>Free</td>
<td>X</td>
<td>referenceehunt</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Toxicology (Diseases Caused by a Toxic Product)</td>
<td>Android</td>
<td>Medical</td>
<td>French</td>
<td>Free</td>
<td>X</td>
<td>SOLUTION-PRO</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>5 minute toxicology consult - poisoned patients</td>
<td>Android</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>YES (RM 409.99 per item)</td>
<td>Skyscape Medpresso Inc</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Antidotos</td>
<td>Android</td>
<td>Medical</td>
<td>Spanish</td>
<td>Free</td>
<td>X</td>
<td>4.5</td>
<td>Sacramento_C</td>
</tr>
<tr>
<td>15</td>
<td>Toxicologia Hoy</td>
<td>Android</td>
<td>Medical</td>
<td>Spanish</td>
<td>Free</td>
<td>X</td>
<td>4.5</td>
<td>Toxicologia Hoy</td>
</tr>
<tr>
<td>16</td>
<td>Antidotes</td>
<td>Android</td>
<td>Medical</td>
<td>Spanish</td>
<td>Free</td>
<td>X</td>
<td>4.3</td>
<td>adamz</td>
</tr>
<tr>
<td>17</td>
<td>Tox Handbook</td>
<td>Android</td>
<td>Medical</td>
<td>English</td>
<td>RM12.99</td>
<td>X</td>
<td>4.8</td>
<td>AAEM/RSA</td>
</tr>
<tr>
<td>18</td>
<td>iTox Urgencias intoxicación</td>
<td>Android</td>
<td>Medical</td>
<td>Spanish</td>
<td>RM98.72</td>
<td>4.8</td>
<td>Adalia Fama</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Official ABT Exam Practice</td>
<td>Android</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>RM 24.99 - RM 119.99 per item</td>
<td>4.4 Pocket Prep, Inc</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>ACEP Toxicology Section Antidote App</td>
<td>IOS</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>X</td>
<td>5.0</td>
<td>American College of Emergency Physicians</td>
</tr>
<tr>
<td>21</td>
<td>Hypertox</td>
<td>IOS</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>YES $3.99</td>
<td>Meditox Pty Ltd</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Poisoning and Drug Overdose</td>
<td>IOS</td>
<td>Medical</td>
<td>English</td>
<td>$69.99</td>
<td>X</td>
<td>4.5</td>
<td>MobiSystems</td>
</tr>
<tr>
<td>23</td>
<td>5 Minute Toxicology Consult Rapid Access Guide</td>
<td>IOS</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>$99.99</td>
<td>Skyscape Medpresso</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Austin Health Toxicology – Clinical Toxicology Guideline</td>
<td>IOS</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>X</td>
<td>Austin Health</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>TOXBASE</td>
<td>IOS</td>
<td>Medical</td>
<td>English</td>
<td>Free</td>
<td>1 year subscription $6.99</td>
<td>1.5 D4t4 Solutions Plc</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Tox Handbook</td>
<td>IOS</td>
<td>Medical</td>
<td>English</td>
<td>$2.99</td>
<td>X</td>
<td>5.0</td>
<td>AAEM Resident and Student Association Inc, The</td>
</tr>
<tr>
<td>28</td>
<td>Poisoning - First Aid First Aid for Children</td>
<td>IOS</td>
<td>Medical</td>
<td>English</td>
<td>$ 1.99</td>
<td>X</td>
<td>Kigorosa UG</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Poisoning &amp; Drug Overdose Info</td>
<td>IOS</td>
<td>Medical</td>
<td>English</td>
<td>free</td>
<td>yes ($59.99)</td>
<td>Skyscape Medpresso Inc</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Poisoning &amp; Drug Overdose Quick Reference</td>
<td>IOS</td>
<td>Medical</td>
<td>English</td>
<td>free</td>
<td>X</td>
<td>AgileMD, Inc.</td>
<td></td>
</tr>
</tbody>
</table>
There are 8 apps offered on both platform: 1) 5 minute toxicology consult - poisoned patients (Android)/ 5 Minute Toxicology Consult Rapid Access Guide (IoS), 2) Austin Health Clinical Toxicology Guideline (Android) / Austin Health Toxicology – Clinical Toxicology Guideline (IoS), 3) TOXBASE, 4) Tox Handbook, 5) Poisoning - First Aid for Children (Android) / Poisoning - First Aid First Aid for Children (IoS), 6) Poisoning & Drug Overdose Info, 7) Poisoning and Drug Overdose and 8) Poisoning & Drug Overdose Ref. (Android) / Poisoning & Drug Overdose Quick Reference (IoS). These apps offer the same features and same company/developer on both platforms. In the marketplace, the apps are grouped into certain categories to help users browse existing apps or discover new ones. 18 apps (82%) are labelled in the “Medical” category, while two apps (9%) in the “Education” category and another two (9%) are in the “Book & Reference” category. 17 (77%) of the apps are in English, while 4 apps (18%) are offered in Spanish and 1 app (5%) in French. 18 apps (90%) are available as free, while another 2 (10%) are paid apps. 6 free apps offered in-app purchases. For Poisoning and drug overdose by MobiSystem, it is offered as a free android app with in-app purchases and paid apps in iOS. Poisoning - First Aid for Children by Kigorosa UG, it is free with no in-apps purchase in Android while it is a paid app in iOS.

While reviewing the description, the researchers try to find out whether the apps mention its targeted users (in specific HCPs). 11 apps (50%) mentioned their apps are targeted for HCPs (Refer Figure 2). Other apps either do not state any specific targeted users. It is beneficial to state the targeted users as part of the material or if the features included in the apps are very specific to the purpose of the targeted users.
To learn the features and functionality of the apps, the 22 apps are further classified into 5 categories: Information Management, Communication and Consulting, Reference and Information Gathering, Patient Management and Monitoring, and Medical Education and Training based on their features (Refer Table 2, and Figure 3). 20 apps contain the features that are defined under the category of reference and information gathering, making this category the most popular category for toxicology apps. Most of the applications developed for toxicology purpose are reference books and guide and it is consistent with Aungst (9) who stated that the mobile device has been transformed into a medical tool for clinical reference and decision making. From 20 apps in this category, 7 apps (35%) are considered as medical textbooks.

Table 2: Apps categorization based on usage and feature

<table>
<thead>
<tr>
<th>Num</th>
<th>APPS NAME</th>
<th>INFORMATION MANAGEMENT</th>
<th>COMMUNICATION AND CONSULTING</th>
<th>REFERENCE AND INFORMATION GATHERING</th>
<th>PATIENT MANAGEMENT AND MONITORING</th>
<th>MEDICAL EDUCATION AND TRAINING</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Poison Rx</td>
<td></td>
<td></td>
<td>√</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>webPOISONCONTROL® Poison App</td>
<td></td>
<td></td>
<td>√</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Poisoning &amp; Drug Overdose Ref.</td>
<td>√</td>
<td></td>
<td>√</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Poisoning and drug overdose</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
<td>√</td>
</tr>
</tbody>
</table>
## Classification of Mobile Apps by Usage and Features

<table>
<thead>
<tr>
<th></th>
<th>Information Management</th>
<th>Communication &amp; Consulting</th>
<th>Reference &amp; Information Gathering</th>
<th>Patient Management &amp; Monitoring</th>
<th>Medical Education &amp; Training</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 2: Apps categorization based on usage and feature

|   | Poison Information Centre | √ | 6 | Poisoning - First Aid for Children | √ | 7 | Poisoning & Drug Overdose Info | √ | 8 | TOXBASE | √ | 9 | Austin Health Clinical Toxicology Guideline | √ | 10 | Toxicology (SyuaLikesApple) | √ | 11 | Toxicology (referencehunt) | √ | 12 | Toxicology (Diseases Caused by a Toxic Product) | √ | 13 | 5 minute toxicology consult - poisoned patients | √ | 14 | Antidotos | √ | 15 | Toxicologia Hoy | √ | 16 | Antidotes | √ | 17 | Tox Handbook | √ | 18 | iTox Urgencias intoxicación | √ | 19 | Official ABT Exam Practice | √ | 20 | ACEP Toxicology Section Antidote App | √ | 21 | Hypertoex | √ | 22 | Toxicology Exam Prep 2017 Edition | √ |

**Figure 3:** Classification of Mobile Apps by Usage and Features
There are 3 apps that match the features for medical education and training (Refer Table 3). “Official ABT Exam Practice” is a self-study app that provides practice tests in two modes: simulation, and study. Questions are reviewed by subject-matter-experts and the content is aligned with actual exams and provide answer explanations for each question. It provides result analysis and examination history. It allows users to customize study sessions and exam requirements to suit their needs. Users can choose total questions, enable timers, and filter examination content. It also has study reminders and exam day countdowns. However, the feature may be different based on the free version or paid version. The free version has limited features, such as limited number of questions, limited exam builder and only provide examination information. The free version provides only 30 practice questions and explanations while there are more than 400 questions for the paid version. The exam customization is available only in the full paid version in addition to answer explanations, references, progress tracking, support and update. This app can be used without internet connectivity.

### Table 3: Reference and Information Gathering Classification

<table>
<thead>
<tr>
<th>Features</th>
<th>Apps Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>medical textbooks</td>
<td>Poisoning and drug overdose</td>
</tr>
<tr>
<td></td>
<td>Toxicology (SyuaLikesApple)</td>
</tr>
<tr>
<td></td>
<td>Toxicology (referencehunt)</td>
</tr>
<tr>
<td></td>
<td>5 minute toxicology consult - poisoned patients</td>
</tr>
<tr>
<td></td>
<td>Antidotos</td>
</tr>
<tr>
<td></td>
<td>Antidotes</td>
</tr>
<tr>
<td></td>
<td>Tox Handbook</td>
</tr>
<tr>
<td>drug reference guides</td>
<td>Poison Rx</td>
</tr>
<tr>
<td></td>
<td>webPOISONCONTROL® Poison App</td>
</tr>
<tr>
<td></td>
<td>Poisoning &amp; Drug Overdose Ref</td>
</tr>
<tr>
<td></td>
<td>Poisoning - First Aid for Children</td>
</tr>
<tr>
<td></td>
<td>TOXBASE</td>
</tr>
<tr>
<td></td>
<td>Austin Health Clinical Toxicology Guideline</td>
</tr>
<tr>
<td></td>
<td>Toxicology (Caused by a toxic product)</td>
</tr>
<tr>
<td></td>
<td>iTox Urgencias intoxicación</td>
</tr>
<tr>
<td></td>
<td>ACEP Toxicology Section Antidote App</td>
</tr>
<tr>
<td></td>
<td>Poison Information Centre</td>
</tr>
<tr>
<td></td>
<td>Hypertox</td>
</tr>
<tr>
<td></td>
<td>Poisoning &amp; Drug Overdose Info</td>
</tr>
<tr>
<td></td>
<td>Toxicologia Hoy</td>
</tr>
</tbody>
</table>
These apps are designed with sections and books chapters that replicate the textbook in a mobile form. 13 apps (65%) are drug reference guide that provides guideline or gives recommendation (Refer Table 3). 3 apps have features for medical education and training (refer Table 4). Some of them include a search feature to help users easily find information that they require. Another interesting feature is the barcode scanner which allows users to scan products to retrieve information.

<table>
<thead>
<tr>
<th>Table 4: Medical Education &amp; Training Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Features</strong></td>
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<tr>
<td>Continuing medical education</td>
</tr>
<tr>
<td>Knowledge assessment tests</td>
</tr>
<tr>
<td>Board exam preparation</td>
</tr>
<tr>
<td>Case studies</td>
</tr>
<tr>
<td>E-learning and teaching</td>
</tr>
<tr>
<td>Surgical simulation</td>
</tr>
<tr>
<td>Skill assessment tests</td>
</tr>
</tbody>
</table>

“Poisonology Exam Prep 2017 Edition” is a self-study and examination preparation app by breaking materials into smaller sets. Each set contains flashcards, matching games, true or false, and multiple choice. It can filter questions by detecting and separating the most difficult questions for user. It keeps track of the user learning process for each set and exam. For the free version, users are given access to hundreds of practice questions & terms, 5 free exam builders, a free matching game and the ability to filter the hardest and weakest questions. For the paid version, users have unlimited access to the exam builder and simulator, including all practice questions & terms prepared by experts for the most current exam. Questions are automatically filtered based on users and progress is tracked for each question and exam. It also encompasses support and update.

“Poisoning and drug overdose” helps users in learning by introducing and expanding vocabulary through a “Word of the day” features. The description of the apps that include learning feature show that it specifically focuses on the self-assessment and exam. It can be a tool for exam preparation and evaluating current skills. Beside the assessment and evaluation, continuous learning is also necessary for HCPs to ensure highly skilled staff and ensure their knowledge is maintained and improved, though there might be difficulties or limitation for the HCPs to attend lectures. Continuous learning using e-learning that includes interactive content could enhance the learning experience of HCPs.

2 apps namely “Poisoning and drug overdose” and “5 Minute Toxicology Consult” in the category of information management have features like writing and dictating notes, audio recording and information and image organization. As for now, the apps include
features for information organization like note taking, creating bookmarks or set favourites to the learning material. For taking notes, the users can input text or use voice. Annotation in the form of a scribble, doodle or text are also the features that could be discovered. These features are useful for reading and revision.

It is notable that none of the selected apps offer features for communication and consulting or patient management and monitoring toxicology. The patient management and monitoring apps are quite common in other health areas. Patient management and monitoring include features such as monitoring patient health, location, rehabilitation or collecting clinical data. Toxicology is an multi-disciplinary area and the study of poison (11), while toxicovigilance is an approach in managing poisoning occurrence. The important step in managing poisoning occurrences is to record and monitor the poisoning cases that happened. Collecting poisoning case details is important in determining the case risk and treatment. These databases are very useful for the reference and guidance or learning material as there are always new products or new toxic from time to time.

Implications and Future Work

Toxicology as any other health area, is a very specific domain. It is a challenge to develop an application that could fit the specific needs of the related HCPs. As shown in the review, in terms of education or training, the available apps are more focused on preparation for exam boards. Although there are many textbooks and drug references available, however it is an opportunity to explore expanding learning features like e-learning and teaching. Simulation or case studies could be offered to promote ongoing, continuous learning for HCPs with the flexibility of using mobile phone. This is where e-learning and mobile apps can be used as the medium to utilize learning delivery or so-called m-learning (12). Developing any type of apps is possible, but the bigger question is to come out with appropriate apps. There is a need to study their requirements in terms of content development and presentation, learning style or features requirement. As emphasized by Lazzara (6), HCPs should be involved in the development process to ensure the correctness and the reliability of the mobile apps as errors in the apps could contribute to the ineffectiveness of the apps and create risks to users.

It is also interesting to explore patient monitoring for poisoning cases. There are many user self-monitoring apps or patient monitoring apps in other health areas. Having this feature in mobile applications will improve accessibility of HCPs to manage and monitor their poisoning cases in the future.

Conclusion

As a conclusion, this paper reviewed 22 toxicology mobile apps in the categories of information management, reference and information gathering, and medical and training. It shows that there is a lack of mobile apps developed for HCPs in this area. As the review has shown, there are many studies that could be conducted for the development of mobile apps that is appropriate for HCPs in area of toxicovigilance especially for HCPs.

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Ethical Clearance: Taken from Universiti Sains Malaysia Ethics Committee with the study protocol code: USM/JEPem/1804210.

Conflict of Interests: No conflict of interests.

References


Stimulation of Male Voice during Pregnancy Results in Higher Expression of Brain Derived Neurotrophic Factor in Cerebellum of Newborn Rattus norvegicus

Nurvy Alief Aidillah¹, Hermanto Tri Joewono², Widjiati³

¹Final year Master of Reproductive Health Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ²Lecture, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ³Professor, Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

Background: Recent studies in the field of Fertomaternal Medicine show that stimulation in the uterus can support brain growth and development. If in previous studies stimulation using several types of music, this study uses human voice which aims to analyze the effect of stimulation of male and female voices on differences in the expression of Brain Derived Neurotrophic Factor (BDNF) in cerebellum of newborn Rattus norvegicus. Methods: This type of research is true experimental with post test only control group design. Rattus norvegicus was given stimulation of men voice and women voice on 6th day until 17th day of pregnancy. The expression of BDNF was examined using immunohistochemistry. Results: The mean ± standard deviation of the expression of BDNF in the cerebellum is 4.20 ± 1.75 (without stimulation), 6.04 ± 1.58 (male voice stimulation), and 5.60 ± 2.43 (female voice stimulation). The results of statistical tests show that there is a significant difference on BDNF expression between the control group and the group stimulated with male voice with a significance value <0.05 of 0.045 Conclusion: Giving stimulation using the male voice during pregnancy shows a significant increase in the expression of BDNF in cerebellum of newborn Rattus norvegicus.

Key words: Stimulation, Pregnancy, Male and female voice, Rattus norvegicus, Cerebellum, BDNF

Introduction

One way to measure the progress of a country, is not only dependent on indicators of economic growth. The Human Development Index (HDI) measures the progress of a country based on its human development dimensions; healthy and long-lived humans, knowledge, and a high standard of living. In 2019, Indonesia was ranked 111. Even though UNDP has classified Indonesia as a country with high HDI, this condition still deserves our attention, because we are still left behind with several developing countries such as the Philippines (106), Thailand (77), and Malaysia (61). Some of these countries outperformed Indonesia in their education indicators so that their human development index scores were better than Indonesia according to UNDP (1).

The high index of education is influenced by the quality of a country’s generation. Several recent findings in the field of fetomaternal medicine state that to obtain a smarter generation, one of which is by enriching the environment during the gestation, especially the development of the fetal brain. Giving stimulation can support brain growth and development by stimulating the formation of synapses or connections between nerve cells and also inhibiting apoptosis or cell death. Apoptosis peaks at around 36-40 weeks of gestation, therefore it is highly recommended to provide optimal and routine stimulation during pregnancy (2–4).
Cerebellum is the second largest part of the brain after the cerebellum; several findings show the cerebellum has a more complex function. Most of the cerebellum is connected with brain association areas related to intelligence, social cognition, and emotional control (5). Brain Derived Neurotrophic Factor (BDNF) is the main neuronal growth factor in the brain that regulates neurogenesis, neuron maturation, survival, and synaptic processes. Although neurogenesis in the hippocampus occurs until adulthood, most neurogenesis occurs in the prenatal and early postnatal periods. The main factor of Neurotrophin that plays a very important role in the learning process, memory, and behavior in the hippocampus is BDNF (6).

A frequency of about 8,000 Hz is very useful for filling (charging) cells in the brain. Music that is rich in high frequencies and has a high rhythm that is similar to the rhythm of the fetal heartbeat and can be linked to simulation and energizing functions (7). In the sound production system in humans, the lungs produce air pressure which then passes through the windpipe (trachea). The pressure then vibrates the vocal cord that is located above the windpipe. The vibration of the vocal cord (opening and closing) produces sound which is then pronounced by mouth. The voice characteristics of each person are different because they are influenced by variations in the shape, length, and thickness of the vocal cord. Therefore, the sounds that sounded are significantly different in each person. The length of the vocal cord for a human is usually between 12 and 24 millimeters (mm), while the thickness is 3 to 5 mm (8).

In music theory, humans have different types of voices, both male and female. The types of voices in male are divided into tenor, baritone, and bass. Meanwhile, female voices are divided into soprano, mezzo-osopran, and alto (9). The results of the study using the Backpropagation Neural Network as a system for identifying the types of human voices show that the algorithm used is very weak to detect the type of alto voice and tend to be good for detecting the type of tenor voice (10).

In recent years, there have been several issues regarding the brain development of infants that are given stimulation during pregnancy, such as a significant increase in the number of cerebral neuron cells of Rattus norvegicus after stimulation of Mozart music (11); Similar studies on BDNF expression obtain a significant increase in BDNF expression with stimulation of Mozart’s music (12). The study uses two different types of human voice characters, namely male voice and female voice as stimulation during pregnancy in experimental animal, Rattus norvegicus, which aims to determine the effect of Ar-Rahman murottal stimulation on the expression of BDNF in cerebellum of newborn Rattus norvegicus.

Materials and Methods

This type of research is the experimental laboratory (true experimental) with a post-test only control group research design. Ethical eligibility was obtained from Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University. The research was conducted at the Animal Experiment Cage and Pathology Laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya, from January to March 2021. The research subjects used were 30 pregnant Rattus norvegicus mothers who were divided randomly into 3 groups, namely the non-stimulated group (P1), the group stimulated with Surah Ar-Rahman by male voice (P2), and the group stimulated with Surah Ar-Rahman by female voice (P3). Stimulation was started from 6th until 17th days of pregnancy in a soundproof box with a sound intensity of 65 dB for 60 minutes at night. Inclusion criteria: Rattus norvegicus mothers who had never been used for previous studies, healthy, body weight of 120-160 g, and give birth 5-9 pups, newborn Rattus norvegicus born surgically in healthy condition. Meanwhile, the criteria for drop out were Rattus norvegicus which was stillborn and had anatomical abnormalities.

Two newborn Rattus norvegicus were selected from each mother with the heaviest and lightest weights. Then sacrificed, the head was weighed and preserved in 10% formalin solution which was then prepared for examination
of BDNF expression by immunohistochemical methods. Antibodies used were primary antibody, anti-BDNF and DAB (3,3-diaminobenzidine tetrahydrochloride). BDNF expression of each subject was assessed according to the modified Remmele method (13), where the Remmele scale index (Immuno Reactive Score / IRS) is the result of the multiplication of the percentage score of immunoreactive cells and the color intensity score of the immunoreactive cells. Data of each subject represented the mean IRS value observed at 5x the field of view (LP) at 400x magnification. The data obtained were then statistically tested using the data normality test with the Shapiro-Wilk test. The data that normally distributed were analyzed using one way ANOVA followed by the Least Significant Difference (LSD) test among the three groups.

Results and Discussions

The results of the normality test using the Shapiro-Wilk test on body weight after treatment are obtained a significance value (p-value) in the control group of 0.712, the group stimulated by male voice of 0.971, and the group stimulated by female voice of 0.958. These results indicate the distribution of parent body weight data in each group is normally distributed. The results of the homogeneity test of variety using the Levene test on data on body weight after treatment are obtained a significance value of 0.237. These results indicate that the variance among the three groups is homogeneous. The results of the normality test using the Shapiro-Wilk test on the treatment group are obtained a significance value (p-value) of fetal head weight in the control group is 0.083, the group stimulated by male voice is 0.243, and the group stimulated by female voice is 0.124. These results indicate that the distribution of data on fetal head weight data in each group is normally distributed. It can be seen in Table 1.

Table 1. The Results of Shapiro-Wilk Normality Test and Levene Variety Homogeneity Test on BDNF Expression in Cerebellum of newborn Rattus norvegicus

<table>
<thead>
<tr>
<th>Group</th>
<th>Significance value (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male Voice</td>
</tr>
<tr>
<td>Control (P1)</td>
<td>0.861</td>
</tr>
<tr>
<td>Male Voice (P2)</td>
<td>0.904</td>
</tr>
<tr>
<td>Female Voice (P3)</td>
<td>0.897</td>
</tr>
</tbody>
</table>

Figure 1 shows the mean and standard deviation of BDNF expression in the cerebellum of newborn Rattus norvegicus in the control group (P1), the group stimulated by male voice (P2), and the group stimulated by female voice (P3).

Figure 1. Graph of Mean and Standard Deviation of BDNF Expression in Cerebellum of newborn Rattus norvegicus in the control group (P1), the group stimulated by male voice (P2), and the group stimulated by female voice (P3).

Table 1. The Results of Shapiro-Wilk Normality Test and Levene Variety Homogeneity Test on BDNF Expression in Cerebellum of newborn Rattus norvegicus

<table>
<thead>
<tr>
<th>Group</th>
<th>Statistic</th>
<th>p-value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (P1)</td>
<td>0.861</td>
<td>0.079</td>
<td>Normal Distribution</td>
</tr>
<tr>
<td>Male Voice (P2)</td>
<td>0.904</td>
<td>0.242</td>
<td>Normal Distribution</td>
</tr>
<tr>
<td>Female Voice (P3)</td>
<td>0.897</td>
<td>0.201</td>
<td>Normal Distribution</td>
</tr>
<tr>
<td>Uji Levene</td>
<td>3.119</td>
<td>0.060</td>
<td>Homogeneous</td>
</tr>
</tbody>
</table>
The results of the homogeneity test of variance using the Levene test on BDNF expression data in the cerebellum are obtained a significance value of 0.060. These results indicate that the variance among the three groups is homogeneous. Based on the results of the normality test and the homogeneity test of variance, the data analysis to test whether there are differences in the treatment groups on BDNF expression in the cerebellum used the Analysis of Variance (ANOVA) test with the advanced Least Significant Difference (LSD) test.

### Table 2. The Results of ANOVA Test on BDNF Expression in Cerebellum of Newborn Rattus norvegicus

<table>
<thead>
<tr>
<th>Variable</th>
<th>Statistic</th>
<th>p-value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF Expression in Cerebellum</td>
<td>2.418</td>
<td>0.108</td>
<td>Not Significant</td>
</tr>
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</table>

The results of ANOVA test on BDNF expression in cerebellum of newborn Rattus norvegicus are obtained a calculated F value of 2.418 with a significance value of 0.108 which indicates that there is no significant difference among treatment groups on BDNF expression in the cerebellum of newborn Rattus norvegicus. The p-value results of the LSD advanced test among the control group (P1), the group stimulated by male voice (P2), and the group stimulated by female voice (P3) can be seen in Table 3.

### Table 3. The Results of LSD Test on BDNF Expression in Cerebellum of Newborn Rattus norvegicus

<table>
<thead>
<tr>
<th>Group</th>
<th>Significance value (p-value)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male Voice</td>
<td>Female Voice</td>
</tr>
<tr>
<td>Control</td>
<td>0.045</td>
<td>0.121</td>
</tr>
<tr>
<td>Male Voice</td>
<td></td>
<td>0.619</td>
</tr>
</tbody>
</table>

The results of LSD test show a significant difference in BDNF expression in the cerebellum of newborn Rattus norvegicus between the group stimulated by male voice and the non-stimulated group (Control). Meanwhile, the control group compared to the group stimulated by female voice shows a less significant difference, it means that there is less significant difference in BDNF expression in the cerebellum of newborn Rattus norvegicus. In the next group, the group stimulated by male voice compared to the group stimulated by female voice, the result is less significant. Although the result in the group stimulated by male voice is higher than the group stimulated by female voice, the difference is less significant or insignificant. Among the three groups, it is known that the group stimulated by male voice is obtained the highest expression results in BDNF expression in the cerebellum of newborn Rattus norvegicus.

Gender affects the size of the vocal cords. The length of the female vocal cords is approximately 12.5 mm to 17.5 mm, whereas in male voice it is from 17.5 mm to 24 mm (14). Because the vocal cords are longer, the pitch of the male voice is lower, therefore the male voice sounds heavier than the female voice. Heavy voices contain more sound intensity. Based on research on the voice identification system using Backpropagation Artificial Neural Network (ANN), the results of the voice identification system tend to be better at detecting the type of tenor voice compared to the type of alto voice, in which the tenor voice is one of male voice types, whereas alto voice is one of female voice types (9). Research with similar results proposing a new feature for automatic gender detection using modified voice contour (MVC), found that the area under the MVC for male speakers is greater than for female speakers (15). This is because male speakers have a higher intensity than female speakers.

Automatic gender detection applications have actually been widely used in the health world, for example in the health care monitoring system (16,17); and the detection of abnormalities in the vocal cords (18,19). Various researches on automatic gender detection (AGD) applications that have been developed have proven that male voices are easier to identify. Therefore, this study proves the theory that male voice is known to have a higher effect as a form of stimulation performed on experimental animal during pregnancy.
The red arrows in Figure 2 show the BDNF expression in the cerebellum which is indicated by the presence of a brown chromogen on the immunohistochemical examination which is observed at 5x the visual field (LP) at 400x magnification.

Figure 2 Comparison of BDNF expressions in the brain of the control group (P1), the group stimulated by male voice (P2), and the group stimulated by female voice (P3).

Conclusion

The stimulation of male voice during pregnancy has a significant effect on increasing the expression of BDNF in cerebellum of newborn Rattus norvegicus because the highest BDNF expression is shown in the group stimulated by male voice. Similar research in other parts of the brain needs to be more developed because this research is the first experiment of giving stimulation using the type of human voice in experimental animal.

Conflict of Interest : None

Source of Funding : Self

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Effect of Thunbergia Laurifolia Lindl. on Oxidative Stress and Blood Cholinesterase in farm Workers Exposed to Pesticides in the Mae Chai District of Phayao Province

Orapin Insuan, Padchanee Sangthong, Pennipat Nabheerong, Surachat Buddhisae, Peerawan Kengsangunsi, Suntorn Prompao, Benchaluk Thongchuai

Abstract

Thunbergia laurifolia Lindl. or Rang Chuet is a plant traditionally used in Thailand for the treatment of toxicity and as an antidote for many poisons. This study aimed to investigate the effect of Rang Chuet capsules on blood chemistry in 39 agricultural workers (21 males, 18 females) during an intensive 8-week spraying program. The following tests were performed: serum butyrylcholinesterase (BChE) and serum malondialdehyde (MDA) levels before and after Rang Chuet capsule supplementation. The results indicate that serum BChE levels were unchanged in male and female workers. However, the levels of serum MDA in male and female workers decreased significantly at p < 0.001 and p < 0.05, respectively. In this study, the phylogenetic relationships within the large tropical and subtropical family Thunbergia were studied using genomic DNA from the fresh leaves of sampled plants. The results showed 99% homology with Thunbergia coccinea. This study indicates that Rang Chuet presents antioxidant activity and could be promoted as a health supplement.

Key words: Rang Chuet, butyrylcholinesterase, malondialdehyde

Introduction

In recent years, many insecticide chemicals have been widely used to increase agricultural production in Thailand. However, many agricultural workers do not understand the correct way to use insecticides; as such, these pesticide chemicals may be deposited in the environment, resulting in health and environmental problems. Cantaloupe, watermelon and lychee are the most common fruits that are widely planted, especially in the north of Thailand. In this study, survey data showed that the farm workers in the Mae Chai District of Phayao Province use many pesticides to control agricultural pests on their farms.
Organophosphate (OP) and carbamate (CB) pesticides are the main pesticides sprayed over this agriculture area. The contamination of pesticide residue may remain on soil particles, water or in food after farm workers have applied it to crops. Many of these chemical residues accumulate in tissues and could build up with harmful effects on the body. Toxic effects are observed with acute and chronic toxicity. OP and CB pesticides are acetylcholinesterase (AChE) inhibitors. During the metabolism of both pesticides, they are converted to an oxon intermediate through an oxidative desulphurization reaction mediated by cytochrome P450 in the liver. The oxon intermediate is a reactive metabolite that binds tightly to the hydroxyl group of the serine residue present in the esteratic region of the cholinesterase active site, located on the post-synaptic membrane. Inhibition of AChE results in the accumulation of acetylcholine, the neurotransmitter acting at cholinergic synapses and neuromuscular junctions in the central and peripheral nervous systems. Some studies have revealed that oxidative stress could be an important component of the mechanism of OP and CB pesticide poisoning. Pesticide exposure over a long period could change molecules and damage DNA, resulting in gene mutation or cancer [1,2]. In addition, some studies have revealed that oxidative stress and lipid peroxidation also occur, leading to DNA single strand breaks caused by reactive oxygen species; this induces apoptosis [3].

*Thunbergia laurifolia* Lindl. (laurel clock or blue trumpet vine) is interesting to study with regard to oxidative stress induced by insecticide exposure. The leaves of this plant have been used in traditional herbal medicine for the treatment of symptoms such as fever and rash [4]. In addition, the reported therapeutic properties include antioxidant, antimicrobial, antiproliferative, hepatoprotective and anti-inflammatory activities, as well as detoxifying properties [5]. The antioxidative properties of *Thunbergia laurifolia* Lindl. and its active compounds have been reported using in vitro and in vivo test systems. Kosai et al., 2015 reported that *Thunbergia laurifolia* Lindl. has anti-inflammatory and antioxidant activity and could repair hepatocellular cells [7]. Moreover, Chan et al., 2011 found the *Thunbergia laurifolia* Lindl. showed excellent antioxidant capacity and reduced hepatocellular damage [8]. *Thunbergia laurifolia* Lindl. contains many active ingredients such as delphinidin-3, apigenin, apigenin-7-O-β-D glucopyranoside, rosmarinic acid, iridoid glucosides, alkaloid, flavonoids, phenolic acid and chlorogenic acid, which also have excellent antioxidant effects [9]. Many reports have revealed that *Thunbergia laurifolia* Lindl. reduces pesticide toxicity in rats. The aim of this study was to evaluate the effect of *Thunbergia laurifolia* Lindl. on cholinesterase activity and its antioxidant activity on pesticide intoxication.

### Materials and Methods

#### Participants

This study was conducted on farm workers in the Mae Chai District of Phayao Province in the north of Thailand during the period from December 2017 to April 2018. The study included the rural population of 39 cantaloupe farm workers (21 males, 18 females). Farmworkers had to be 20-70 years old.

#### Data collection

Participants were interviewed by the research staff using a questionnaire. The information collected included personal formation, occupational history and behaviours at work and at home.

#### Administration of Rang Chuet

Rang Chuet capsules were purchased from Ouay Un Osoth Co., Ltd. The participants were treated with Rang Chuet capsules (two 250 mg capsules), before meals three times per day. They were treated with Rang Chuet capsules for 60 days.

#### Sample collection

After overnight fasting, 3 mL of whole blood with EDTA and 7 mL of clotted blood were collected. Serum was separated for malondialdehyde (MDA) assessments. Clotted blood samples were centrifuged for 15 minutes.
at 3,000 rpm at room temperature, then the serum was separated and stored at -20°C until analysis.

**Determination of butyrylcholinesterase in serum**

Butyrylcholinesterase (BChE) was measured in serum following a modified method described by Ellman et al., 1961 [10]. First, 3,000 µL of 5, 5-dithiobisnitrobenzoic acid solution was used for setting zero, then 20 µL of diluted sample was added and mixed gently. After that, 5% butyryl thiocholine iodide (50 µL) was added and mixed. The absorbance of the solution was measured at 405 nm. BChE was calculated and reported in U/L.

**Thiobarbituric acid reactive substances (TBARS) assay**

Lipid peroxidation levels in serum were measured following a modification of the methodology described by Santos et al., 1980 [11]. The sample (80 µL) was mixed with 0.2% BHT in in 240 µL 0.44 M methanol, then H₃PO₄ and 160 µL 0.6% TBA were added. After heating for 30 minutes at 95-100°C and cooling, the absorbance of the solution was measured at 540 nm.

**Plant molecular evaluation using the NucleoSpin® Plant II kit**

Genomic DNA of fresh leaves of Rang Chuet and the powder of Rang Chuet capsules were extracted using the NucleoSpin® Plant II kit and the extracted DNA was used as the DNA template for PCR amplification. The PCR products were amplified using designed primers. These primers were universal primers complementary to the chloroplast DNA of *Thunbergia laurifolia* Lindl. The PCR products were visualised by agarose gel electrophoresis containing 3 µL ethidium bromide in 1% (w/v) under a UV transilluminator. The PCR products were purified using a NucleoSpin Gel and PCR clean-up kit before sequencing. Finally, the sequences were aligned using the database in GenBank® (http://www.ncbi.nlm.nih.gov/genbank/).

**Statistical Analysis**

Graph Prism Version 8 was used to assess the levels of cholinesterase and MDA in farm worker sera.

**Results**

**Butyrylcholinesterase levels in serum**

The blood BChE levels were slightly decreased in female farmworkers and considerably increased in male farmworkers (Figure 1). The BChE levels before Rang Chuet treatment were 5,012 ± 1,364.0 U/L and 5,299 ± 1,381.0 U/L in female and male sprayers, respectively. The BChE levels after Rang Chuet treatment were 4,605 ± 668.8 U/L and 5,490 ± 1,395.0 U/L in female and male sprayers, respectively. The results demonstrate that BChE activity was not significantly impacted in sprayers.

**Malondialdehyde levels in serum**

The MDA levels after Rang Chuet treatment were significantly lower in both male and female farmworkers at p < 0.001 and p < 0.05, respectively. The MDA levels before Rang Chuet treatment were 5.69 ± 2.81 mM and 8.80 ± 3.61 mM in female and male sprayers, respectively. The MDA levels after Rang Chuet treatment were 5.13 ± 2.14 mM and 6.58 ± 2.24 mM in female and male sprayers, respectively (Figure 2).
Figure 2: Malondialdehyde levels before and after Rang Chuet treatment in male and female farmworkers (*p<0.05, **p<0.01, ***p<0.001).

Plant molecular identification

The purified PCR products from fresh leaves Rang Chuet and the powder of Rang Chuet capsules were analysed (Figure 3) and the nucleotide sequences of the trnL-trnL intergenic spacer and trnL-trnF intergenic spacer were determined using the dye terminator technique (First BASE Laboratories Sdn Bhd, Malaysia). The nucleotide sequences were analysed by a biological sequence alignment editor program (BioEdit). The results illustrate that the sequence analysis of the trnL-trnF intergenic spacer from fresh leaves Rang Chuet and the powder of Rang Chuet capsules compared with the reference data in the NCBI database showed 99%, 99%, 99% and 98% homology with *Thunbergia coccinea* (GenBank® accession number KT075030.1), *Thunbergia grandiflora* (GenBank® accession number KT075034.1), *Thunbergia laurifolia* (GenBank® accession number KT075036.1) and *Thunbergia erecta* (GenBank® accession number KT075032.1).
Discussion

In this study, the objective was to investigate the effect of *Thunbergia laurifolia* Lindl., commonly known as Rang Chuet in Thailand. It has several therapeutic effects, especially as an antidote for many xenobiotic poisons. OP and CM insecticides are well-known to have toxic effects by inhibiting cholinesterase activity in plasma, red blood cells and brain tissue. This study demonstrated that OP and CM insecticides result in a significant increase in MDA formation, which may be associated with the chemical structure of organophosphate pesticides [12].

In addition, OP insecticides, as inhibitors of AChE and BChE, induce the production of ROS and oxidative tissue damage [13]. In this study, the activity of AChE and BChE were not significantly increase. Tayeh et al., 2018 illustrated that Rang Chuet has antioxidant activity and neuroprotective activity in animals exposure to OP insecticides [14]. The data from this study show unchanged cholinesterase enzyme levels after treatment with Rang Chuet. However, the levels of cholinesterase were in the normal range in all participants, possibly due to uncontrolled factors such as subject performance when spraying, personal hygiene and frequency of spraying by each farm worker. In this study, treatment with Rang Chuet did not affect cholinesterase levels. OP pesticide exposure causes oxidative stress resulting in MDA generation, as well as decreased glutathione levels [15,16]. There is also a significant decrease in catalase...
and superoxide dismutase\textsuperscript{[17]}. People who are exposed to insecticides show evidence of DNA damage via lipid peroxidation reactions and significantly increased MDA levels\textsuperscript{[18]}. In this study, the blood MDA levels in male and female participants were significantly decreased after Rang Chuet treatment. \textit{T. laurifolia} leaves contain phenolic compounds, caffeic acid, apigenin and flavonoids, which are rich in antioxidant activity\textsuperscript{[6]}. Interestingly, blood MDA levels in farmers using chemical pesticides have been found to be significantly increased when compared with farmers using organic pesticides\textsuperscript{[19]}. Our research team suggests initiating a campaign to reduce the use of chemical pesticides or provide information for individualised health intervention, in order to reduce health effects in farmers.

The results of plant identification from the fresh leaves of Rang Chuet in the local area and the powder of Rang Chuet capsules using the NucleoSpin® Plant II kit found that the nucleotide sequences on the \textit{trnL-trnF} intergenic spacer regions showed 99\% homology with \textit{Thunbergia coccinea} (GenBank® accession number KT075030.1) and \textit{Thunbergia grandiflora} (GenBank® accession number KT075034.1). In addition, Wongakson et al., 2015 elevated the phytogenic relationships within the \textit{Thunbergia spp.} using sequences from the chloroplast \textit{psbA-trnH}, \textit{trnL-trnF} intergenic spacers and second nuclear internal transcribed spacer (ITS2)\textsuperscript{[20]}. Therefore, the utilisation of fresh leaves of \textit{T. laurifolia} yield a comparable result as a capsule supplementation processed from the plant.

In conclusion, Rang Chuet supplements significantly reduce blood MDA levels and did not affect the daily life of participants. Prospective studies are needed on a larger sample of farm workers to improve the statistical power and measure more biomarkers in the antioxidant system.

**Conflict of Interest:** The authors declare no conflicts of interest.

**Funding:** The authors are grateful for the research funding provided by the Faculty of Allied Health Sciences, University of Phayao, Phayao, Thailand.

**Acknowledgements:** The Faculty of Allied Health Sciences, University of Phayao, Phayao, Thailand supported this study. We are grateful to the Department of Medical Technology, Faculty of Allied Health Sciences, University of Phayao and the Department of Chemistry, Faculty of Science, Chiang Mai University for providing the equipment and facilities.

**Ethical approval:** This research design was approved by the Ethics Committee of the University of Phayao.

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Dental Panoramic Radiographs as Early Signs of Osteoporosis in Pre and Post Menopausal Women – An Observational Study

P.E. Chandra Mouli1, Madhavan Prasad2, T. Gopalakrishnan3

1Professor and Head, Department of Oral Medicine and Radiology, 2Reader and Incharge, Department of Pedodontics, 3Reader, Department of Oral Pathology And Microbiology, Sri Venkateswara Dental College and Hospital, Thalambur, Chennai. Tamil Nadu

Abstract

Osteoporosis is defined as “a skeletal disorder characterized by low bone mass and micro architectural deterioration of bone tissue leading to enhanced bone fragility, with consequent increase in fracture risk”. An osteoporotic fracture is an outcome of trauma to bone of compromised strength, commonly first occurring in the vertebral bodies and distal radius, both sites composed predominantly of medullary (trabecular) bone.

Osteoporosis is an extremely common disease affecting most women during their lifetime. Osteoporosis reduces bone density, affecting the bone mass and strength without altering the basic chemical composition. Declining estrogen levels during and after menopause lead to increase bone resorption and increased urinary excretion of calcium. Thus, estrogen deficiency plays a primary role in osteoporosis, accounting for up to one half of the bone lost during a women’s lifetime.

Oral signs of osteoporosis might be manifested by excessive alveolar ridge resorption, tooth loss, chronic destructive periodontal disease, referred maxillary sinus pain, or fracture.

Radiographic evaluation of post-menopausal women and patients with advanced renal failure demonstrated the loss of cortical bone at the mandible.

The purpose of the study is to evaluate relationship between various oral signs the probability of this relationship excising in certain patients and to access cortical bone thickness measurements using panoramic radiographs.

Keywords Osteoporosis · Panoramic radiography · Bone density · Fragility fracture · Mandible

Introduction

In human beings, the loss of bone mass with increasing age is a universally observed phenomenon. Human bones decrease in density and increase in porosity beginning at about third decade of life. Osteoporosis is a term used to describe a significant age-related deficiency in the bone mass with a potential or structural failure.

Osteoporosis is defined as “a deficiency of bone tissue, per unit volume of bone”1. Osteoporosis is a generalized disease in bone mass, is a public health problem of middle aged and elderly women2. Although it may effect as a result as many different metabolic bone disorders, the disease dramatically accelerates after the age of menopause and in women whose ovaries have been removed. Therefore, evaluation or dental radiographs for osseous changes might be useful measures to screen for osteoporosis. Osteoporosis reduces bone density, affecting the bone mass and strength without altering the basic chemical composition. Declining estrogen levels during and after menopause lead to increase bone resorption and increased urinary excretion of calcium.

Corresponding Author:
Dr. T. Gopalakrishnan
Reader, Department of Oral Pathology and Microbiology, Sri Venkateswara Dental College and Hospital, Thalambur, Chennai. Tamil Nadu
Mob- 9940570533
Thus, estrogen deficiency plays a primary role in osteoporosis, accounting for up to one half of the bone lost during a women’s lifetime.

Oral signs of osteoporosis might be manifested by excessive alveolar ridge resorption, tooth loss, chronic destructive periodontal disease, referred maxillary sinus pain, or fracture.

Postmenopausal osteoporosis is usually evaluated in the region of the lumbar and thoracic spine. Thoracic spine fractures are reported to comprise 50% to 70% of all spinal fractures. Thoracic spine fracture can be related to certain oral signs; the possibility of latent osteoporosis might prompt dental practitioners to refer these for medical evaluation. The fracture risk assessment tool FRAX was developed by the World Health Organization (WHO) to evaluate fracture risk in men and women.

Radiographic evaluation of post-menopausal women and patients with advanced renal failure demonstrated the loss of cortical bone at the mandible. The purpose of the study is to evaluate relationship between various oral signs and to access cortical bone thickness measurements using panoramic radiographs.

Skeletal mass in old age is proportional to the skeletal mass at maturity, indicating that infant and childhood calcium intake may play an important role in the occurrence and severity of the disease in later years. Measurement of the mandibular inferior cortical width is made bilaterally on panoramic radiographs at a site below the mental foramen.

Dentists may be able to refer postmenopausal women with suspected spinal osteoporosis for bone densitometry on the basis of dental panoramic radiographs with diagnostic performance similar to that of osteoporosis screening tools based on questionnaires.

Aims and Objectives

Assessment of osteoporosis in mandible in pre and postmenopausal women with history of estrogen use, hysterectomy, oophorectomy and without history of estrogenic use, hysterectomy, oophorectomy.

Materials and Methods

This is a cross sectional hospital-based study conducted in the outpatient Department of Oral Medicine and Radiology, which was designed to assess Osteoporosis in mandible in pre and postmenopausal women with history of estrogen use, hysterectomy, oophorectomy and without history of estrogen use, hysterectomy, oophorectomy.

Includes premenopausal and postmenopausal women patients reporting to Dental Hospital, seeking dental treatment. The study consists of 105 patients and is divided into 3 groups. Group A consists of 35 post-menopausal women with history of hysterectomy, oophorectomy and estrogen use. Group B consists of 35 post-menopausal women without history of hysterectomy, oophorectomy and estrogen use. Group C is the control group and consists of 35 pre-menopausal women.

Measurement of Mandibular Cortical Width:

Measurement of mandibular cortical width was made bilaterally on the radiographs at the site of the mental foramen according to the study (Fig.1). We drew a line parallel to the long axis of the mandible and tangential to the inferior border of the mandible and constructed a line perpendicular to this tangent intersecting the inferior border of mental foramen, along which the mandibular cortical width was measured with a calliper. The mean cortical width on both sides of the mandible was used in this study.

Usefulness of the MCW in screening for osteoporosis

In osteoporotic patients, bone resorption occurs chiefly in the Haversian and Volkmann canals, which are the nutrient canals of the cortical bone. The cortical bone becomes thinner when adjacent canals finally conglutinate. This phenomenon is clearly detected in the mandibular inferior cortex on panoramic radiographs. The mandibular angle and the area below the mental foramen are reported to be useful sites for measuring...
the inferior cortical width of the mandible. However, the MCW is considered to be a simpler and more useful indicator given the magnification of panoramic radiographs, the reproducibility of the measurement, and the attachment of the masseter and medial pterygoid muscles which may influence the results.\(^7\)

A detailed history of the patient and thorough clinical examination was done and findings recorded in the enclosed proforma. Selected patients’ blood was estimated for Serum Calcium, Serum Phosphorus and Serum Alkaline Phosphatase. Chi square test, P value, and ANOVA analysis were made for the statistical purpose.

**Results**

105 female subjects were included in the study. **Group A** consists of 35 post-menopausal women with history of hysterectomy, oopherectomy and estrogen use. **Group B** consists of 35 post-menopausal women without history of hysterectomy, oopherectomy and estrogen use. **Group C** is the control group and consists of 35 pre-menopausal women. (TABLE 2, TABLE 3, GRAPH 1 AND GRAPH 2)

![Fig 1. Measurement of Mandibular Cortical Width](image)

Mandibular cortical width (between the white arrows) is measured bilaterally on the panoramic radiograph at a site below the mental foramen.

<table>
<thead>
<tr>
<th>Time since Menopause</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of subjects</td>
<td>%</td>
<td>No of subjects</td>
</tr>
<tr>
<td>0-10</td>
<td>22</td>
<td>62.86</td>
<td>28</td>
</tr>
<tr>
<td>11-20</td>
<td>7</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>21-30</td>
<td>6</td>
<td>17.14</td>
<td>-</td>
</tr>
<tr>
<td>31 &amp; above</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(\chi^2 = 6.22\)  \(p\) value = 0.03
Shows distribution of subjects according to Time since Menopause.

Of the 35 subjects in Group A, 22 (62.86%) subjects were between 0-10 yrs; 7 (20%) subjects were between 11-20 yrs and 6 (17.14%) were between 21-30 yrs.

Of the 35 subjects in Group B, 28 (80%) subjects were between 0-10 yrs and 7 (20%) subjects were between 11-20 yrs.

The distribution of subjects according to Time since Menopause shows significance with p value ≤ 0.03.

### Table-2 - Distribution of subjects according to Age Group

<table>
<thead>
<tr>
<th>Age(yrs)</th>
<th>Group A</th>
<th></th>
<th>Age(yrs)</th>
<th>Group B</th>
<th></th>
<th>Age(yrs)</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of subjects</td>
<td>%</td>
<td></td>
<td>No of subjects</td>
<td>%</td>
<td></td>
<td>No of subjects</td>
</tr>
<tr>
<td>40-50</td>
<td>8</td>
<td>22.90</td>
<td>40-50</td>
<td>9</td>
<td>25.71</td>
<td>10-20</td>
<td>8</td>
</tr>
<tr>
<td>51-60</td>
<td>13</td>
<td>37.14</td>
<td>51-60</td>
<td>20</td>
<td>57.14</td>
<td>21-30</td>
<td>22</td>
</tr>
<tr>
<td>61-70</td>
<td>10</td>
<td>28.57</td>
<td>61-70</td>
<td>5</td>
<td>14.28</td>
<td>&gt;30</td>
<td>5</td>
</tr>
<tr>
<td>71-80</td>
<td>2</td>
<td>5.714</td>
<td>71-80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>&gt;80</td>
<td>2</td>
<td>5.714</td>
<td>&gt;80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$$\chi^2 = 111.72 \quad p \text{ value } = 0.001 \quad F = 278.1$$

GRAPH 1
Table -3: Distribution of subjects according to Cortical Width (Mean)

<table>
<thead>
<tr>
<th>Cortical Width(mm)</th>
<th>Group A</th>
<th></th>
<th>Group B</th>
<th></th>
<th>Group C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of subjects</td>
<td>%</td>
<td>No of subjects</td>
<td>%</td>
<td>No of subjects</td>
<td>%</td>
</tr>
<tr>
<td>1-2</td>
<td>1</td>
<td>2.86</td>
<td>1</td>
<td>2.86</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.1-3</td>
<td>6</td>
<td>17.14</td>
<td>4</td>
<td>11.43</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.1-4</td>
<td>15</td>
<td>42.86</td>
<td>11</td>
<td>31.43</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.1-5</td>
<td>13</td>
<td>37.14</td>
<td>15</td>
<td>42.86</td>
<td>3</td>
<td>8.57</td>
</tr>
<tr>
<td>5.1-6</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>11.43</td>
<td>21</td>
<td>60</td>
</tr>
<tr>
<td>&gt;6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>31.43</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 79.09 \quad p \text{ value} = 0.001 \quad F = 75.7 \]
Discussion

The number of teeth missing increases as age increases in both Group A and Group B and there was no significant change in Group C.

Shape of cortex shows significance in both Group A and Group B, cortex was severely eroded between 51-60 years in Group A and between 61-70 years in Group B.

Cortical width was reduced below the normal(3mm) in Group A; but not in Group B although p value was significant in both groups. This is due to inadequate number of subjects in Group B and unavailability of subjects between 71-80 and above 80 years.

The number of teeth missing, Shape of cortex, Cortical width are useful parameters for assessment of osteoporosis. This coincides with the previous studies by Harry V. Daniel⁸

Cortical erosion is severe with subjects who are underweight in group A.

The number of missing teeth was significant in group A and not significant in group B.

Cortical shape showed 63% erosion in group B and only 50% in group A. This may be due to unavailability of subjects in group A.

Cortical width was significant in group A and not significant in group B. This is due to unavailability of patients in group B who are underweight.

Serum calcium, phosphorus and alkaline phosphatase levels were on the lower side of normal limits and were not useful in our study. This may be due to limited sample size and laboratory standards. John L. Stock et al have also proved this in his study⁹.

In our study, The bone density and the mineral levels are normal for pre menopause women and for post menopause women both the serum mineral levels and the bone density was less than the normal, because of which the general dental health of the post menopause women will not be good¹⁰,¹¹,¹²,¹³.

Cortical width was clearly significant in group A and is not significant in group B. This may be due to unavailability of subjects with time since menopause between 21-30 years in group B¹⁴,¹⁵,¹⁶.

Conflict of Interest: Conflict of Interest Declared None.

Ethical Clearance- Taken from ethical committee of RAGAS DENTAL COLLEGE AND HOSPITAL CHENNAI.

Source of Funding- Self

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Administrative Factors and Organization Climate Affecting the Performance of Health Personnel at the Emergency Department in Community Hospital

Padcharin Phomdonko¹, Prachak Bouphan², Nuttapol Yotha³, Surachai Phimha⁴

¹Master of Public Health Student in Public Health Administration, Faculty of Public Health, KhonKaen University, Thailand, ²Associate Professor in Department of Public Health Administration, Health Promotion, and Nutrition, Faculty of Public Health, KhonKaen University, Thailand, ³Muang Phon Sub-District Health Promoting Hospitals, KhonKaen Province, Thailand, ⁴Lecturer of Department of Public Health Administration, Health Promotion, and Nutrition, Faculty of Public Health, Khon Kaen University, Thailand

Abstract

This is a cross-sectional analytical study aimed to identify administrative factors and organizational climate affecting the performance of health personnel at the emergency department in community hospital Roi-Et province, Thailand. The study samples selected randomly from Emergency Department were 137 health personnel. Stratified random sampling technique was applied to recruit the respondents from the total of 229 health personnel. Similarly, 12 key informants were selected for the qualitative study. In-Depth interview guidelines was applied to conduct qualitative study. The content validity of the questionnaire was evaluated by three experts yielding an IOC value more than 0.50 in all questions. The Cronbach’s alpha coefficient of the questionnaire was 0.98. Data were collected between 23rd December 2020 to 12th January 2021. Data analysis was performed by descriptive statistics and inferential statistics at a significant level at 0.05.

The study results showed that the administrative factors such as money, organizational climate such as dimension of warmth, a dimension of responsibility and dimension of identity were affecting the performance of health personnel (49.6 % (R²=0.496, p-value<0.001). In conclusion, four factors are affecting the performance of health personnel. Hence those factors should be addressed, and administration should create the climate which can address the budget, working objective and goal for the development of the organization.

Keywords: Administrative factors, Organization Climate, Performance of Health Personnel, Emergency room

Introduction

The standard nursing service is provided to the accident-related victims and patients in emergency who are injured or suffering from critical illnesses whose physical and mental systems are affected. It is assessed by the severity of the illness, the decision to provide the first aid, and use medical equipment and pharmaceutical supplies to improve patients’ lives as well as monitoring the parameters of changes in patients after providing nursing services. Moreover, it can help patients in urgent situations safely, and reduce illnesses from complications. The standard of ambulance and emergency service consists of 8 standards: 1) Nursing Therapeutic in Emergency Medical Service2) Nursing Therapeutic in Emergency Department3) Continuing Care4) Health Promotion5) Protection of health conditions and risk prevention6) Providing information

Corresponding author:
Surachai Phimha
Lecturer, Department of Public Health Administration, Health Promotion, and Nutrition, Faculty of Public Health, KhonKaen University, Thailand.
E-mail: suraphi@kku.ac.th
and health knowledge7) Protection of patient rights and 8) Nursing Documentation [1]. In order to perform successfully for Health workers in the emergency room, they need to consider many factors to support the jobs; however, one of the most important factors is a management factor which affects the optimal use of resources and provides smooth work and achieve the objectives. It consists of 6 compositions which are 1) Man 2) Money 3) Material 4) Management 5) Time and 6) Technology [2]. Moreover, the atmosphere in the organization is considered to be important to the performance of the health workers in emergency accident organization. It also influences and inspires behavior, and able to perceive the organizational atmosphere both directly and indirectly. It has 9 dimensions which are 1) Structure 2) Responsibility 3) Warmth 4) Support 5) Reward 6) Conflict 7) Standard 8) Identity and 9) Risk [3].

Nowadays, there are 19 community hospitals in Roi Et province, Thailand and there are 229 public health professionals are working under the nursing department working in the accident-emergency room at community hospitals in Roi Et. The Emergency Accident Unit is one of the most high-risk units of the hospital as well as having lots of workload. In the past studies, the Nursing Therapeutic in Emergency Medical Service standard found that some personnel with less than three years of working did not attend the Resuscitation Competency Development Program in order to have the skills and confidence in helping patients and injured patients properly. For the Nursing Therapeutic in Emergency Department still faces some problems with insufficient manpower in some emergency departments and inconsistent with the calculation of workload Full Time Equivalent (FTE) [4-5]. From the mentioned problem, the researcher is interested in the management and atmosphere factor in the organization which affect the public health workers in the emergency accident unit at community hospitals in Roi Ed in order to ensure safety and reduce risk for the personnel. The result of this research will be used as a guideline for planning the development of service quality to reach the needs of the people in order to create confidence and satisfaction in the service sustainably.

**Research Objective**

The aim of the study was to investigate administrative factors and organization climate affecting the performance of health personnel at the emergency department in community hospital Roi-Et province.

**Research Methodology**

This study was an analytical Cross-sectional Study.

**Population and sample size**

The study population was 229 health personnel at the emergency department in community hospital Roi-Et Province [4-5]. Calculating the population size for multiple linear regression analysis to test the hypothesis by using the formula of Cohen [6] as follow

\[
N = \frac{\lambda(1-R_{AB}^2)}{R_{AB}^2-R_{AB}^2} + \frac{w}{\lambda} \\
\text{when} \quad \lambda = \lambda_L \quad \left(1/v_L - 1/v_U\right) \quad (\lambda_L - \lambda_U) \\
\text{Formula} \quad ..........(1)
\]

A total of 137 study respondents were identified.

**Sampling method**

Stratified random sampling method was used by dividing the population into groups of 19 community hospitals and comparing the sample sizes to the population of registered nurses and Advanced Emergency Medical Technician who were providing the service at
the emergency department in community hospital Roi-Et Province. Finally, a total of 137 respondents were recruited.

**In-depth Interview random sampling**

A group of people who provided information in an in-depth interview to verify quantitative data were chosen from health personnel at the emergency department in community hospital Roi-Et Province. 12 Key Informants were selected.

**The research tools**

Two types of data collection tools were used:

**Set one** was a questionnaire that consists of 5 parts:  **Part one** was the question of personal characteristics consisting seven questions.  **Part two** was the question of administrative factors which consists of 30 questions.  **Part three** was the question of organization climate with 45 questions.  **Part four** was the question of performance of health personnel at the emergency department that consists of 41 questions. Use of rating scale five levels in part two, three and four.

The scoring criteria for comments are as follows: The highest level, high level, intermediate level, low level, and lowest level are five point, four point, three point, two point, and one point, respectively. Part five is a question about problems, and suggestions about performance of health personnel at the emergency department in community hospital.

**Set two** was an In-Depth Interview Guideline based on the information from the quantitative research. The least average questions are used for in-depth interviews to confirm, support and explain the quantitative data. The researcher defined the issue in three parts:  **Part one** was an in-depth interview guide about administrative factors.  **Part two** was an in-depth interview guide about Organization climate.  **Part three** was an in-depth interview guide about performance of health personnel at the emergency department in community hospital in Roi Et province.

**Quality of research tools**

Both sets of tools were approved by three experts in terms of content validity then Item Objective Congruence: IOC was analyzed. It was found that all of items had IOC at the level of 0.67-1.00. The questionnaires were pretested in 30 health personnel at the emergency department in community hospital in Khon Kean Province, Thailand. The Cronbach’s Alpha Coefficient was 0.95 for administrative factors, 0.97 for the organizational climate, 0.97 for the performance at the emergency department and the overall questionnaire reliability was 0.98 respectively.

**Data Analysis**

The researcher analyzed the data by using descriptive statistics; frequency, percentage, mean, standard deviation, median, minimum and maximum value, and inferential statistic; Pearson’s correlation coefficient, and stepwise multiple regression analysis.

**Research Results**

1. Personal characteristics and the performance of health personnel at the emergency department in community hospital

Personal Characteristics and the performance of health personnel at the emergency department in community hospital Roi-Et province Thailand were as follows: Most of the sample were female, 120 people (87.6%), aged between 21-30 years, 65 people (47.4%) with an average age of 34.36 years (SD = 9.94 years, Min = 22 years old, Max = 58 years old). Most of them graduated with a bachelor’s degree or equivalent, 84 people (61.3%), married 68 people (49.6%), and most of them were registered nurses, 106 people (77.4%). There were 92 respondents working for 10 years or less (67.1%) with a median of 7 years (Min = 1 year, Max = 35 years), and 105 (76.6%) emergency accident workers had received on-the-job training.

2. Administrative factors and organizational climate affecting the performance of health personnel at the emergency department in community hospital
The analysis results showed that independent variable that affects the performance of health personnel at the emergency department in community hospital Roi-Et Province Thailand. That is selected into the equation is the variables that were statistically significant at level 0.05 and independent variables that were not selected into the equation were variables that were statistically significant greater than 0.05 with variables selected into the sequencing equation as follows: organization climate: warmth (p-value <0.001), organization climate: responsibility (p-value = 0.002), organization climate: identity (p-value = 0.005) and administrative factors: money (p-value = 0.048). Therefore, four independent variables can predict the performance of health personnel at the emergency department in community hospital at 49.6% (Table 1).

Study results: Multiple Linear Regression equation which is a prediction equation of the performance of health personnel at the emergency department in community hospital as follows:

\[
Y = 1.559 + (0.286) \text{(Organization climate: warmth)} + (0.257) \text{(Organization climate: responsibility)} + (0.229) \text{(Organization climate: identity)} - (0.123) \text{(Administrative factors: money)}
\]

Table 1. Statistics about Stepwise Multiple Regression Analysis the performance of health personnel at the emergency department in community

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>Beta</th>
<th>T</th>
<th>P -value</th>
<th>R</th>
<th>R²</th>
<th>R² adj</th>
<th>R² change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Organizational climate:</td>
<td>0.286</td>
<td>0.316</td>
<td>3.343</td>
<td>&lt;0.001</td>
<td>0.633</td>
<td>0.401</td>
<td>0.397</td>
<td>0.401</td>
</tr>
<tr>
<td>Warmth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Organizational climate:</td>
<td>0.257</td>
<td>0.309</td>
<td>3.124</td>
<td>0.002</td>
<td>0.677</td>
<td>0.458</td>
<td>0.450</td>
<td>0.057</td>
</tr>
<tr>
<td>Responsibility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Organizational climate:</td>
<td>0.229</td>
<td>0.252</td>
<td>2.881</td>
<td>0.005</td>
<td>0.694</td>
<td>0.481</td>
<td>0.469</td>
<td>0.023</td>
</tr>
<tr>
<td>Identity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Administrative factors:</td>
<td>-0.123</td>
<td>-0.150</td>
<td>-1.999</td>
<td>0.048</td>
<td>0.705</td>
<td>0.496</td>
<td>0.481</td>
<td>0.015</td>
</tr>
<tr>
<td>Money</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Constant 1.559, F = 32.531, p - value < 0.001, R = 0.705, R² = 0.496, R² adj = 0.481

**Conclusion and Discussion**

The Stepwise Multiple Regression Analysis found that independent variable affecting the performance of health personnel at the emergency department in community hospital were organization climate; Warmth, Responsibility, Identity, and administrative factors; Money can predict 49.6% for the performance of health personnel at the emergency department in community hospital.
The organization climate in warm dimension of emergency department of community hospital can reduce stress and anxiety which are definitely important to the workers. The warmth needs to be able to support the energy and the relationship to build the great rapport, and it will affect the better organization climate. This result of the study is related to the previous study of the performance of health personnel at the emergency department\(^{[10-11]}\). The organization climate in responsibility dimension is the performance of health personnel at the emergency department in community hospital. It can be responsible with working freely, responsible to themselves, and make a strong decision to solve problems which can create learning on their own working. This result of the study is related to the previous study of the performance of health personnel at the emergency department\(^{[10-14]}\). The organization climate in identity dimension of health personnel at the emergency department is important to the part of supporting energy and relationship in the workers and it importantly emphasizes being a part of the organization. Therefore, it will lead to the rapport and influence on efficiency perform. This result of the study is consistent with the previous study of the performance of health personnel at the emergency department\(^{[10,13,15-17]}\). However, the situation of emergency department and individual of Health Personnel which is different in each hospital, the result of this study inconsistent with the results of the study found that the organization climate; warmth, and identity can’t predict the performance of health personnel\(^{[12]}\).

The administrative factors in terms of money play an important role to manage manpower, materials and necessary equipments which are the prerequisite for the creative environment of the management. The budget is the crucial of factor for the performance to reach the objectives\(^{[2]}\). However, the result of the study found that the coefficient regresses in this factor has a negative value, and it can explain that the money budget affects the performance at the emergency accident unit inversely. It may be, because of the administrative factor’s money is under the control of hospital accounting which have to pass many checking processes. This result is related to the previous study of the performance of health personnel at the emergency department\(^{[17]}\). However, the budgeting support depends on the needs and policies of organization, the result of this study inconsistent with the results of the study found that the administrative factors in money can’t predict the performance of health personnel at the emergency department in community hospital\(^{[18-19]}\).

The in-depth interviews found that the performance of health personnel at the emergency department, it found that the emergency accident unit performance is one of the standards which assess the public health workers’ performance. Besides competencies in each profession to assess the standard of the emergency accident unit, moreover the studied hospital has been set the individual criteria for assessing which is able to assess the individual performance. Therefore, the patient will reach the standard and safety service due to the fact that the performance of the staff is more effective.

In conclusion, the organization climate; warmth, responsibility, identity, and administrative factors; money affect the performance of health personnel at the emergency department in community hospital, so they should be supported and developed the organization climate with mostly considering the budget, work objective and goal, moreover should encouraging by giving rewards and certifying the performance’s ability including having the criteria for clear judgement to encourage the motivation and confidence for working.

Acknowledgements: The author would like to thank all at the emergency department in community hospital and health personnel who participated in this study.

Ethical Clearance: This study was approved by the Khon Kaen University Ethics Committee for Human Research on December 15, 2020 number HE 632258.

Conflict of Interest: No conflicts of interest declare.

Source of Funding: Self-funding
References


Awareness and Attitude of Forensic Odontology among Undergraduate Dental Students in Kabul University of Medical Sciences, Afghanistan

Palwasha Seraj1, Mohammad Hassan Hamrah2, Farhat Homayoun3, Ali Maisam4, ElahaSomaya Ghafary5, Sepideh Hosseini2 Maryam Khosrozadeh2

1Department of Forensic Medicine, School of Dentistry, Kabul Medical University Sciences, Kabul, Afghanistan, 2Department of Pediatric Dentistry, Tehran University of Medical Sciences, Tehran, Iran, 3School of Dentistry, Kunduz University, Kunduz, Afghanistan, 4Department of Endodontic, School of Dentistry, Kabul Medical University Sciences, Kabul, Afghanistan, 5Department of Periodontology, School of Dentistry, Kabul Medical University Sciences, Kabul, Afghanistan

Abstract

Aims and Objectives: The aim of the study is to assess the knowledge, attitude, and practice of forensic odontology among undergraduate dental students at Kabul university of medical science, Kabul, Afghanistan after adding this subject in their curriculum

Material and Methods: This cross-sectional study is conducted among 150 undergraduate dental students of Kabul University of medical sciences aged 18 and 23 years during the period from December 2020 to February 2021. A questionnaire of Abdul et al. (2019) study was used; the questionnaire consisted of 19 questions that assessed the knowledge, attitudes, and practice of the participants. The questionnaire is divided into three parts: knowledge, attitude and practice. The collected data were analyzed using SPSS-24 software, chi-square test.

Results: Ninety percent of the participants were aware of the branch of science called forensic dentistry. All the included participants welcomed the idea of adding a module on forensic odontology to the current undergraduate curriculum. Almost 88.7% of the participants were aware that dental record help for identification of deceased person’s age however, The most of undergraduate dental students (90.7%), claim that they have lack of the knowledge related forensic odontology. Most of participants were willing to maintain dental record in their future clinics.

Conclusion: Our study showed that the knowledge, attitude, and practice in undergraduate dental students are better since it’s added into curriculum of Kabul University of medical science. However, there are no workshops, seminars, and continuing dental education programs for dental students. There is a need for further exposure and formal training to bring awareness among all health-care providers.

Keywords: Knowledge and Attitude, Forensic odontology, Students

Introduction

Forensic odontology can be defined as a branch of dentistry by the Federation Dentaire International (FDI) which in interest of justice, deals with the proper manipulation and examination of dental evidence with the proper evaluation, and presentation of dental findings(1).

Forensic odontology is a challenging and attractive branch of forensic science that includes the use of dental science in identifying deceased people by comparing
pre- and post-mortem records. Forensic odontology focuses largely on the teeth and involves assisting in the identification of deceased individuals and criminals and the specialists should have basic skills and knowledge about forensic odontology and be aware of how to manage dental records, dental DNA analysis, radiography, dental morphology and anatomy.

Teeth are resilient to conditions such as high temperatures, immersion and decomposition and are classified as one of the primary identifiers by Interpol. Moreover, teeth are the cheapest, quickest, and easiest methods of human identification when compared to fingerprinting and DNA.

Dental records play an important role in cases where corpses have been severely damaged, impossible, or difficult to identify. This is attributed to the fact that dental tissues and dental restorations are resistant in nature, which make them the most reliable source of DNA, that will help for any individual identifying case. Despite the importance of this field in criminal justice, the literature shows that forensic odontology has been underestimated in many countries. Furthermore, there is no universal acceptance path for forensic odontology education and requirement in each country.

The aim of this study was conducted to assess undergraduate dental students’ knowledge, attitude, and practice of Kabul University of medical science in Kabul, Afghanistan after adding this subject in their curriculum.

**Materials and Methods**

This cross-sectional study is conducted among 150 undergraduate dental students of Kabul University of medical sciences aged 18 and 23 years during the period from December 2020 to February 2021.

A questionnaire of Abdul et al. (2019) study was used; the questionnaire consisted of 19 questions that assessed the knowledge, attitudes and practice of the participants. The questionnaire written in English and Persian language distributed among 150 undergraduate dental students. All included participating was willing to participate in the research process. Males 60 (40%) and females 90 (60%) were included. Information was collected from demographic data of participant following: age, gender, education level. The questionnaire is divided into three parts: knowledge, attitude and practice. The collected data were analyzed using SPSS-24 software, chi-square test.

**Results**

A total of 150 undergraduate dental students, Males 60 (40%) and females 90 (60%) aged between 18 and 23 years were included. The questionnaire was self-administered by a convenience. According to descriptive findings, 58% of respondents were over 21 years old and 42% were under 21 years old.

<table>
<thead>
<tr>
<th>Table 1. Frequency of age and sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>&lt;21</td>
</tr>
<tr>
<td>&gt;21</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
</tbody>
</table>
Question 1-9 were knowledge-based, questions on forensic odontology. (Table 2)

Q1: In our study, 90% of the participants were aware of the branch of science called forensic dentistry and about 10% of the participants were unaware of it.

Q2: 69% of undergraduate dental students were aware that teeth serve as a source of DNA.

Q3: Ninety-six percent of the participants were aware that forensic dentistry helps to investigate criminals and dead persons.

Q4: 76.6% of the participants were aware of age estimation in children and adults by an eruption pattern of teeth. The remaining participants answered that histological and biochemical methods were used to identify the dental age.

Q5: Almost 88.7% of the participants were aware that dental record help for identification of deceased person’s age. However, 10.7% of the participant were thinking fingerprint can help in this situation.

Q6: Nearly, 46.7% of the participants were not aware they mark don’t know option, only 26.7% of participants were able to mark the correct answer.

Q7: Most of the study participants (73.3%) were aware of teeth bite mark pattern significance.

Q8: Proportion of this study participants marks the source of their knowledge book and internet respectively.

Q9: The most of undergraduate dental students (90.7%), claim that they have lack of the knowledge related forensic odontology.

Table 2. Question 1-9 knowledge-based, on forensic odontology

<table>
<thead>
<tr>
<th>Question</th>
<th>Components</th>
<th>Frequency</th>
<th>% percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Do you know about forensic odontology as a branch in dentistry?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>135</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>2. Can teeth serve as source of DNA?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>104</td>
<td>69.3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Don’t know</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td>3. Is forensic odontology useful in identifying criminals and the dead people?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>144</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>5</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Don’t know</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>4. How do you identify the dental age in children and adults?</td>
<td>Eruption patterns and calcification</td>
<td>115</td>
<td>76.6</td>
</tr>
<tr>
<td></td>
<td>Histological methods</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Biochemical methods</td>
<td>10</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Don’t know</td>
<td>1</td>
<td>0.7</td>
</tr>
</tbody>
</table>
Cont... Table 2. Question 1-9 knowledge-based, on forensic odontology

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. How will you identify a deceased person’s age and gender in mass disasters like fire, stampede and accidents?</td>
<td>110</td>
<td>40</td>
</tr>
<tr>
<td>Dental records</td>
<td>133</td>
<td>88.7</td>
</tr>
<tr>
<td>Fingerprints</td>
<td>16</td>
<td>10.7</td>
</tr>
<tr>
<td>Don’t know</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lipology</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Cheiloscopy</td>
<td>40</td>
<td>26.7</td>
</tr>
<tr>
<td>Dermatoglyphics</td>
<td>10</td>
<td>6.6</td>
</tr>
<tr>
<td>Don’t know</td>
<td>70</td>
<td>46.7</td>
</tr>
<tr>
<td>7. Are you aware of the significance of bite mark pattern of teeth?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>110</td>
<td>73.3</td>
</tr>
<tr>
<td>No</td>
<td>40</td>
<td>26.7</td>
</tr>
<tr>
<td>8. What is the source of your knowledge about forensic dentistry?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Books</td>
<td>59</td>
<td>39.3</td>
</tr>
<tr>
<td>Internet</td>
<td>50</td>
<td>33.3</td>
</tr>
<tr>
<td>I don’t have knowledge</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Scientific articles/journals</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>Workshops</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>Seminars</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Undergraduate lectures</td>
<td>34</td>
<td>22.7</td>
</tr>
<tr>
<td>9. Do you think your knowledge and awareness about Forensic odontology is enough?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>No</td>
<td>136</td>
<td>90.7</td>
</tr>
<tr>
<td>Don’t Know</td>
<td>8</td>
<td>5.3</td>
</tr>
</tbody>
</table>
Questions 10–13 were attitude-based questions on forensic odontology (Table 3)

Q10: 63.3% of participant replied that they are willing take forensic dentistry as a diploma or postgraduate course if introduced. However, 36.7% study population did not show interest.

Q11: About 53.3% of students reported their interest to select forensic dentistry as a profession and 46.7% of student refused the question.

Q12: Nearly, eighty percent of this study participant showed interest to attend in workshops and seminars in forensic odontology but, 20 percent ignore it.

Q13: Almost 86.7% of participants were willing to maintain dental record in their future clinics.

Table 3. Questions 10–13 attitude-based on forensic odontology

<table>
<thead>
<tr>
<th>Question</th>
<th>Components</th>
<th>Frequency</th>
<th>% Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>10. Are you willing to take courses in forensic odontology, if introduced as a diploma or postgraduate course?</td>
<td>Yes</td>
<td>95</td>
<td>63.3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>55</td>
<td>36.7</td>
</tr>
<tr>
<td>11. Are you interested to join forensic odontology as a profession?</td>
<td>Yes</td>
<td>80</td>
<td>53.3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>70</td>
<td>46.7</td>
</tr>
<tr>
<td>12. Are you interested to participate in workshops and seminars in forensic odontology?</td>
<td>Yes</td>
<td>120</td>
<td>80.0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>30</td>
<td>20.0</td>
</tr>
<tr>
<td>13. Do you will maintain dental records in your clinic?</td>
<td>Yes</td>
<td>130</td>
<td>86.7</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>20</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Questions 14–19 were practice-based questions on forensic odontology (Table 4)

Q14: About 76.7% of participants were aware that child abuse can identify according all above option which includes physical injuries, scars, clothing, and behavioral changes.

Q15: Over 50% of participants were in favor of reporting to police, however less than 50% of participants were willing to report for their parents.

Q16: Almost 98.7% of participants had not attended in any formal training related to forensic odontology.

Q17: All of included participant agreed that, forensic odontology is as part of their curriculum.

Q18: About 73.3% of the participants agreed to have limited resources to study forensic dentistry in Afghanistan.

Q19: Only 46.7 percent of the participants were aware that the dentists have to testify as an expert witness in the court of law with forensic dental evidence and 53.3% of them were not aware of it.
Table 4. Questions 14-19 practice-based on forensic odontology

<table>
<thead>
<tr>
<th>Question</th>
<th>Components</th>
<th>Frequency</th>
<th>%frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>14. How will you identify physical/neglected/sexual/psychologically abused child patient?</td>
<td>Physical injuries</td>
<td>26</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td>Behavioral changes</td>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Clothing</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Any scars</td>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>All the above</td>
<td>115</td>
<td>76.7</td>
</tr>
<tr>
<td></td>
<td>Don’t know</td>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td>15. What action would you take, if you identify child abuse?</td>
<td>Inform police</td>
<td>90</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>Inform parents</td>
<td>60</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>Take no action</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16. Do you have any formal training related to forensic odontology?</td>
<td>Yes</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>148</td>
<td>98.7</td>
</tr>
<tr>
<td>17. Do you have forensic odontology as part of your curriculum or course outline?</td>
<td>Yes</td>
<td>150</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18. Do you think Afghanistan has very limited resources/equipment to study forensic science?</td>
<td>Yes</td>
<td>110</td>
<td>73.3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>40</td>
<td>26.7</td>
</tr>
<tr>
<td>19. Are you aware that you can testify as an expert witness in the court to present forensic dental evidence?</td>
<td>Yes</td>
<td>70</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>80</td>
<td>53.3</td>
</tr>
</tbody>
</table>

**Discussion**

This study was conducted to assess undergraduate dental students’ knowledge, attitude, and practice of Kabul University of medical science in Kabul, Afghanistan and gives an insight into the significance of this field in reducing the crime rate, illegal birth rates, illegal immigrants, and identification of individuals in mass disasters and pilgrimages in Afghanistan.

There are many studies carried out on forensic odontology in other countries, but no study was conducted in the Afghanistan among dental students. Therefore, this is the first study of forensic odontology in Afghanistan, which targets undergraduate dental students. However, the limitations of this study should
be considered when interpreting the findings; the sample utilized was drawn from one educational institution, therefore, it does not represent all dental students in Afghanistan.

Our study showed that the knowledge, attitude, and practice in undergraduate dental students are better since it is added into curriculum of Kabul University of medical sciences. These findings were consistent with the results of Al-Azri et al (10) in Australia showed that knowledge Students and dentists are well versed in forensic dentistry and many of them are aware of the field of work and the type of specialization in this field.

In the present study revealed that 90% of the participants were aware of the purpose of dentists in mass disaster, which, in contrast to other studies that showed only 31.6% and 59.4% awareness in this expression the adding forensic accounting course to their curriculum. However, the participants were reported their lack of seminars and workshops regards forensic odontology. Furthermore, the poor information regarding Lipology and Cheiloscopy could be due to a lack of awareness and insufficient knowledge about the aspects and use of these techniques.

Dental records are considered as an essential aid in identification of persons who are victims of an illegal act. The dental record might act as a future reference for the dentist when needed, but unfortunately, it is not constantly preserved for a forensic purpose (11). In the current study, most of dental students agreed that maintaining dental records is an essential component in human identification. This insufficient knowledge highlights the necessity for proper education and further training (12). Dentists should know not only the importance of preparing an accurate dental record but also the importance of preserving these records. Almost half of the students reported that their source of information in forensic odontology is from the Media. Media plays a definitive role in creating awareness among the public (13). The same holds true for the present study. Additionally, journals and seminars continue to remain as one of the most reliable and correct sources of this knowledge. However, the focus should be on the worrying finding that the majority of students in this study think that they do not have adequate knowledge or confidence in handling forensic dental cases. However, 100% of the students welcomed the idea of adding a module on forensic odontology to the current undergraduate curriculum.

This study provides a baseline for the upcoming studies in the Afghanistan. Findings from this study might be considered as a reference for endorsing the area of forensic odontology in the undergraduate training of dental students. Thus, the undergraduate program must be improved by including preclinical lectures on forensic odontology, followed by clinical training and a field slips to forensic departments. This suggests that These recommendations might have a positive impact on students’ knowledge and awareness concerning forensic odontology and Students should be aware of this specialty and be encouraged to participate in research and identification teams and consider it as their specialty. Further studies should be carried out at other dental colleges in Afghanistan.

**Conclusion**

Our study showed that the knowledge, attitude and practice in undergraduate dental students are better since it’s added into curriculum of Kabul University of medical science. However, there are no workshops, seminars, and continuing dental education programs for dental students. There is a need for further exposure and formal training to bring awareness among all health-care providers.

**Conflicts of Interest:** Non

Research involving Human Participants and/or Animals: The study protocols were approved by the Regional Committee for Medical Research Ethics.

**Funding:** No finding has been received for the conduct of this study and/or preparation of this manuscript.
References


Menstrual Blood Stem Cell Preservation

Parul Saini1, Bharti Sachdeva1

1Assistant Professor, Faculty of Nursing, SGT University, Gurgaon

Abstract

Unlike past days menstrual blood has been considered as the rich source of self-rejuvenating stem cells. These cells can multiple rapidly and helps in various cosmetic and regenerative surgeries. They can easily be used same as that of stem cells derived from umbilical cord and bone marrow.

Key Words: Menstrual Blood Banking, Stem cell, Regenerative capacity.

Introduction

Menstrual blood stem cell banking has been established as an important field of research and is progressing day by day. Earlier menstrual blood was considered as unsanitary waste of human body but latest researches have shown that menstrual blood is rich in stem cells and they have the ability to multiple and differentiate into any kind of cells.

Over the years menstrual stem cells are used in various regenerative and cosmetic surgeries.

Stem cells derived from menstrual blood are rich in mesenchymal stem cells. These cells have high survival rate after transplant as they are immunologically immature and can successfully contribute in cell cycle. Menstrual stem cells have same regenerative capacity as that of umbilical cord blood and bone marrow. They are considered as powerful tools for repairing as they can multiply rapidly and can differentiate into many other types of cells such as cardiac, neural, bone, fat and cartilage. They can easily be collected non-invasively without ethical concerns.

Sources of stem cells:

There are two main sources of stem cells are embryonic stem cell and adult stem cells. Embryonic stem cells can be collected from human embryos whereas adult stem cells can be derived from bone marrow, umbilical cord, menstrual blood cells, placental tissue, endometrium, peripheral blood stem cells.4 Adult cells found in bone marrow collected through invasive painful procedure.5

Collection and storage:

Menstrual blood cell collection procedure is hassle free and can be done easily at home. It can be collected using a medical-grade silicone cup instead of a sanitary pad or tampon. On the heaviest flow day, a silicone cup is inserted into the vagina for collecting 20 millilitres of blood. 15 ml to 20 ml of menstrual blood could easily yield between 10 million to 100 million Mesenchymal Stem cells. After that collected blood is poured into collection kit and sent to menstrual blood bank laboratory where it is processed, frozen and stored. It is completely painless and non-invasive procedure. The menstrual stem cells are cryogenically preserved in overwrapped vials which is closely monitored all the times for its safety and future usage.6

Advantages:

- Easily accessible, non-invasive and painless procedure.
- Highly potential to replicate into bone, skeletal, cartilage muscle cells
- More proliferative and multiply for longer duration without damaging DNA.
Can be administered easily through a standard IV line.

It can be preserved multiple times at any age.

**Uses:**

Menstrual stem cells are used in Heart Failure & Post Myocardial Infarction as they help to secrete certain angiogenic and trophic factors that assist in activation and regeneration of cardiac stem cells. They can also be used in treatment of stroke as these cells migrate to the infarct site and secrete neurotrophic factors. They are also beneficial in several other conditions like atherosclerosis, Diabetes, Rheumatoid arthritis, Parkinson’s disease and Alzheimer’s disease etc. As the chances of immune rejection are minimal as female patients are using their own cells hence it has broad scope in the near future.

**Menstrual blood stem cell banks:**

In India, stem cell banking services are mainly provide by Privately- held banks, Government- owned (Public) banks and Community stem cell bank. Life cell international Pvt. Ltd. is the first community stem cell bank in India. It has two high-tech labs located at Chennai (Tamil Nadu) and Gurugram (Haryana). It has dual storage facility.

**Conclusion:** Menstrual derived stem cells have shown regenerative properties in prevention and control of various disorders like stroke, diabetes, Myocardial infarction and many other neuro degenerative diseases. Diverse therapeutic research on mesenchymal stem cells would greatly contribute to treat variety of diseases. In summary, more clinical research on menstrual derived stem cells preservation and its clinical use can make these cells a preferred type of stem cells over umbilical cord and bone marrow stem cells.

**Discussion**

Stem cells are used to treat variety of diseases and disorders. Menstrual derived stem cells are considered to have tremendous therapeutic potential. Naoko Hida et al (2008) stated that menstrual derived mesenchymal stem cells have potential to restore impaired cardiac functioning. Ian A White et al (2016) have also stated Mesenchymal stem cells are capable of differentiation into cardiac cells. In another study Borlongan et al. concluded that menstrual derived stem cells successfully improved ischemic stroke among rats in vitro.

**Ethical Clearance** - Not applicable

**Source of Funding** - Self

**Conflict of Interest** - Nil

**References**

9. Hida N, Nishiyama N, Miyoshi S et al. Novel...
cardiac precursor-like cell from human menstrual blood derived mesenchymal cells. Stem Cells 2008; 26: 1695-1704.

Case Report

Psychoeducation on Adherence to Treatment of Schizophrenia Patient During Covid-19 Pandemic Era

Patria Yudha Putra1, Izzatul Fithriyah2

1General Practitioner, Psychiatry Resident, Faculty of Medicine, 2Psychiatrist, Assistant Professor, Department of Psychiatry, Faculty of Medicine, Universitas Airlangga/ Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

Abstract

Background: The outbreak of Covid-19 is really disrupting people’s thought and their ability to face the problem clearly and their mental health could be disturbed. They will go under their circumstance of fear, start to panic, and leads to poor decision making. This fear and anxiety could affect and lead to any negative behavioural changes of patients to the belief of being infected by avoiding going to the hospital as a source of contagion place.

Methods: Case study from an inward patient, adult woman that suffered from schizophrenia for more than 10 years and got injection of antipsychotic routinely once a month. During the early time of Covid-19 pandemic, the patient and her family are afraid of coming to the hospital to get her injection. After one month of not getting the injection, she started to relapse again and hospitalized for 10 days.

Results: The difficulty faced in handling this case is to provide an understanding of the patient and family about adherence on her continuity treatment. Therefore, by giving a psychoeducation using a telemedicine is considered to the most suitable way to handle this patient to gain more insight about her willingness back to get the monthly treatment during the pandemic situation.

Keywords: Schizophrenia, Covid-19, Adherence of Treatment, Psychoeducation, Telemedicine, Telepsychiatry

Introduction

Schizophrenia is part of a psychotic disorder which is mainly characterized by a loss of understanding of reality and a loss of insight. (1) consisting of a complex, heterogeneous group of cognitive and behavioural syndromes, which may originate from brain development disorder caused by genetic or environmental factors, or both. (2) Schizophrenia occurs all over the world, affects about 1 percent of the global population that lasts along their life. (3)

The incidence rate of Schizophrenia is about 1.5 per 10,000 people and the onset of people getting schizophrenia is around 18 to 25 years old for men and around 25-35 years old for women to be diagnosed with. (4)

According to Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5), there are specific criteria for schizophrenia over a period of 1 month such as delusions, hallucinations, unorganized speech, unorganized or catatonic behavior and...
negative symptoms. Patients with schizophrenia also experience cognitive impairment like patients have poor performance to control their cognitive functions. 

World Health Organization (WHO) declared that 2020 is a year of Coronavirus disease 2019 (Covid-19). The spreading of this virus contributed globally in just some weeks, the Covid-19 epidemic had spread to over 100 countries that matched to the definition of pandemic.

Coronavirus comes from a family of *Coronaviridae* virus and contains four structural proteins: spike (S), envelope (E), membrane (M) and nucleocapsid (N). The spike glycoprotein will infect the human body by attaching the membrane of the host cell and started to make symptoms like common cold or attack respiratory organ. This family of virus also responsible to the other global pandemic virus such as, Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). 

The mode of transmission of the Covid-19 virus is mainly spread via respiratory droplets. Basic reproduction number of the human transmission of Covid-19 is more contagious than other viruses such as SARS, MERS, H1N1 or Ebola and the value of reproduction number of Covid-19 reported is 3.6 compared to the other pandemic virus is 2.7. Thus, it proved that Covid-19 is highly contagious. This basic reproduction number could be reduced by encouraging people to restrict travel that are not essential and do the physical distancing. To prevent the spreading of Covid-19, people are asked to maintain their standard of hygiene. The public health authorities strongly advised people to wear a face mask wherever they go and do the physical distancing by not taking activities outside their houses if is not very necessary during the outbreak. Also, the government has done several non-pharmaceutical interventions by closing some places that people could gather in a large number, such as shopping centres, public areas, or restaurants. They also minimizing the office hours to minimize the spreading of the virus.

The outbreak of Covid-19 is really disrupting people’s thought and their ability to face the problem clearly and their mental health could be disturbed. Their reaction could be varies, some believe their life is in a danger situation, some believe it is just a theory conspiracy or they will believe the misleading rumours. With this outbreak happens, most people will react with confusion and anxiety. They will go under their circumstance of fear, start to panic and leads to poor decision making.

People with fear of Covid-19 are afraid to go outside their house, especially people with poor mental health and living in region with high reported Covid-19 cases. Their ability to think clearly is disturbed so they will become afraid to go to the hospital and this will ruin their long-term treatment. The long-term medication and hospitalization for patients with psychotic symptoms could decrease their motivation. They take medication pills or get an injection day by day passively in the hospital in a long time aimlessly could lower their adherence of medication, especially in the patients with schizophrenia, they usually have a lower insight than other patients. It is difficult to gain an insight of the patient especially with schizophrenia about their adherence of medication and stay continue with her treatment.

This review is aimed to give a management to the patient with mental health illness during Covid-19. They have to be provided with a very clear about information, situation, strategies to face the unstable living condition in order to fulfil their treatment on time. Psychoeducation is one of the methods to improve the adherence of the treatment given to psychotic patients.

**Case Report**

The patient was a 28-year-old female. Patient was angry 2 days before admitted to the hospital. The patient was late getting the treatment because the patient was forbidden by her husband to come to the hospital for fear of the spread of the Covid-19 virus which was increasingly widespread. The patient could tell that
she was taken to the hospital because suddenly she could not control her anger, the patient felt unable to control her emotions so that the patient was angry both to her husband as well as to her mother, felt restless and slammed things nearby. She also could not sleep well for 2 days before being admitted to the hospital, became restless and paced around the house. She was taken by his family to the emergency room Dr.Soetomo General Hospital Surabaya because her mother saw the patient getting angry and agitated. The patient also said that she was the Guardian of God to save the world that was currently sick and through her, the patient wanted to prevent human from going into hell. The patient also said that she had many similarities with famous Indonesian artists.

The patient said she had to go home soon because the patient has many thousands of employees who are waiting for her to return to work. She said that currently she was working on the making of protective gown project in the Covid-19 pandemic season and had donated a large set of them to many hospitals in East Java that made her contacted directly by the president and given a grand prize of Holy pilgrimage program in 2025. The President contacted patients through direct message on Instagram. She also heard a whispering voice that emerge clearly in the left ear which says that the voice of whisper said that the patient must die, the patient feels that the voice is the voice of the devil and she did not know before. Besides that, she also heard a different whispering voice that arises from her right ear. The voice from the right ear says that the patient is in good health, and the voice that the patient believed that the voice came from of a good genie and wants to save the patient. These sounds appeared continuously in turn at any time during the last 2 days before being admitted to the hospital and disappear when the patient closed her ears. Because these sounds often disturb the patient and make the patient confused and ultimately made the patient unable to control her emotions and the patient became angry.

The patient first experienced a mental disorder since she was in the high school. At that time, she often got bullying by her classmates. Since then, she often had become frightened, often seeing the shadow of the devil and hear of whispering genie. She had previously been treated at MenurMental Hospital for more than 10 times inpatient care because of her mental illness. She had been hospitalized in Psychiatric Ward Dr Soetomo General Hospital 2 times, in 2015 and 2017. Patients routinely got Fluphenazine 25 milligram injection via intramuscular since 2016 and routine given to her every 1 month. The patient never had any serious illness that brought her to the hospitalized. She never had any problems with any substance abuse or using any drugs before and never had smoking any cigarettes.

The patient was diagnosed with Schizophrenia Disorder because her symptoms met the diagnosis of criteria such as unorganized speech, grandiose and bizarre idea, delusion, and hallucinations that persists one month. During hospitalized, patient got several psychopharmacological, Trifluoperazine 2x5mg tablet orally every 12 hour, Depakote 2x250mg tabletorally every 12 hour and Lorazepam 1x1mg tablet orally every 24 hours. For 10 days treatment of psychopharmacology given orally, the patient wet showed better progress of her clinical status. She got injection of Fluphenazine 25 milligram intramuscular again after she was discharged from the hospital.

Discussion

Covid-19 is causing high morbidity and mortality and most of the governments has decided to lockdown almost all the nation’s aspect including economics and politics. People are not able to go to work anymore, many people loses their job and these situations make people started to worry and become fear of their future. (17,18)

The fear and anxiety of being infected to the Covid-19 has increased more since people are exposed to the digital media platform (news, broadcast message, some influencer’s thought, rumoured news), their perception of facing the pandemic and their obsessive thought of being less control from preventing the virus. Having heard about the increasing number of mortality
of Covid-19 made people confused to do their plan and
did not want to work and do activities like they usually
did.(19,20) This fear and anxiety could affect and lead
to any negative behavioural changes of patients to the
belief of being infected by avoiding going to the hospital
as a source of contagion place. (21,22) This condition could
disrupt patient’s understanding and decision-making to
keep on their treatment program. (23)

The adherence of treatment depends on the patient’s
willingness to receive and keep following the doctor’s
suggestions. There are many types of the incompliance
of treatment that patients usually do like, not using the
doctor’s prescribed as advised, the patients missed the
appointments and not attending the follow up sessions.
(24)

Bad adherence of treatment is the main cause of
relapse of the disease and increasing patient’s frequent
hospitalized. Education to the patient’s insight really
could help minimize the relapse and recurrence of the
patient’s disease so does minimize the treatment cost.
Medical employees here, including doctors, nurses and
other hospital staffs need to give psychoeducation to
the patient for being aware of their illness and know
their symptoms further. (24) Besides, the medical staffs
are required to give a nice, improved and secured
hospital environment especially during the pandemic so
it expected to reduce the patient’s fear to come to the
hospital. (25)

The Intervention of psychoeducation for patients
with schizophrenia disorders shows some improvements
outcome to the patient especially for very long-term
inward patient. A Good psychoeducation from a doctor
will determines a great improvement in some areas of
their knowledge and adherence to their psychiatric
medication to ensure that their ongoing medication
after discharge from the hospital. It will become
very important to every doctor to give an explainable
psychoeducation to the patient and family about the
diagnosis and the following treatment. (16)

Medical staffs here need to gain concern about
delivering active psychological and psychiatric
intervention for those who needs help during this
pandemic era of Covid-19. We are all cannot just sit and
wait until this pandemic over. The long-term treatment of
psychotic inward patients must go on and telepsychiatry
and use of digital media believed could be used helping
the treatment to continue during this era. (26)

Telemedicine is one of the healthcare services
methods that gives solution of bringing healthcare
service within distance between patients and doctors
especially during the pandemic era, using digital media
technologies involving information and communication.
In the psychiatry department, this service known as
Telepsychiatry. (27)

Telemedicine is an interesting tool for psychiatrist
because psychiatric care is not necessary to meet the
patients physically. The verbal and visual assessment
could be easily done via telepsychiatry. Doctor and
patient could face to face directly via their digital media
platform such as, laptop, tablet and phones without
any restrictions (28). They are only having to switch on
their camera during the consultation so rapport and
expressed face could be obtained and helping doctor to
determine their treatment. This service is very useful for
both patients and doctors during this pandemic because
doctors can still provide assistance and treatment to
patient in a distance while still maintain to minimize the
risk of spreading infection of Covid-19. (27)

Even telepsychiatry sounds especially useful to
the psychiatrist to continue giving the psychiatric care
during the pandemic but telepsychiatry also have some
difficulties that both doctor and patients feel. Doctors
feel that using this technology they hard to detect non-
verbal cues during their videoconference also they could
not build the relationship between doctors and patients
as good as they meet directly. (27)

The needs of telepsychiatry to the inward patient is
giving the supportive psychotherapy and psychoeducation
by phone or video during the pandemic and to follow
up the condition of the patient. (29) One of the aims of
giving psychoeducation to this patient by telepsychiatry
is to gain more insight about patient’s willingness to
get the monthly treatment. Long-acting injection of antipsychotics that given to the patient is every 4 weeks. Compared to the oral antipsychotics, proven that long acting injection have more stability in the blood system, more consistent bioavailability and more predictable on adherence treatment and also have lower rate of relapse.

This patient was getting better condition since she got long-acting injection of antipsychotics. She never relapsed again of her disorder and always got her long-acting injection once a month from 2018 until 2020. Because of her family’s fear of Covid-19, her adherence getting bad and relapse again. By giving right choice of psychoeducation that is telepsychiatry to this patient, from the moment she was discharged from the hospital and always encouraging and reminding her and her family to keep on her medication by taking long-acting injection on the inward clinic every month and doctor gives the consultation via videoconference to minimize her time in the hospital during time of injection.

**Conclusion**

Patients with chronic schizophrenia disorder that had a treatment with long-acting injection antipsychotics must have a good adherence of obeying the procedure of treatment to stabilize their conditions. There are some psychosocial interventions that recommended, one of them that suggested is psychoeducation. A combination of giving a long-acting treatment and psychoeducation intervention for patients with schizophrenia disorder will improve the patient’s condition especially for very long-term inward patient. During the era of Covid-19, many people are afraid of coming to the hospital like they usually did it before. This is a normal situation, but medical staffs must gain their concerns to the continuation of patient’s treatment. Telemedicine is one of the healthcare services methods that gives solution of bringing healthcare service within distance between patients and doctors especially during the pandemic era by using the digital media technologies.

**Conflicts of Interests:** None

**Funding:** Nil

Ethical clearance: The studies were reviewed and approved by Institutional Ethics Committee of Faculty of Medicine, Universitas Airlangga-Dr. Soetomo General Hospital. Verbal informed consent was obtained from the subject/patient and was witnessed. This confirms that the participant has consented to the inclusion of material pertaining to herself, that she acknowledge that she cannot be identified via the manuscript and that the participant has been fully anonymised by the author.

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Key Success Factors and Motivation Affecting the Care of Patients with Tuberculosis of Health Personnel in Primary Care Units

Phatthraphon Chowong¹, Prachak Bouphan², Nakarin Prasit³, Surachai Phimha⁴

¹Master, ²Associate Professor, in Department of Public Health Administration, Health Promotion, and Nutrition, Faculty of Public Health, KhonKaen University, Thailand, ³Sisa Laloeng Health Promoting Hospital, Nakhon Ratchasima Province, Thailand, ⁴Lecturer of Department of Public Health Administration, Health Promotion, and Nutrition, Faculty of Public Health, KhonKaen University, Thailand

Abstract

This research is a cross-sectional descriptive aimed to study key success factors and motivation affecting the care of patients with tuberculosis of health personnel in primary care units Nongbualamphu province, Thailand. The populations were 89 health personnel and 12 key informants. The content validity of the questionnaire was evaluated by three experts with an IOC greater than 0.50 and Cronbach’s Correlation Coefficient was 0.97 and In-Depth interview guidelines for the qualitative. Data was collected between 4th January 2021 and 25th January 2021.

The results of the study showed that five factors including; 1) motivation factor in responsibility 2) Hygiene Factor in policy and management 3) key success factor in the startup in areas that are ready and willing to participation 4) Hygiene Factor in a relationship with supervisors, subordinates, co-workers and 5) Hygiene Factor in salary and compensation affecting and could joint predict the care for tuberculosis patients in primary care at 75.0 percentage (R² = 0.750, p-value <0.001). In conclusion, key success factors and motivation affecting the care of patients with tuberculosis of health personnel in primary care units. Therefore, the health personnel should be developed in terms of work priorities, working process that led to the intention to work, awareness of their roles and responsibilities Including promoting participation in policymaking on tuberculosis work.

Keywords: Key success factors, Care of patients with Tuberculosis, Primary care units

Introduction

Thailand is the center of Southeast Asia and many people are traveling to Thailand. The ASEAN Economics Community leads to people movement into the country, therefore vulnerable to problems of various diseases, including emerging diseases and recurrent disease[1]. It increases the spread of infectious diseases including tuberculosis a serious contagious disease. The World Health Organization(WHO) classifies Thailand as one of 14 countries with a high burden of tuberculosis according to the WHO reported in 2019[2]. In 2018, there were many patients registered for tuberculosis treatment in Thailand (New tuberculosis patients and recurring) 85,029 cases were covered by active case finding and registered for treatment[3]. Tuberculosis is still a major public health problem in Thailand and tuberculosis patient’s care also important.

Taking care of patients with tuberculosis by using Patient-Centered Care (PCC) which is a holistic care service that aligns with the lifestyle of the patient and understands the various contexts of the patient[4]. To ensure the care and treatment of tuberculosis patients from public health personnel at all stages is defined details of measures and requirements into five measures, including 1) prevention and control of tuberculosis, 2) search and screening, 3) treatment, 4) management, and
5) care and treatment of Multidrug-resistant tuberculosis (MDR-TB)[5].

Nong Bua Lam Phu province has 89 primary care facilities comprising 83 sub-district health promoting hospitals and 6 primary care units to provide tuberculosis patients care. The total number of tuberculosis cases in 2017 - 2019 was 505, 482, and 495 cases, representing the incidence at 107.10, 90.65, and 104.15 per 100,000 population, respectively. The problem in tuberculosis care was unable to follow-up, drug resistance. The admission patient has become more severe because most of the registered tuberculosis patients are elderly and having a common disease[6]. It led to the study in key success factorsbase on Supawongtheory[7] and Herzberg’s theory of motivation factors which classify into two main dimensions was motivation factor and Hygiene Factor[8] which affecting the care of patients with Tuberculosis of health personnel in primary care units.

Objective

The study aimed to investigate Key Success Factors and motivation affecting the care of tuberculosis patients of health personnel in primary care units Nong Bua LamPhu province, Thailand.

Research methodology

This research is a cross-sectional descriptive research

Population

The population was 89 health personnel in primary care units who responsible for the care of tuberculosis patients under the public health office of Nong Bua Lam Phu province, Thailand. The researcher studied the entire population.

The sample group for an In-Depth Interview was 12 health personnel responsible for the care of tuberculosis patients under the public health office of Nong Bua Lam Phu province, Thailand.

Research instruments

There were two sets of data collection tools in this research

Set 1: There are five parts questionnaires: Part 1. Personal characteristics, Part 2. Key success factors consist of 30 questions, Part 3. Motivation factor (motivation factor and Hygiene Factor) consists of 59 questions, Part 4. Care for TB patients consists of 24 questions. The parts 2 to part 4 using the approximation scale there is a choice of answers with five scoring levels[9] and Part 5. In the open-ended questions which the respondent proposes problems, and suggestions in caring for patients with tuberculosis.

Set 2: In-Depth Interview, there are three parts: Part 1. key success factors, Part 2. Motivation factors, Part 3. Care for tuberculosis patients of health personnel in the primary care unit.

The measurement: Interpretation of the key success factor rating score, motivation score, and the care for patients with tuberculosis of health personnel in primary care was classified by rating scale in 5 levels which were the most, most, medium, low, and lowest then calculate in average score.

Quality of research tools

Both tools were tested for content validity by three experts who found that each question has a consistency index greater than 0.50. Then, the questionnaire was examined, corrected, and improved by experts and. The questionnaire was tryout with 30 health personnel in Bueng Kan province, Thailand who have similar performance characteristics. The Cronbach’s Alpha Coefficients found that the accuracy value reliability of key success factor variable was 0.93, Motivation factor was 0.95, care for tuberculosis patients was 0.86, and overall was 0.97.

Data Analysis

Data analysis was performed by descriptive statistics: frequency, percentage, mean, standard
deviation, median, minimum, and maximum values, and inferential statistics; Pearson’s correlation coefficient statistics and stepwise multiple regression analysis with a statistical significance level of 0.05.

**Results**

1. **Personal characteristics**

   The population characteristics found that most of the study population was female, 67 people (75.3%), aged between 31-40 years, 44 people (49.4%) with an average age of 36.93 years (S.D. = 8.46, Min=23 years old, Max=60 years old), most of them have marital status (couples) of 49 people, (55.1%). They have education in bachelor’s degree, 73 people (82.0%), most of the income (per month) between 15,001 - 25,000-baht, amount 52 people (58.1%) with an average income of 23,872.47 Baht (S.D. = 11369.30).

2. **Key success factors and motivation affecting the care of patients with tuberculosis of health personnel in primary care units**

   Independent variables affecting care tuberculosis patients of health personnel in primary care unit which selected into the equation is significant variables statistical at level 0.05. The variables selected in the equation were 1) motivation factor in responsibility (p-value <0.001) 2) Hygiene Factor in policy and management (p-value =0.001) 3) key success factor in the startup in areas that are ready and willing to participate (p-value =0.001) 4) Hygiene Factor in a relationship with supervisors, subordinates, co-workers(p-value =0.008) and 5) Hygiene Factor in salary and compensation(p-value <0.030), so five independent variables were affecting and predictable for the care of tuberculosis patients of health personnel in primary care unit at 75.0 percentage(Table 1). The Multiple Linear Regression equation is a prediction equation of the care of patients with Tuberculosis of health personnel in primary care units as follows:

   \[
   Y = 0.410 + (0.315) \text{ (Motivation factor in responsibility)} \\
   + (0.299) \text{ (Hygiene Factor in policy and management)} \\
   + (0.218) \text{ (Key success factor in the startup in areas that are ready and willing to participation)} \\
   + (0.205) \text{ (Hygiene Factor in a relationship with supervisors, subordinates, and co-workers)} \\
   - (0.121) \text{ (Hygiene Factor in salary and compensation)}
   \]

   **Table 1: The key success factors and motivation affecting the care of patients with tuberculosis of health personnel in primary care units by stepwise multiple linear regression analysis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>Beta</th>
<th>T</th>
<th>P - value</th>
<th>R</th>
<th>R2</th>
<th>R2adj</th>
<th>R2change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Motivation factor in responsibility</td>
<td>0.315</td>
<td>0.332</td>
<td>4.315</td>
<td>&lt; 0.001</td>
<td>0.746</td>
<td>0.556</td>
<td>0.551</td>
<td>0.556</td>
</tr>
<tr>
<td>2. Hygiene Factor in policy and management</td>
<td>0.299</td>
<td>0.341</td>
<td>3.310</td>
<td>0.001</td>
<td>0.823</td>
<td>0.677</td>
<td>0.669</td>
<td>0.121</td>
</tr>
<tr>
<td>3. Key success factor in the startup in areas that are ready and willing to participation</td>
<td>0.218</td>
<td>0.233</td>
<td>3.344</td>
<td>0.001</td>
<td>0.845</td>
<td>0.714</td>
<td>0.704</td>
<td>0.037</td>
</tr>
<tr>
<td>4. Hygiene Factor in a relationship with supervisors, subordinates, and co-workers</td>
<td>0.205</td>
<td>0.238</td>
<td>2.723</td>
<td>0.008</td>
<td>0.858</td>
<td>0.735</td>
<td>0.723</td>
<td>0.021</td>
</tr>
<tr>
<td>5. Hygiene Factor in salary and compensation</td>
<td>-0.121</td>
<td>-0.176</td>
<td>-2.207</td>
<td>0.030</td>
<td>0.866</td>
<td>0.750</td>
<td>0.735</td>
<td>0.015</td>
</tr>
</tbody>
</table>

   Constant 0.410, F = 49.843, p – value < 0.001, R = 0.866, R2 = 0.750, R2adj = 0.735
Conclusion and Discussion

The results of the study showed that 1) motivation factor in responsibility 2) Hygiene Factor in policy and management 3) key success factor in the startup in areas that are ready and willing to participation 4) Hygiene Factor in a relationship with supervisors, subordinates, and co-workers and 5) Hygiene Factor in salary and compensation affecting and could joint predict the care for tuberculosis patients in primary care at 75.0 percentage.

The motivation factor in responsibility is the important factor in the fundamentals of health workers in tuberculosis work. Due to responsibility the satisfaction that arises from being assigned to a new task and having the power to take full responsibility monitored. It is a sequence of work, the intention to perform the job and lead to motivation and responsibility to make the job successful. The results of this research are consistent with the research that has been studied and found that motivation in responsibility incentive factors affect the performance of health personnel in primary care units[10-12].

The Hygiene Factor in policy and management which represent the organizations management, goal setting, and providing opportunities to participate in policymaking to achieve clarity of the policy, rules, and regulations until the operator has a real understanding. The results of this research are consistent with the research was found that the Hygiene Factor in policy and management had an effect on the performance of public health personnel in the primary care unit[13-14].

The key success factor in the startup in areas that are ready and willing is a very important factor for promoting participation for public health personnel to work on tuberculosis in the community area. Tuberculosis patient care needs to build cooperation from the community and various organizations in the community by encouraging people and take part in tuberculosis care with understanding, moreover, having the department of public health as a mentor will support a project plan for tuberculosis implementation. The results of this research are consistent with the research that has been studied and found that key success factors in the startup in areas that are ready and willing affect the performance of public health personnel in the primary care unit[15-17].

Hygiene Factor in a relationship with supervisors, subordinates, and co-workers which is the tuberculosis operations on communication to contact in verbal or non-verbal that express a good relationship with each other, understanding to network partners, stakeholders. The results of this research are consistent with the research that has been studied was found that Hygiene Factor in a relationship with supervisors, subordinates, and co-workers affect the performance of public health personnel in the primary care unit[11, 18].

The Hygiene Factor in salary and compensation, in the primary care unit the other benefits or privileges from tuberculosis work were limit. The disbursement is quite difficult because the process is complicated and requires a lot of paperwork this makes it a problem and obstacle for the operator in making financial disbursement. The results of this research a consistency with research that has been researched and found that Hygiene Factor in salary and compensation affect the performance of public health personnel in the primary care unit[10, 14].

The qualitative data found that the primary care monitoring of tuberculosis work from the district-level authorities limit causing no continuity of operations. The suggestion is there should be supervision and monitoring for TB work from related persons at the mentor hospital level and district level. Continuously, monitoring, supervising, giving advice, providing guidance on the work that is correct as the same standard. They should integration between multidisciplinary teams of each department together, such as making a joint plan or sharing administrative resources, information exchange to those involved.

The health personnel low motivation to work because risk of tuberculosis infection and it cannot drive career advancement. the suggestion is providing special rewards for risks arising from operations to encourage the morale of the working staff.
In conclusion, the motivation factor in responsibility, the Hygiene Factor in policy and management, relationship with supervisors, subordinates, and co-workers, salary and compensation, and key success factor in the startup in areas that are ready and willing to participation affecting the care of patients with tuberculosis of health personnel in primary care units. Therefore, the health personnel should be developed in terms of work priorities, working process that led to the intention to work, awareness of their roles and responsibilities Including promoting participation in policymaking on tuberculosis work.

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Ethical Clearance: The data collected after being certified by the Human Research Ethics Committee of KhonKaen University on December 19, 2020 number HE632268.

Conflict of Interest: No conflicts of interest to declare.

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Determination of Learning Styles among Nursing Students

Pooja Gill¹, Parul², Yogesh Kumar³, Jyoti Sarin⁴

¹Nursing Tutor, ²Associate Professor, Department of Mental Health Nursing, ³Professor, Department of Child Health Nursing, ⁴Dean-Principal, Maharishi Markandeshwar College of Nursing, Maharishi Markandeshwar Deemed to be University, Mullana, Ambala, Haryana, India

Abstract

Nursing education furnishes students with the learning qualities required for their profession and also makes students lifelong learners. Aim of the study was to evaluate the learning styles among nursing students. A quantitative approach with descriptive design was applied. A total of 122 students were recruited by purposive sampling from two nursing colleges, selected by convenient sampling. In addition, tools used for the study were structured questionnaire for sample characteristics and Kolb’s Learning Style Inventory. Learning styles were assessed after collecting baseline data. Results indicated that the most preferred learning style was turned out to be Accommodator (35%). Findings concluded that there is a diversity of learning styles among nursing students as the students were having all the types of learning styles.

Keywords: Learning styles, Nursing students, Baccalaureate.

Introduction

The nature of education is in the midst of a transformation. Education has traditionally been viewed as the means to convey information and students as identical empty vessels to fill with information. Nursing education furnishes students with the learning qualities required for their profession. Knowledge and practice are not static always; they are going to change at every step in education.¹

Learning style is defined as “The way each learner begins to concentrate on, process and retain new and difficult information”. Learning style theories assume that all may learn in different ways and at different levels. There are theories and models which focus on aspects such as talents, sensory modalities, cognitive and/or learning and thinking processes.²

Learning styles are not “fixed” and can be modified depending on the teaching styles and teaching methods. Teachers have several options for managing their diversity of learners about learning styles accommodate particular styles provide creative mismatches or provide a variety of instructional approaches so that learners are both accommodated and mismatched at the same time.³

Within the nursing career, realistic and theoretical knowledge need to be distinctly consolidated. Practicing in clinical field may be very critical for undergraduate nursing college students to increase themselves professionally. This is the core responsibility of the teacher to provide an individualized learning environment, so that the students can link up the theoretical knowledge to practical skills. The teacher also acts as a role model for students and is seen as pivotal to student learning in clinical setting.⁴

Knowing students’ learning style preferences enables teachers to use more effective and a wide range of teaching methods and to choose better educational tools for a particular student.⁶

Corresponding Author:
Ms. Pooja Gill,
Nursing Tutor (Mental Health Nursing) Maharishi Markandeshwar Deemed to be University
Mullana- 133203, Ambala- 133001, Haryana, India Ph. No.: 8569827527, Email: gjasmine884@gmail.com
Materials And Methods

The study used quantitative approach with descriptive design. The study was conducted in two nursing colleges. A total of 122 students were selected as study participants by purposive sampling. The tools used for the study consisted of selected variables regarding study participants characteristics, Kolb’s Learning Style Inventory to assess the preferred learning styles. Reliability of the tools was established by split-half method for Kolb’s Learning Style Inventory (0.71-0.89). The data collection was done during the period of November to December 2019. Pilot study was conducted before final study. Data collection was started by collecting the baseline data and then assessment of learning styles. Descriptive statistics were used to analyse the data by SPSS 20 version.

Results

The Kolomogorov- Smirnov test was applied to check the normality of data distribution and it was concluded that the data was normally distributed with the value (p= 0.36). Hence parametric tests were applied for the study.

Sample characteristics

The baseline data was collected from participants through sample characteristics which conclude that variables were homogenous. Nearly half (46.90%) of students were from the age group of 19-21 years. Majority of (73.30%) the students were enrolled in B.Sc. nursing course. More than half (68.80%) of the students were females. Seventy three (73.3%) of the nursing students were belonged to Hindu religion. All (100%) the nursing students were unmarried. More than half (66.7%) of the nursing students had 61-75% marks in previous university examination. Nearly all the nursing students (96.6%) were not having any additional qualification. Majority of the (73.3%) nursing students involved in extracurricular activities. All the nursing students (100%) had previous experience in psychiatric ward. Majority (87.5%) of the nursing students were not having any history of mental illness in their family.

Learning style preferences

The most preferred learning style was found to be Accommodator (34.78%) followed by Diverger learning style (32.60%) and assimilator learning style (32.60%). Astonishingly no students were having converger learning style preference.

Fig.1.Bar diagram showing Frequency Distribution in Terms of Preferred Learning Styles among nursing students.
Discussion

Mismatching of students’ learning style preferences avails better benefit to the students. The study results depicts that there are variety of learning style preferences among nursing students.

In this study, 122 nursing students were enrolled and were homogenous in terms of baseline characteristics. The findings of the study highlighted that there is a wide range of different learning styles among nursing students.

In addition, there has been a predominance among nursing students in gaining knowledge related to learning styles at some point of the years of Bachelor of Science in nursing (Nair MA. 2016). There has been a trend of accommodator and diverging learning style as most preferred learning style in the undergraduate nursing college students.

In addition, understanding learning styles could be considered as a key component of managing classroom teaching strategies (Parul, C. Vikas. 2015). The most preferred learning was reflector followed by theorist; pragmatist and activist preferred learning style. It focussed that nursing students prefer to range between different learning styles.

A research study conducted by (Aina-popoola S, Hendricks CS 2012) also admire that divergers are most preferred learning styles followed by assimilators, accommodators and convergers among first year undergraduate nursing and midwifery university students.

Implications

Nursing Education

Nursing educator on the institutional level need to recognize the variations among college teaching techniques. Nursing students getting aware about their own learning style preference would inculcate better thinkers, hassle-solvers, lifelong newbie’s and skilful in peer and self-assessment. It also helps them to collect skills that aid expert improvement. Educators need to design lectures around a trouble-fixing model or discussion method version and lead the class via the invention method after applying appropriate learning styles.

Nursing Administration

Administrators also can form a climate of utilization of most newly research pertaining of learning styles and teaching methods as it helps tutors/educators in understanding the learning styles. Nursing administrators can provide in-service training programme for the nurse educators on assessing desired studying varieties of nursing college students and also inspire them to apply teaching getting to know techniques consistent with their preferred gaining knowledge of styles that suitable to the unique learner.

Strengths and Limitations

The strengths of the study were that it was conducted in two different nursing colleges and the response rate was high. Almost all the students were included besides who were not present at the time of data collection. As with the strengths, the study lacked few points as the sample size was quite small due to time constraints. A similar design can be govern on large sample. It would be a great breakthrough in understanding the learning styles among nursing students.

Conclusion

In the nutshell, the nursing students possessed different preferred learning styles. It depicts the preference of nursing students varied and not all of them pan out in one learning style only. This emerged as an immense need to inculcate teaching learning strategies that are formulated according to preferred learning styles.

Conflict of Interest

Please check the following as appropriate:

- All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b)
drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

- The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

- The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript:

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**Ethical Clearance:** Taken from Communication Of Decision Of the Committee (IEC) (Ethical no. IEC-1519). Study is registered under CTRI/2020/06/025645.

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Hepatitis-B Screening among Health Care Worker – a Cross Sectional Study

Praf ul Damor¹, Siddaram Sarate²
¹M.Sc Nursing, Manikaka Topawala Institute of Nursing, CHARUSAT, Gujarat, ²Assistant Professor and HOD, Community Health Nursing, Manikaka Topawala Institute of Nursing, Charusat, Gujarat

Abstract

Introduction and Background: Hepatitis B virus (HBV) is known to be an infectious disease affecting the liver and transmitted through contaminated blood and body fluids. It also poses a huge danger to patients. Health employees are more likely to become infected as a result of interaction with infected patients. It begins with individuals coming into contact with someone who has been infected with a hep-b infection with contaminated blood, exposed, wounds, or body fluids. Hepatitis B infection is typically a self-limiting disease characterized by acute inflammation and hepatocellular necrosis, with a 0.5-1 percent case-fatality rate and a higher rate among health care workers than among the general population.

Objective: The objective of the study is to detect infection of Hepatitis-B among healthcare workers.

Methodology: In the analysis, the design of non-experimental cross-sectional research was used. A total of 100 samples were obtained from the chosen hospital using a convenient non-probability sampling technique. Rapid kit (I-care for HBsAg Rapid Screen Test) was performed among health care workers for the identification of hepatitis B.

Result: Descriptive and inferential statistics were used to interpret the results. The study showed that 5(5%) participants were tested positive for hepatitis B. Chi Square used to find association between attribute variables and hepatitis B infection. There was significance difference found among blood transfusion ($\chi^2=0.718$, p<.000), tattooing on body ($\chi^2=10.809$, p < .004), history of hepatitis –B infection in family ($\chi^2=33.518$, p <.000) at 0.05 level of significance.

Conclusion: The risk of infection with hepatitis B is higher among healthcare staff. While it decreases the risk of infection with the use of hep-B vaccine, there is still considerable potential for change, as many healthcare staff remain unvaccinated. There is also a need for well-planned and consistent HBV screening and vaccination policies for healthcare staff, especially those at higher risk of exposure to blood or other potentially infectious materials.

Keywords: Hepatitis –B, health care worker, detection.

Introduction

Hepatitis B is a significant and growing problem for public health. Hep-B virus is transmitted via the blood, sperm, or other sources of human body fluid to an infected person. It is not spread by sneezing or coughing or sharing needles through sexual contact. HBV spread very rapidly by needles and syringes contaminated with tainted blood. An individual is at increased risk for viral hepatitis through intravenous drug sharing.¹

Viral hepatitis caused 1.45 million deaths worldwide in 2013, reflecting a 63% increase in the mortality burden between 1990 and 2013.² Hepatitis (HBV) is estimated to affect at least 257 million people worldwide and 27 million people living with multiple hepatitis B virus infection (WHO 2016), an estimated 887,000 deaths (WHO 2015). There are 56.6 million people...
(WHO 2016) affected by hepatitis in India.

In India, 40 million people are chronically contaminated with hep-B and between 6 and 12 million people are chronically contaminated with hep-C, according to current estimates. HEV is the most important factor in the spread of viral hepatitis, while hep-B is more common among young children. Most cases reported as acute liver failure are due to hep-B. [4]

Healthcare workers (HCWs) who are often in contact with blood and other body fluids are at greater risk of developing blood-borne infections such as HBV, HIV and hepatitis C infections during their work. Approximately two million HCWs are observed worldwide and approximately 70,000 are getting infected with HBV virus per year. As per WHO global disease burden has shown that 37 per cent of HBV between HCWs is due to serious injuries from occupational experience. More than 90% of this contamination occurs in developing countries. [5]

Preventive hep B obstruction vaccination is common in many countries for clinical workers, but is still not used in a variety of low asset environments. Weak resistance reactions to HBV immunization due to, for example, current viral infections or diabetes have been documented. Therefore, in addition to the compulsory immunization of HCWs, the WHO plans to research immune responses to the vaccine. [6]

In this study, researcher aims to detect hepatitis-B amid health care workers working in selected hospitals.

**Material and Methods**

The cross-section design with a quantitative approach has been used. A total of 100 health care staff between the ages of 18 and 58 were drawn from hospital settings. Data was collected by means of a fast kit and a socio-demographic Performa. Blood samples obtained by means of a syringe and precautions were taken during the process to ensure protection. The reliability of the instrument was tested through a calibration review in the hospital.

The duration of the analysis had been 30 days. Descriptive statistics such as frequency distribution, standard deviations and inferential statistics such as the chi-square test were used to interpret the data, and the purpose of the analysis was clarified to ensure better cooperation during the data collection process.

**Tool for data collection**

- Socio demographic and attribute variable.
- Rapid test kit used to detection of hepatitis-B among health care worker

  - The selection of the sample from the sample population is carried out by syringe and needle and the HBsAg Rapid Test is a direct binding test for the visual detection of hepatitis B surface antigen (HBsAg) in serum, plasma and whole blood.
  - The sample is collected in red tube (vacutioner) blood is collected from the participants and store in cold chain at temperature (between+2 to +8 degree) until the time it is used.

**Study participants and size:** Sample size determine by power analysis calculated based on previous studies.

\[
N = \frac{4pq}{L^2}
\]

Where \( p = 0.087 \), \( q = 0.913 \), \( L = 0.017 \)

\[
= \frac{4 \times 0.087 \times 0.913}{0.017 \times 0.017} = \frac{0.317}{0.000289} = 1097
\]

Calculation sample size for this study was 1097, due to time constraint and global pandemic situation investigator consider 100 participants for the study.

**Ethical Clearance:** Permission was gained from CHARUSAT Institutional Ethical Committee, Charotar University of Science and Technology, Gujarat, India
Result

Table 1 shows Data on detection of Hepatitis B infection among health-care-worker

- The data was analysed by using descriptive and inferential statistics such as Chi-square were used.

**Table 1: The 5(5%) participants were tested positive for hepatitis B.**

<table>
<thead>
<tr>
<th>Hepatitis Infection</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Positive</td>
<td>5</td>
<td>5.0</td>
</tr>
<tr>
<td>a. Negative</td>
<td>95</td>
<td>95.0</td>
</tr>
</tbody>
</table>

Staff nurses, lab technicians and class IV participants are included as participants in this research. The majority of study participants have worked experience of more than 5 years. There is a major difference between the attribute variable, such as blood transfusion, body tattooing, and the history of hepatitis B infection. Chi square used to find a relation between the attribute variable.

**Table 2: Findings related to association of attribute variables and hepatitis B**

<table>
<thead>
<tr>
<th>Sr no</th>
<th>Attribute variable</th>
<th>Chi square</th>
<th>Df</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
<td>3.016</td>
<td>3</td>
<td>.389</td>
</tr>
<tr>
<td>2</td>
<td>Gender</td>
<td>0.009</td>
<td>1</td>
<td>.926</td>
</tr>
<tr>
<td>3</td>
<td>Educational status</td>
<td>1.610</td>
<td>2</td>
<td>.447</td>
</tr>
<tr>
<td>4</td>
<td>Marital status</td>
<td>0.651</td>
<td>1</td>
<td>.420</td>
</tr>
<tr>
<td>5</td>
<td>Monthly family income</td>
<td>0.096</td>
<td>1</td>
<td>.619</td>
</tr>
<tr>
<td>6</td>
<td>Place of residence</td>
<td>1.464</td>
<td>1</td>
<td>.481</td>
</tr>
<tr>
<td>7</td>
<td>Years of experience</td>
<td>3.605</td>
<td>3</td>
<td>.819</td>
</tr>
<tr>
<td>8</td>
<td>Taken hap-B vaccine</td>
<td>1.681</td>
<td>2</td>
<td>.431</td>
</tr>
<tr>
<td>9</td>
<td>Do you use contraceptive in multiple relationship</td>
<td>0.786</td>
<td>2</td>
<td>.675</td>
</tr>
<tr>
<td>10</td>
<td>Factor convince to take vaccination</td>
<td>4.123</td>
<td>3</td>
<td>.249</td>
</tr>
<tr>
<td>11</td>
<td>Under blood transfusion</td>
<td>0.718</td>
<td>1</td>
<td>.000(S)</td>
</tr>
<tr>
<td>12</td>
<td>Tattooing on body</td>
<td>10.80</td>
<td>2</td>
<td>.004 (S)</td>
</tr>
<tr>
<td>13</td>
<td>Any history of hep-B infection in family</td>
<td>33.518</td>
<td>1</td>
<td>.000(S)</td>
</tr>
<tr>
<td>14</td>
<td>Discard of needle in hospital.</td>
<td>0.458</td>
<td>1</td>
<td>.499</td>
</tr>
<tr>
<td>15</td>
<td>Proper segregation in hospital</td>
<td>0.396</td>
<td>.529</td>
<td>.529</td>
</tr>
<tr>
<td>16</td>
<td>Injured from piercing or dental carries</td>
<td>1.078</td>
<td>1</td>
<td>.299</td>
</tr>
</tbody>
</table>
Table2 suggests that there was significance difference found with this attribute variable such as blood transfusion ($\chi^2 = 0.718, p < .000$), (χ$^2$=10.809, p < .004), history of hepatitis B infection in family ($\chi^2=33.518$, p <.000) at 0.05 level of significance. There was no any significance noted in the other demographic variable.

Conclusion

This research was performed only on 100 samples (health care worker). Thus 5(5 per cent) participants were tested positive for hepatitis B. Significant difference was found between attribute variables such as blood transfusion ($\chi^2=0.718$, p<.000), body tattooing ($\chi^2=10.809$, p<.004), history of hepatitis B infection in the family ($\chi^2=33.518$, p<.000) at 0.05 level of significance.

Financial support and sponsorship: Self

Conflict of Interest: There is no conflict of interest

References


Effect of Air Pollution on Health of Community People–A Descriptive Approach

Praful Damor¹, Abhay Pattan², AmbilyV.Vijayakumar³
¹Lecturer, Community Health Nursing Parul Institute of Nursing, Limda, Wagodia Gujarat, ²Assistant Professor and HOD,Community Health Nursing, Parul Institute of Nursing, Charusat, Gujarat, ³Lecturer, Community Health Nursing, Parul Institute of Nursing Waghodia, Gujarat

Abstract

Background: Air pollution is a global and one of the serious environmental hazards because it causes lot of damage to living and non-living things in the environment. Air pollution is a problem which emitted various gases in the air because of burning of wood etc. Air pollution cause not so serious in the past as it is now because of growing population, rapid industrialization, use of automobiles, modern agricultural activities etc.¹

Objectives:

1. To assess the knowledge regarding effects of air pollution on health among community people Gujarat.
2. To assess the attitude regarding effects of air pollution on health among community people in central Gujarat
3. To find out the association between knowledge score regarding effects of air pollution on health with selected socio demographic variables.

Methodology: A quantitative research approach at survey design was adopted to conduct the study at central part of Gujarat. Sample comprised of 60 community people. Sample was selected by convenience sampling technique. The data obtained was analyzed and interpreted in terms of the objectives of the study. A structured questionnaire was used to assess the knowledge and likert scale was used to assess the attitude on effects of air pollution on health among community people.

Result: The findings of the study show that 53% female and 47% male samples, highest percentage 91% are belongs to hindu,1% muslim,3% Cristian,4% are others, majority of people were age group of 26-35years(33%),15-25years(20%),36-45 years (20%),above 45 years (26.6%),43% people received up to secondary level education,91% people are Hindu, 71% people are self-employed, 18.3% people are living in nuclear family,45% people having less than 5000rs income,70% people living in pakka housing,56.6% get knowledge regarding effects of air pollution on health from T.V . 2. The score of the study is mean (16.1) and standard deviation is (3.46).

Conclusion: Overall knowledge and attitude about the effects of air pollution on health was average which suggested that people having average knowledge regarding effects of air pollution Group.

Keywords: Air pollution, health, descriptive approach

Introduction

The term air pollution can be referred when there is an excess of foreign matter in the air. That can be harm full to the man and his environment, the phenomenon called “pollution” is an inescapable consequence of the presence of man and his activity. the term “air pollution” signifies the presence in the ambient (surrounding) atmosphere of substances (e.g.: gases, mixtures of gases
an particulate matter) generated by the activities of man in concentration that interfere with human health, safety or comfort, or injurious to vegetation and animals another environmental media resulting in chemicals entering the food chain or being present in drinking water and there by constituting additional source of human exposure. The direct effect of air pollutants on plants, animals, and soil can influence the structure and function of eco system, including self-regulation ability, there by affecting the quality of life.3

Pollution is the contaminants into an environment that causes instability, disorder, harm or discomfort to the ecosystem i.e. physical systems or living organisms. Pollution can take the form of chemical substances, or energy, such as noise, heat, or light energy. Pollutants, the elements of pollution, can be foreign substances or energies, or naturally occurring; when naturally occurring, they are considered contaminants when they exceed natural levels. Pollution is often classed as point source or nonpoint source pollution.4 Air pollution is a significant risk factor for a number of pollution-related diseases and health conditions including respiratory infections, heart disease, and COPD, stroke and lung cancer.5

The health effects caused by air pollution may include difficulty in breathing, wheezing, coughing, asthma and worsening of existing respiratory and cardiac conditions. These effects can result in increased medication use, increased doctor or emergency room visits, more hospital admissions and premature death.
The human health effects of poor air quality are far reaching, but principally affect the body’s respiratory system and the cardiovascular system. Individual reactions to air pollutants depend on the type of pollutant a person is exposed to, the degree of exposure, and the individual’s health status and genetics. The most common sources of air pollution include particulates, ozone, nitrogen dioxide, and sulphur dioxide. Children aged less than five years that live in developing countries are the most vulnerable population in terms of total deaths attributable to indoor and outdoor air pollution.

Material and Methods
Researcher overall plan for obtaining answers to research questions or for testing the research statement is referred as the research design.A descriptive design was adopted for the study. In this study the base measure was structured questionnaire method was used to assess the knowledge of the people of the age group between 15 to 45 years.

Tool for data collection
· Socio demographic and attribute variable.
· The researcher obtained written permission from the consent authority of prior of data collection. Data collection date was heldon. The purpose of study was explained to the samples and written informed consent was obtained. Their response was assessing the status of air pollution.

· Individual explanation was given regarding structured Knowledge questionnaires to assess the knowledge regarding effects of air pollution on health.Knowledge test was checked. The average time taken 60 minutes. All people gave good cooperation during data, investigation, analyzed the data and made findings.

Study participants and size: Sample size determine by power analysis calculated based on previous studies.
N = \frac{4pq}{L^2}

Where \( p = 0.087 \)
\( q = 0.913 \)
\( L = 0.017 \)

\[
= \frac{4 \times 0.087 \times 0.913}{0.017 \times 0.017} = 0.317 = 1097
\]

Calculation sample size for this study was 1097, due to time constraint and global pandemic situation investigator consider 64 participants for the study.

**Result**

**SECTION 1: FINDINGS RELATED TO ANALYSIS OF DEMOGRAPHIC CHARACTERISTICS OF PEOPLE.**

**Table 1: Frequency and percentage wise distribution of people according to demographic variable**

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Demographic variables</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>AGE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>15 to 25year</td>
<td>12</td>
<td>20%</td>
</tr>
<tr>
<td>b.</td>
<td>26 to 35year</td>
<td>19</td>
<td>31.66%</td>
</tr>
<tr>
<td>c.</td>
<td>36 to 45year</td>
<td>12</td>
<td>20%</td>
</tr>
<tr>
<td>d.</td>
<td>More than 46years</td>
<td>17</td>
<td>28.33%</td>
</tr>
<tr>
<td>2.</td>
<td>SEX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Male</td>
<td>28</td>
<td>46.6%</td>
</tr>
<tr>
<td>b.</td>
<td>Female</td>
<td>32</td>
<td>53.3%</td>
</tr>
<tr>
<td>3.</td>
<td>EDUCATION</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Primary</td>
<td>08</td>
<td>13.3%</td>
</tr>
<tr>
<td>b.</td>
<td>Secondary</td>
<td>26</td>
<td>43.3%</td>
</tr>
<tr>
<td>c.</td>
<td>Highersecondary</td>
<td>19</td>
<td>31.3%</td>
</tr>
<tr>
<td>d.</td>
<td>Graduation</td>
<td>07</td>
<td>11.66%</td>
</tr>
<tr>
<td>4.</td>
<td>RELIGION</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Hindu</td>
<td>55</td>
<td>91.66%</td>
</tr>
<tr>
<td>b.</td>
<td>Muslim</td>
<td>01</td>
<td>1.66%</td>
</tr>
<tr>
<td>c.</td>
<td>Christian</td>
<td>02</td>
<td>3.33%</td>
</tr>
<tr>
<td>d.</td>
<td>Other</td>
<td>02</td>
<td>3.33%</td>
</tr>
<tr>
<td>5.</td>
<td>OCCUPATION</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Own business</td>
<td>20</td>
<td>33.33%</td>
</tr>
<tr>
<td>b.</td>
<td>Labourwork</td>
<td>15</td>
<td>25%</td>
</tr>
<tr>
<td>c.</td>
<td>Job</td>
<td>13</td>
<td>21.66%</td>
</tr>
<tr>
<td>d.</td>
<td>Other</td>
<td>12</td>
<td>20%</td>
</tr>
</tbody>
</table>
Cont... Table 1: Frequency and percentage wise distribution of people according to demographic variable

<table>
<thead>
<tr>
<th></th>
<th>TYPE OF FAMILY</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nuclear</td>
<td>43</td>
<td>33.33%</td>
</tr>
<tr>
<td></td>
<td>Joint</td>
<td>11</td>
<td>18.3%</td>
</tr>
<tr>
<td></td>
<td>Extended</td>
<td>04</td>
<td>6.66%</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>02</td>
<td>3.33%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>FAMILY INCOME PER MONTH</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a. Below 5000 rupees</td>
<td>27</td>
<td>45%</td>
</tr>
<tr>
<td></td>
<td>b. 5001 to 10,000 rupees</td>
<td>20</td>
<td>33.33%</td>
</tr>
<tr>
<td></td>
<td>c. 10,001 to 20,000 rupees</td>
<td>13</td>
<td>21.66%</td>
</tr>
<tr>
<td></td>
<td>d. Above 20,001 rupees</td>
<td>00</td>
<td>00%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>TYPE OF HOUSE</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kachhahouse</td>
<td>08</td>
<td>13.33%</td>
</tr>
<tr>
<td></td>
<td>Pakka house</td>
<td>44</td>
<td>73.33%</td>
</tr>
<tr>
<td></td>
<td>Semi pakkahouse</td>
<td>08</td>
<td>13.33%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>PREVIOUS INFORMATION ABOUT EFFECTS OF AIR POLLUTION ON HEALTH</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a. Massmedia</td>
<td>34</td>
<td>56.66%</td>
</tr>
<tr>
<td></td>
<td>b. Healthworkers</td>
<td>10</td>
<td>16.33%</td>
</tr>
<tr>
<td></td>
<td>c. Newspaper</td>
<td>11</td>
<td>8.33%</td>
</tr>
<tr>
<td></td>
<td>d. Noinformation</td>
<td>05</td>
<td>8.33%</td>
</tr>
</tbody>
</table>

Section 2-Analysis And interpretation of the knowledge and attitude score of the regarding effect of air pollution on health

Table-2 Percentage of knowledge and attitude score of subject regarding effect of the air pollution on health

<table>
<thead>
<tr>
<th>Areas of analysis</th>
<th>Total score</th>
<th>Total Score obtained</th>
<th>Mean % of scores of subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge</td>
<td>1440</td>
<td>966</td>
<td>16.1%</td>
</tr>
<tr>
<td>Attitude</td>
<td>3000</td>
<td>1882</td>
<td>31.36%</td>
</tr>
</tbody>
</table>

SECTION-3: Findings related to association between the knowledge score with demographic variables.

For the age calculated chi square ($x^2$) 1.255 was less than the tabulated value of chi square ($x^2$) 7.82 at the 3 degree of freedom at 0.05 level of significance there for, there was no significant association for the knowledge score among the samples.

For the sex the calculated chi square ($x^2$) 4.218 was less than the tabulated value of chi square ($x^2$) 3.84 at the 1 degree of freedom at 0.05 level of significant there for was significant association for the knowledge score among the samples.

For the education the calculated chi square ($x^2$) 0.426
was less than tabulated value of chisquare \( x^2 \) 7.82 at the 3 degree of freedom at 0.05 level of significant there for, there was no significant association for the knowledge score among the samples.

For the religion the calculated chisquare \( x^2 \) 10.874 was less than tabulated value of chisquare \( x^2 \) 7.82 at the 3 degree of freedom at 0.05 level of significant there for, there was significant association for the knowledge score among the samples.

For the occupation the calculated chi square \( x^2 \) 5.442 was less than tabulated value of chi square \( x^2 \) 7.82 at the 3 degree of freedom at 0.05 level of significant there for, there was no significant association for the knowledge score among the samples.

For the type of house the calculated chisquare \( x^2 \) 3.907 was less than tabulated value of chisquare \( x^2 \) 7.82 at the 3 degree of freedom at 0.05 level of significant there for, there was no significant association for the knowledge score among the samples.

For the family income the calculated chi square \( x^2 \) 3.603 was more than tabulated value of chi square \( x^2 \) 7.82 at 3 degree of freedom at the level of significant there for, there was significant association for the knowledge score among.

For the type of house the calculated chisquare \( x^2 \) 3.535 was less than tabulated value of chisquare \( x^2 \) 7.82 at the 2 degree of freedom at 0.05 level of significant there for, there was no significant association for the knowledge score among the samples.

For the previous information the calculated chi square \( x^2 \) 1.740 was less than tabulated value of chi square \( x^2 \) 7.82 at the 3 degree of freedom at 0.05 level of significant there for, there was no significant association for the knowledge score among the samples.

**Conclusion**

The present studies assess the knowledge and attitude regarding effects of air pollution on health among the community people, on the basis of the study the following conclusion were made: the air pollution among the community people are high rather than other residing people.

And they are getting more respiratory problems due to the air pollution.

**Financial support and sponsorship:** Self

**Conflict of interest:** There is no conflict of interest

**Bibliography**

Risk Factors of Stunting in Children Aged 6-59 Months: A Case-Control Study in Horticulture Area

Prayudhy Yushananta¹, Mei Ahyanti¹, Yetti Anggraini¹
¹Assistant Professor, Tanjungkarang Health Polytechnic

Abstract

Background. Stunting is a critical public health problem in Indonesia because it affects cognitive and physical development and contributes to child mortality. This study aims to identify risk factors for stunting in children aged 6-59 in the horticultural area. Methods. A case-control study was conducted to compare previous exposure between stunted children and non-stunted children. Measurements and interviews were conducted with 160 participants (120 controls and 40 cases), including mothers or caregivers. SPSS was used for X² statistical analysis, multiple logistic regression, and odds ratios. Results. The study identified four risk factors for stunting: children who were born short (AOR = 17.57; 95% CI: 5.02-61.51), LBW (AOR = 4.35; 95% CI: 1.38-13, 78), and got a low protein intake (AOR = 4.96; 95% CI: 1.22-20.26). Significantly, a relationship between stunting and access to sanitation was also found (AOR = 6.06; 95% CI: 1.25-29.35). Conclusion. The risk factors for stunting in children aged 6-59 are related to nutrition during pregnancy and the child’s quality of food. Nutrition interventions should emphasize improving the nutritional status of pregnant women and children and women empowering to affect access to resources and allocations for children’s nutrition.

Keyword: Stunting, birth length, LBW, horticulture, under-five

Introduction

Malnutrition is a critical public health problem for children under five in developing countries, including Indonesia. Malnutrition is due to many interrelated factors and has detrimental health effects in the short and long term 1,2. Malnutrition will affect children’s cognitive and physical development, increase the risk of infection, and significantly contribute to child morbidity and mortality 3-6. The high indicators of malnutrition in a country reflect children’s low nutritional status and health under five 2,7. Three extensively recognized indicators of children’s nutritional status are stunting, wasting, and underweight, and stunting indicates chronic malnutrition form 1,2,6,8-11. A stunted child if their height for age is more than two standard deviations below the median of the World Health Organization (WHO) 2005 2,12.

Stunting is the best measure of malnutrition in childhood, a predictor for long-term morbidity and mortality, and long-term societal costs 13. Children who suffer from stunting will grow into adults at risk of obesity, glucose tolerance, coronary heart disease, hypertension, osteoporosis, decreased performance, and productivity 2,5,6,10,11,13,14.

Globally, in 2025, malnutrition contributes to at least half of all deaths each year in children under five 7,13,15. In 2025, estimating 127 million will be stunted 16. Prevalence was greater in developing countries, especially in South Asia and Africa 15-17. In Indonesia, the stunting prevalence was 30.8%, consisting of 11.5% very short and 19.3% short.
Many factors are associated with stunting. Several studies reported socioeconomic inequality, geographic differences, practices of feeding, food insecurity, education, and childhood morbidity, infection, and environmental 3–6,11. Stunting is also associated with micronutrient deficiencies, such as protein, iron, zinc, calcium, and vitamins D, A, and C 15. There are limited research reports on risk factors for stunting, especially in horticultural farming areas. In the study area (Liwa City), the risk factors for stunting in children aged 24-59 months have not been studied. It is crucial to identify risk factors for stunting to overcome the problem of stunting and its consequences. The study aimed to identified risk factors for stunting among children under five in horticultural farming areas.

Methods

A case-control study was conducted in Liwa City, West Lampung Regency, to compare previous exposures between stunted children (cases) and non-stunting (controls). This research was conducted after obtaining approval from the Health Research Ethics Committee, Tanjungkarang Health Polytechnic(No.261/KEPK-TJK/V/2020). Guided by the Helsinki protocol, informed consent was taken, and data handling was confidential. No risk of harm would be to the participants, and participants have the right to withdraw during the study. All study procedures were described before the interview.

The study was conducted from July to August 2020. Children aged 6-59 months with a mother or caregiver who lived for at least six months in the study area were included. Children without mothers or caregivers, children who appeared to have physical disabilities, children whose exact age was unknown were excluded from the study. Controls selected from the case’s nearby neighbor who was of the same age. If multiple controls are found, they are randomly selected. The exposure considered was parenting (32.9%). Assuming 95% CI, 90% power, control to case ratio 3:1, the total sample size is 160 (120 controls dan 40 cases).

Data were collected from measurements and interviews using a questionnaire. Data were entered into SPSS (24.0) after checked for completeness, edited, coded. The analysis used the Chi-square, Crude OR, and Multiple Logistic Regression analysis.

## Results

A total of 160 (120 controls and 40 cases) children aged 6-59 months and their mothers or caregivers participated in the study. Nobody dropped out during the study period, so the participation rate was 100%. The number of samples was boys and girls almost equal (Table 1), and most were in the 6-23 month age group (73.13%). The majority of mothers or caregivers have completed junior high school (71.25%), they do not work (60.0%), and the family income is low (81.25%).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex of childs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>22 (55,0)</td>
<td>54 (45,0)</td>
<td>0,361</td>
</tr>
<tr>
<td>Male</td>
<td>18 (45,0)</td>
<td>66 (55,0)</td>
<td></td>
</tr>
<tr>
<td>Age of childs (months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-23</td>
<td>29 (73,5)</td>
<td>88 (73,3)</td>
<td>1,000</td>
</tr>
<tr>
<td>24-59</td>
<td>11 (27,5)</td>
<td>32 (26,7)</td>
<td></td>
</tr>
</tbody>
</table>
Although the majority was normal (Table 2), we found about 19 (47.5%) of children in the case group and 4 (3.3%) in the control group were born stunted. There were also 13 (32.5%) children in the case group and 9 (7.5%) in the control group born with low birth weight. Complete immunization was obtained by about 36 (90.0%) children in the case group and 92 (76.7%) in the control group. Almost all (90.63%) children in the case and control groups received adequate protein intake. However, around 24 (60%) in the case group and 59 (49.2%) lacked parenting.

Table 2. Health and child feeding characteristic

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case (n=40)</th>
<th>Control (n=120)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth length</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>19 (47,5)</td>
<td>4 (3,3)</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Normal</td>
<td>21 (52,5)</td>
<td>116 (96,7)</td>
<td></td>
</tr>
<tr>
<td>Birth weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>13 (32,5)</td>
<td>9 (7,5)</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Normal</td>
<td>27 (67,5)</td>
<td>111 (92,5)</td>
<td></td>
</tr>
<tr>
<td>Immunization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incomplete</td>
<td>4 (10,0)</td>
<td>28 (23,3)</td>
<td>0,110</td>
</tr>
<tr>
<td>Complete</td>
<td>36 (90,0)</td>
<td>92 (76,7)</td>
<td></td>
</tr>
<tr>
<td>Protein intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>10 (25,0)</td>
<td>5 (4,2)</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Adequate</td>
<td>30 (75,0)</td>
<td>115 (95,8)</td>
<td></td>
</tr>
<tr>
<td>Parenting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack</td>
<td>24 (60,0)</td>
<td>59 (49,2)</td>
<td>0,315</td>
</tr>
<tr>
<td>Normal</td>
<td>16 (40,0)</td>
<td>61 (50,8)</td>
<td></td>
</tr>
</tbody>
</table>
Almost all children in the case group (95.0%) and the control group (97.5%) were found in homes with access to safe drinking water. However, about 7 (17.5%) children in the case group and 4 (3.3%) in the control group were found in homes without access to healthy sanitation, as Table 3 shows.

### Table 3. Environmental characteristic

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case (n=40)</th>
<th>Control (n=120)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
</tr>
<tr>
<td>Acces to safe drinking water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-acces</td>
<td>2 (5.0)</td>
<td>3 (2.5)</td>
<td>0.793</td>
</tr>
<tr>
<td>Acces</td>
<td>38 (95.0)</td>
<td>117 (97.5)</td>
<td></td>
</tr>
<tr>
<td>Acces to health sanitation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-acces</td>
<td>7 (17.5)</td>
<td>4 (3.3)</td>
<td>0.007</td>
</tr>
<tr>
<td>Acces</td>
<td>33 (82.5)</td>
<td>116 (96.7)</td>
<td></td>
</tr>
</tbody>
</table>

Only 4 of the 12 variables associated with stunting (p <0.05) were shown from multiple logistic regression analysis (Table 4). Interaction tests were also carried out, but none of them showed interactions between variables. The fit model is shown by the Homers and Lemeshow test obtained (p-value = 0.253).

The proportion of children born shortly was significantly higher in the case group than in the control group. Low birth length (boy less than 46.1 cm, and girl less than 45.6 cm) was found to be a risk factor for stunting (adjusted odds ratio (AOR) = 17.57; 95% confident interval (CI): 5.02-61.51). Birth weight less than 2500 grams was also a risk factor for stunting (AOR = 4.35; 95% CI: 1.38-13.78). The proportion of children with low protein intake was higher in the case group than in the control group (AOR = 4.96; 95% CI: 1.22-20.26). We also found a statistically significant relationship between house access to sanitation and stunting (AOR = 6.06; 95% CI: 1.25-29.35). This study found that the dominant variable related to stunting was the low birth length.
Table 4. Risk factor for stunting

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case (n=40)</th>
<th>Control (n=120)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth length</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>19 (47,5)</td>
<td>4 (3,3)</td>
<td>26,24 (8,11-84,89)</td>
<td>17,57 (5,02-61,51)</td>
</tr>
<tr>
<td>Normal</td>
<td>21 (52,5)</td>
<td>116 (96,7)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Birth weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>13 (32,5)</td>
<td>9 (7,5)</td>
<td>5,94 (2,30-15,33)</td>
<td>4,35 (1,38-13,78)</td>
</tr>
<tr>
<td>Normal</td>
<td>27 (67,5)</td>
<td>111 (92,5)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Protein intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>10 (25,0)</td>
<td>5 (4,2)</td>
<td>7,67 (2,43-24,12)</td>
<td>4,96 (1,22-20,26)</td>
</tr>
<tr>
<td>Aquate</td>
<td>30 (75,0)</td>
<td>115 (95,8)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Access to health sanitation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-access</td>
<td>7 (17,5)</td>
<td>4 (3,3)</td>
<td>6,15 (1,69-22,3)</td>
<td>6,06 (1,25-29,35)</td>
</tr>
<tr>
<td>Access</td>
<td>33 (82,5)</td>
<td>116 (96,7)</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Discussion

Of all the factors studied, the length of birth showed the dominant risk factor for stunting in horticulture farming areas. The results confirm Islam, that low birth length and LBW are relationships with stunting. Birth length is associated with low maternal nutritional intake during pregnancy, which is influenced by low family economic status and food insecurity in the family.

Food insecurity in the family results in a decrease in the variety and the nutritional value of food consumed. It will sustainably affect the family’s nutritional status, including child development. In pregnant women, which impacts stunted babies’ birth, Access and availability of food for the poor combine poverty problems, lack of permanent jobs, low and irregular cash income, and limited purchasing power, and closely related to low education levels.

In addition to impaired motor and verbal development, an increase in degenerative diseases, morbidity, and mortality, a further concern of stunting is the disruption of cognitive development. Most child early malnutrition did not finish high school and work as manual laborers. Impaired cognitive development and learning achievement will reduce work productivity to hinder economic growth, increase poverty, and widen inequality in a country.

Multivariate analysis showed that four variables were significantly associated with the incidence of stunting. If related to the child’s life span, it has caused the mother’s nutritional status during pregnancy. These
results explain the concept of stunting in the first 1000 days of life. The role of mothers is critical in facilitating interventions through strengthening their nutritional status during pregnancy and breastfeeding.

Malnutrition in pregnancy results from a low average intake of protein, fat, total energy, and often insufficient micronutrients such as folate, Fe, Ca, and Zn. Malnutrition in pregnant women affects disruption of intra-uterine growth due to LBW, stuntedness, perinatal mortality. Linear growth failure is mostly caused in the intra-uterine period due to an inadequate diet.

Malnutrition in pregnancy is detected from anemia. Anemia is a condition characterized by an abnormal decrease in the total mass of red blood cells caused by blood loss due to acute or chronic bleeding, destruction of red blood cells, and insufficient red blood cell production. Anemia is a risk for pregnant women in agricultural areas due to the chronic impact of pesticide exposure. Sanitation access is associated with increased exposure to microbes and infectious diseases, especially diarrhea. Fecal-oral pathways are water, food, vectors, and vectors.

Control with a nutrition approach for the first 1,000 days by promoting healthy behaviors, breastfeeding, nutrition during pregnancy includes micronutrient supplementation, breastfeeding, and disease prevention will reducing child malnutrition, especially chronic malnutrition in the form of stunting. Nutrition sensitive interventions must also highlight a fundamentally important factor that indirectly impacts mothers’ and children’s nutrition, namely women’s empowerment. Empowerment of women is a process of improving women’s institutions and status, affecting household access to resources, including allocations for children’s health and nutrition.

**Conclusion**

This study found four factors associated with stunting among children aged 6 to 59 months in horticulture farming areas, length at birth, LBW, protein intake, and access sanitation. Of the four variables, it indicates malnutritional during pregnancy. Therefore, it needs intervention and nutrition programs for pregnant women, including micronutrient supplementation and fortification. It also empowers women in the family to affect household access to resources, including allocations for children’s health and nutrition.

**Conflict of Interest:** Authors declare no conflict of Interest in this study

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23. Kinshella MLW, Moore SE, Elango R. The missing focus on women’s health in the First 1,000 Days approach to nutrition. Public Health Nutrition. 2020;


A Prospective Study of Burn Deaths with Special Reference to Manner of Death and Duration of Survival

Priyamvada Kurveti Verma¹, Rajendra Kumar Morya², Dheeraj Singh Verma³

¹Associate Professor, ²Ex-Resident, ³Demonstrator; Department of Forensic Medicine and Toxicology, Gandhi Medical College, Bhopal, Madhya Pradesh, Presently ²Assistant Professor, Department of Forensic Medicine and Toxicology, Government Medical College, Shivpuri, Madhya Pradesh

Abstract

Around 0.2 million people are admitted in hospital due to burns, of which about 5000 people die every year. Extent of Total Body Surface Area involved is an important parameter, that affects the prognosis in case of burn injury. The majority of fire related deaths are accidental, usually due to carelessness. Suicidal burns are mostly seen in domestic situations and in females. Homicidal burns are rare; mostly accomplished to take revenge or to conceal crime. So, this study was carried out with the aim to estimate the total body surface area affected & duration of survival / hospital stay and to find out the manner of death in burn cases.

It was found that 79.00% cases had burn injuries covering 60–100% of the total body surface area (TBSA). Maximum percentage of burns was seen in females (30.00% cases) with TBSA of 80-90%. 95% cases were hospitalized with 75.00% having the hospitalization period of 1 – 10 days and maximum number of cases (33%) survived for a period of 3 to 5 days. In most of the cases (64.00%), the manner of death was accidental followed by suicidal (34.00%), with only 2% cases of homicidal burns.

Keywords: burn deaths, manner of death, total body surface area, duration of survival.

Introduction

Burns are a serious health problem having physical, emotional and psychological impact on sufferers. More than 20 lakhs people sustain burn injuries, nationwide every year. Nearly, 0.5 million people were treated as outdoor patients. Around 0.2 million people are admitted in hospital, of which about 5000 people die every year. Extent of Total Body Surface Area involved is most important parameter that affects the prognosis in case of burn injury. There is marked fluid loss resulting in shock, in case more than 20 per cent of the body is affected and usually more than 50 per cent is fatal. Generally speaking, involvement of about one-third (33%) of total body surface area has grave prognosis and about 50% involvement of total body surface area is expected to be fatal in present Indian circumstances. However, with recent advances in modalities of treatment, prognosis is expected to improve the prognosis of injury.

Deaths from burns are usually accidental but may be of suicidal or homicidal origin. The majority of fire related deaths are accidental and there is typically abundant collateral evidence, from police and fire brigade investigations, to exclude suicide or homicide. These deaths are usually the result of carelessness, in allowing clothing to brush against fires, in manipulating matches or other lighted objects such as cigarettes, maintaining faulty electrical and heating appliances, as well as being unable to effectively combat or escape a fire.

Alcoholics and other individuals under the influence of drink or drugs are at risk. Occasionally a natural
disease such as epilepsy or a myocardial infarction may cause the victim to collapse onto a heater, starting a fire; the same natural disease may explain failure to escape the fire.\textsuperscript{13}

Suicide by burns is mostly seen in domestic situations like bedroom, bathroom or kitchen. Self immolation as protest against some social injustice is also common. It is common in females especially amongst newlyweds because of marital disputes and inflammable material being very much in reach. Generally fuel is poured on head and it reaches other part of body tricking downwards. Hence, the body catches fire all at a time. Smell of fuel will be present particularly in scalp hairs and clothing worn at the time of incidence. Soles may be spared, but the percentage of area involved is generally more than 60%.

Homicidal burns are relatively rare and seen in cases where paraffin or some other inflammable material is thrown over victim and his/her clothing then set alight\textsuperscript{14}. It is not unusual for murderer to try to dispose off the body of the victim by fire to conceal crime. At times, some people may cause burn injuries on a dead body and then produce it before the police to support a false charge of murder against his adversary. In both the cases, care should be taken to distinguish between ante mortem and postmortem burns to show that the victim was or was not alive at the time of fire.\textsuperscript{8}

Hence, this autopsy based cross sectional study was planned with the aim to estimate the total body surface area affected & duration of survival / hospital stay and to find out the manner of death in burn cases.

**Material and Methods**

This prospective study was conducted in the Department of Forensic Medicine and Toxicology, at Gandhi Medical College & Hamidia Hospital, Bhopal for a period of 1.6 years. Cases of burns either admitted or directly brought dead to the institute, cases with proper hospital records and cases of spot death due to burns were included. Out of all those, 100 cases were selected by simple random sampling, for the study.

Detailed information regarding the demographic profile of the cases, date, time and place of incidence, duration of hospital stay, cause and manner of death was gathered using a questionnaire; from the case dairies of police that included the inquest papers and dying declaration of deceased, hospital records, autopsy reports and history given by relatives and police accompanying the body.

To estimate the extent of body surface area affected by burns, \textbf{Rule of Nines}\textsuperscript{15} given by Alexander Wallace (Fig. 1A)\textsuperscript{15} was applied; the body being divided into different areas, each representing nine per cent. Head including face and neck 9%, Chest, front & Back each 9%, Abdomen, front & Back each 9%, Upper limbs, Right & Left each 9%, Lower limbs, right & Left each 18%, External genitalia 01%, Total = 100%. For children (less than 15 years of age) and Infants, \textbf{Lund and Browder chart}\textsuperscript{9} was used (Fig. 1B).\textsuperscript{9}
Results And Discussion

On analyzing the collected data, following observations were made:

Figure-1 depicts Estimation of total body surface area in Adults and Children (A- Rule of Nine and B- Lund and Browder)⁹,¹⁵

I. Distribution of cases according to Total Body Surface Area (TBSA) affected by Burns

Graph-1: Distribution of cases according to Total Body Surface Area affected by Burns
In the present study, majority i.e. 79 (79.00%) cases, had burn injuries covering 60– 100% of the total body surface area (TBSA). Maximum percentage of burns was seen in females i.e. 30 (30.00%) cases with 80-90% TBSA; as compared to males where 20 (20.00%) cases with 50-70 % TBSA were seen. TBSA of 35% (minimum in this study) was seen in males and 100% TBSA seen in females.

This observation was somewhat similar to the study of Harish D et al (2013)\(^7\) where 22% (85) victims had 61- 70% TBSA, followed by 71- 80% TBSA in 17% (64) cases i.e. majority of the victims (about 40%) had burn injuries covering 61– 80% of the total body surface area. Memchoubi and H. Nabachandra (2007)\(^12\) observed that in 73.84% cases, >80% body surface area was involved. Mazumder A and Patowary A (2013)\(^11\) observed in most of the victims the burn injury covers 90-100% of the total body surface area followed closely by 50-60% of the total body surface area involvement.

Chawla R et al (2011)\(^4\) found that in males, maximum 10% cases suffered burns to the extent of 0-50%, followed by 8% to the extent of 81-90% TBSA. In females, maximum 26% cases fell in the category of 91-100% of TBSA. Gowri S et al (2012)\(^6\) reported that 39% of males with total burn surface area <19% whereas in 40% females, total burn surface area was >61%.

Chaudhary BL et al (2013)\(^3\) in their study reported that 96 (46.37%) cases had total body surface area of burn between 90 to 100% followed by 80-90% in 29 (14.00%) cases. Buchade D et al (2011)\(^2\) observed that 51 to 75% burns found in133 (56.12%) cases. Mangal HM et al (2007)\(^10\) noted that 232 cases (77.33 %) had >50 % of body surface area, while in 48 cases (16 %) 40-50 % body surface area was burnt and in only 20 cases (6.67%) the involved body surface area was <40%.

### II. Distribution of cases in accordance with Duration of Hospitalization (Survival)

<table>
<thead>
<tr>
<th>Duration</th>
<th>Number of Cases</th>
<th>Percentage of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bought Dead</td>
<td>2</td>
<td>2 %</td>
</tr>
<tr>
<td>&lt;24 hours</td>
<td>11</td>
<td>11 %</td>
</tr>
<tr>
<td>1-3 days</td>
<td>27</td>
<td>27 %</td>
</tr>
<tr>
<td>3-5 days</td>
<td>33</td>
<td>33 %</td>
</tr>
<tr>
<td>5-7 days</td>
<td>6</td>
<td>6 %</td>
</tr>
<tr>
<td>7-10 days</td>
<td>9</td>
<td>9 %</td>
</tr>
<tr>
<td>10-15 days</td>
<td>4</td>
<td>4 %</td>
</tr>
<tr>
<td>15-30 days</td>
<td>6</td>
<td>6 %</td>
</tr>
<tr>
<td>&gt;30 days</td>
<td>2</td>
<td>2 %</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

95 cases were hospitalized (95%), two cases were of spot death and 3 cases were declared brought dead at casualty. Majority i.e. 75 (75.00%) cases had the hospitalization period of 1 – 10 days. 11 (11.00%) cases succumbed to death in less than 24 hours. Maximum duration of hospitalization after sustaining burns was 45 days.
Harish D et al (2013)\(^7\) made similar observation that majority of patients i.e. 102 (24%) surviving for more than a week before succumbing to the death and 31 (8%) cases died within the 1\(^{st}\) 24 hours of sustaining burn injuries. Chaudhary BL et al (2013)\(^3\) observed in their study that 150 (72.46%) cases of burns died in hospital after admission during course of treatment while 57 (27.53%) cases died on the spot and declared brought dead in hospital. In 42 cases (20.28%) period of survival after sustaining burn injury was between 7 to 15 days followed by 39 (18.84%) cases where survival period was 3 to 7 days. The maximum period of survival was 59 days in a single case.

On the contrary, Memchoubi and H. Nabachandra (2007)\(^12\) in their study observed that 49.23% victims died within an hour of sustaining burns whereas 21.53% cases survived for more than 1 week. Chawla R et al (2011)\(^4\) reported that 40% cases died within few minutes to 24 hours of sustaining burns, 24% cases died within a week and 20% cases died within 2 weeks. 12% cases died within 3 weeks and beyond.

### III. Distribution of cases on the basis of Manner of Infliction of burns

![Graph-2: Distribution of cases on the basis of Manner of Infliction of burns](image)

On the basis of history, police investigations and postmortem findings, it was observed that in most of the cases i.e. 64 cases (64.00%), the manner of death was accidental, followed by suicidal in 34 cases (34.00%). Out of 34 suicidal cases, 33 (97.00%) cases had alleged history of pouring of kerosene and then setting fire. Homicidal burns were seen in 2 cases, who were newly married females.

This finding of the present study is similar to Chaudhary BL et al (2013)\(^3\) who observed accidental burns in 72.94% cases, followed by suicidal in 17.39% and homicidal in 9.66% cases. Buchade D et al (2011)\(^2\), also found that most common manner of the burn was accidental in 147 (62.02%) cases, followed by suicidal in 62 (26.16%) and homicidal in 28 (11.82%) cases. Mangal HM et al (2007)\(^10\) conducted their study on 300 cases and observed that in most of the burn victims, the manner of death was accidental in 183 cases (61%), followed by suicidal in 105 cases (35%) and homicidal in only 12 (4%) cases. Similar observations were made by Das KC (1998)\(^5\) and Bangal RS (1995)\(^1\).
It is very difficult to state that a burn injury is accidental or suicidal or homicidal in nature, until and unless an eye witness is there. At times, the only way to prove homicidal nature is the Dying Declaration, which is very difficult to obtain either due to unconsciousness of victim, negligence or lack of knowledge on the part of the police. The higher number of accidental deaths especially in females may be due to their involvement in domestic cooking work responsible for accidental cases; and suicidal incidents might be because of marital mal-adjustment resulting in suicidal or bride burning cases owing to dowry disputes.

**Conclusion**

From the present study, it is evident that maximum number of cases (79.00%), had burn injuries covering 60–100% of the total body surface area (TBSA), with TBSA of 80-90% seen predominantly in females. 75.00% cases had the hospitalization period of 1 – 10 days. 11.00% cases succumbed to death in less than 24 hours. Maximum duration of hospitalization after sustaining burns was 45 days. The manner of death was accidental in majority (64.00%), followed by suicidal (34.00%) and least numbers were homicidal (2%).

Similarly, Harish D et al (2013)\(^7\), Memchoubi and H. Nabachandra (2007)\(^12\), Mazumder A and Patowary A (2013)\(^11\) in their study also reported TBSA of more than 60% in maximum cases. Chawla R et al (2011)\(^4\) and Gowri S et al (2012)\(^6\) also observed maximum % TBSA in females. Chaudhary BL et al (2013)\(^17\) also noted survival/ hospitalization period of 7-15 days in majority of the cases, quite similar to the present study. Buchade D et al (2011)\(^2\), Mangal HM et al (2007)\(^10\), Das KC (1996)\(^3\) and Bangal RS (1995)\(^1\) in their respective studies, also found accidental burns to be the most common manner of death.

With advanced burn care, survival rate in patients less than 60% TBSA of burns and without any significant comorbidities, have improved up to a great extent. But still there’s a long way to go to achieve the targets. Educating masses regarding safety precautions to be taken while handling fire; and counseling by experts to control the in- built aggression in individuals and to inculcate awareness; is the need of the hour.

**Conflict of Interest :** None

**Source of Funding:** self with assistance from the institute.

**Ethical Clearance:** The study protocol was approved by the Institutional Ethics Committee of Gandhi Medical College, Bhopal.

**References**

11. Mazumdar A, Patowary A. A Study of Pattern of


Knowledge of Oral and Dental Health Impacts the Oral Hygiene Index Simplified (OHI-S) of Primary School Children

Pudentiana Rr RE¹, Tedi Purnama¹, Emini¹, Siti Nurbayani Tauchid¹, Neni Prihatiningsih²

¹Lecturer, Departement of Dental Health Ministry of Health Polytechnic Jakarta I, Indonesia,
²Dental Therapist of Agidental Clinic Modrland Tangerang City, Indonesia

Abstract

Primary school children are a vulnerable group to oral and dental diseases. Less of knowledge on dental health will lead to bad dental and oral hygiene status resulting in debris and calculus. The OHI-S is an ideal dental and oral hygiene check tool for assessing the oral hygiene of primary school children. This study aims to analyze the relationship of knowledge of dental and oral health to Oral Hygiene Index Simplified in elementary school children. Method: this type of analytic observational study with cross sectional design. The research was conducted on the research carried out in class III students of SDN Perigi 03 Pondok Aren, South Tangerang City. Data collection with a questionnaire on dental health knowledge and Oral Hygiene Index Simplified. Data analysis using Chi-Square. Result: Knowledge of oral health to Oral Hygiene Index Simplified indicates that the p-value 0.023 (p < 0.05). Conclusion: there is a significant relationship between knowledge of dental and oral health to Oral Hygiene Index Simplified in primary school children.

Keywords: Knowledge, oral health, oral hygiene index simplified

Introduction

Oral and dental health is a part of body health that cannot be separated from one another because oral health will affect the health of the body. Maintenance of oral hygiene is an effort to improve oral health. The role of the oral cavity is very large for human health and well-being. In general, a person is said to be healthy, not only in a healthy body but also in the oral cavity and teeth. Therefore, oral health plays a very important role in supporting the health of one’s body. Dental and oral health issues are an important concern in health development, one of which is the vulnerability of school-age children to dental health problems.¹–⁵

Several studies in Indonesia prove that the dental and oral hygiene status of elementary school children is in the poor category. Among them, Alhamdaet al proved that the dental and oral hygiene status of students in the 12-year-old SDN Bukittinggi City was included in the medium category with an OHI-S average of 1.52; Research by Mawuntu et al proved the OHI-S index in St. Catholic Elementary School children Agustinus Kawangkoan 60% medium category.⁶,⁷

In other countries it also shows that the dental and oral hygiene status of elementary school children is in the moderate category. This is evidenced Denloye cit. Yohanes et al in Nigeria on children aged 13-15 years as outlined in his journals proved that the Debris Index (DI) was 1.57 and the Calculus Index (CI) was 1.48 with an average Oral Hygiene Index Status (OHI-S), for men reaching 3.09 and for women reaching 2.94 which is classified as mild to moderate.⁸ From the research above, it can be concluded in Indonesia and in the world, it shows that school-age children maintain their oral health and have poor oral hygiene status.

One of the main factors affecting oral and dental hygiene is behavior. Behavior that can influence the
development of caries is about how to maintain healthy teeth and mouth and behavior is strongly influenced by knowledge. Behavior based on correct knowledge will last longer than behavior without knowledge, including knowledge about how to maintain proper dental health will greatly affect the incidence of caries.6,9

This is evidenced by the results of research by Sari et al. (2015) showing that knowledge of oral health is related to the oral hygiene index, where the better the level of knowledge, the better the OHI-S index. Another study, Yohanes et al. (2013) showed that children who had good knowledge had a 2.2 times chance of having good oral and dental hygiene status.8,10

Children 6 to 12 years of age, at this stage, they often show the best in their lives, so dental health for them is one of the things that can motivate learning. This is of course every child’s dream, sometimes they feel embarrassed when a friend taunts them because their teeth are yellow. So at this time, the sense of responsibility for schoolwork and homework will be more visible. Therefore, children can be taught how to maintain oral health in more detail so that it will create a sense of responsibility for their own hygiene.11,12

Method

This research is an analytic observational study with a cross-sectional design. The research was conducted in third grade students of SDN Perigi 03 PondokAren, South Tangerang City. The research sample was taken by total sampling technique, namely as many as 51 respondents. The independent variable in this study was knowledge of dental health and the dependent variable was the dental hygiene status of elementary school children (OHI-S).

Data collection on dental health knowledge was measured using a questionnaire containing 15 questions about knowledge of dental health, while dental hygiene status was measured by standard dental hygiene checks for primary school children, namely Oral Hygiene Index Simplified (OHI-S). The stages of data collection activities were carried out in 3 stages: first the respondents were asked to sign an informed consent. The second provides a questionnaire about dental health knowledge.

The third performs an OHI-S index examination by applying a disclosing solution using a cotton pellet on the index tooth if there is staining on the tooth it means that there is debris on the tooth and if at the time the tooth is examined using a sonde and there is a rough surface it shows the tooth. there’s calculus. Then the results of the examination are recorded on the examination sheet.

Data analysis was performed using the SPSS statistical program for univariate analysis and presented in the form of a frequency distribution. After that was done bivariate analysis with chi-square to measure the relationship of dental health knowledge with the Simplified Oral Hygiene Index in primary school children.

Result

Table 1. Frequency distribution of dental and oral health knowledge

<table>
<thead>
<tr>
<th>No.</th>
<th>Knowledge</th>
<th>N</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Good</td>
<td>11</td>
<td>21.6</td>
</tr>
<tr>
<td>2</td>
<td>Enough</td>
<td>27</td>
<td>52.9</td>
</tr>
<tr>
<td>3</td>
<td>Less</td>
<td>13</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>51</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1 shows that respondents have good knowledge criteria of 11 children (21.6%), 27 children (52.9%) have enough knowledge criteria, and 13 children (25.5%) are categorized as less of knowledge.
Table 2. Frequency distribution of Oral Hygiene Index Simplified

<table>
<thead>
<tr>
<th>No.</th>
<th>OHI-S</th>
<th>N</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Good (0.0 - 1.2)</td>
<td>9</td>
<td>17.6</td>
</tr>
<tr>
<td>2</td>
<td>Moderate (1.3 - 3.0)</td>
<td>40</td>
<td>78.4</td>
</tr>
<tr>
<td>3</td>
<td>Bad (3.1 - 6.0)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2 shows that respondents who have a score range of 0.0 to 1.2 with good criteria are 9 children (17.6%), respondents who have scores in the range 1.3 to 3.0 are 40 children (78.4%) including moderate criteria, and in the range of scores of 3.1 to 6.0 a number of 2 children (4%) are considered bad criteria.

Table 3. Results of the chi-square analysis of dental health knowledge with Oral Hygiene Index Simplified

<table>
<thead>
<tr>
<th>Knowledge</th>
<th>Oral Hygiene Index Simplified</th>
<th>Total</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Moderate</td>
<td>Bad</td>
</tr>
<tr>
<td>Good</td>
<td>1 9.1</td>
<td>10 90.9</td>
<td>0 0</td>
</tr>
<tr>
<td>Enough</td>
<td>8 29.6</td>
<td>19 70.4</td>
<td>0 0</td>
</tr>
<tr>
<td>Less</td>
<td>0 0</td>
<td>11 84.6</td>
<td>2 15.4</td>
</tr>
<tr>
<td>Total</td>
<td>9 17.6</td>
<td>40 78.4</td>
<td>2 3.9</td>
</tr>
</tbody>
</table>

Table 3 results of the chi-square analysis of dental health knowledge against Oral Hygiene Index Simplified indicates that the p-value is 0.023 (p <0.05), which means that there is a significant relationship between knowledge of dental health and Oral Hygiene Index Simplified.

Discussion

The results of the research data were obtained respondents11 children (21.6%) had good dental health knowledge, 27 children (52.9%) had enough knowledge and 13 children (25.5%) had less knowledge. Knowledge is a very important domain for the formation of one’s actions. Knowledge of dental and oral health is very important for the formation of actions to maintain dental and oral hygiene. Maintaining dental hygiene is one way to improve dental health.11,13 The results of this study are not much different from the results of research by Restuaastuti et al regarding knowledge and behavior of dental health among students of SDN 174 Muara FajarPekanbaru, it is known that 25 students (41%) have insufficient knowledge of dental health and as many as
25 students (41%) have bad behavior regarding brushing their teeth.

The results of the research data showed that respondents who had good OHI-S criteria were 9 children (17.6%), respondents who had moderate OHI-S criteria were 40 children (78.4%) and a number of 2 children (4%) had OHI-S bad criteria. This is possible due to the less of knowledge of dental health maintenance, so with relevant research. Research by Mawuntu et al on the description of the dental hygiene status of St. Catholic Elementary School students, Agustinus Kawangkoan. Obtained from 65 children who were examined using the OHI-S index showed that 37% of respondents were in the good category; 60% moderate category; and 3% bad category.

The result research about dental health knowledge against Oral Hygiene Index Simplified indicates that the p-value is 0.023 (p <0.05), which means that there is a significant relationship between knowledge of dental health and dental health Oral Hygiene Index Simplified. The results of this study agree with the research. This is evidenced by the results of research by Sari et al. showing that knowledge of oral health is related to the oral hygiene index. Another study, Yohanes et al. also stated that it showed knowledge of dental and oral health was related to the oral hygiene index of children.

Knowledge of oral and dental health is formed from a person’s behavior. Other studies also show a correlation between knowledge and oral health behavior. Remembering the magnitude of the influence of behavior on the degree of dental and oral health, a special approach is needed in shaping positive behavior towards dental health. A positive attitude will affect the intention to participate in activities related to this and a person’s attitude is closely related to the knowledge he/she receives, especially dental health. Knowledge is the basis for the formation of a behavior. A person is said to be less knowledgeable if he is in a state of being unable to recognize and explain and analyze a situation. If the level of knowledge is higher.

**Conclusion**

Based on the research results, it can be concluded that there is a significant relationship (p <0.023) between knowledge of dental health and Oral Hygiene Index Simplified

**Acknowledgement:** This study was done by self-funding from the authors. The authors thank to all partisipants and research assistans

**Conflict of Interest:** The authors declare that they have no conflict interests.

**Ethical Clearance:** All participants were signed the informed consent prior to the data collection.

**References**

8. Yohanes IG, Pandelaki K, Mariati NW. Hubungan pengetahuan kebersihan gigi dan mulut dengan status kebersihan gigi dan mulut pada siswa SMA
The Difference of Plasma Prothrombin Time Value in the Production of Adsorbed Plasma Using Various Concentrations of Barium Sulphate in Dr. Soetomo General Hospital, Surabaya, Indonesia

Putu Devi Oktapiani Putri¹, Paulus Budiono Notopuro²

¹Resident, Clinical Pathology Specialization Program, Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia, ²Lecturer, Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia

Abstract

Detection of a factor deficiency in the case of coagulation prolongation is very important, in this case a factor deficient plasma is required. This research provides a simpler method to produce factor deficient plasma, using barium sulphate as an adsorbent with Prothrombin Time (PT) of more than 60 seconds as a target. The aim of this study is to analyze the difference of PT value in various concentrations of barium sulphate. Pooled normal plasma (PNP) was made from citrated blood and basic PT value was measured. Nine plain tubes were prepared with 1 mL of PNP for each tube. Barium sulphate 200-1000 mg/ml plasma was then added to each tube. Finally, PT value of each tube was measured. The presence of barium sulphate <800 mg/ml plasma made no significant changes in PT. Conversely, the presence of ≥800 mg/ml plasma increased PT more than 5 times compared to normal PT. Adsorbed plasma lack vitamin K dependent factors because barium sulphate binds to α-polypeptide, and removes all vitamin K dependent factors. The barium sulphate concentration used to make adsorbed plasma in this research was different with previous research. It was found that barium sulphate with ≥800 mg/ml plasma can significantly change the PT value, and effectively reach the PT target of adsorbed plasma as a basic reagent for substitution study. In conclusion, different concentrations of barium sulphate used resulted in different PT values. Optimal concentration of barium sulphate to make adsorbed plasma was ≥800 mg/ml plasma.

Keywords: Adsorbed plasma, barium sulphate, plasma prothrombin time.

Introduction

Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) examinations are routine tests performed to evaluate coagulation function. There are more than 40,000 PPT and APTT examinations at RSUD Dr. Soetomo per year with results that extend by 28%¹,². In order to determine whether the coagulation abnormality is caused by a lack of coagulation factors or the presence of an inhibitor, a mixing test with normal pooled plasma can be performed. If a coagulation factor deficiency is found, a substitution test can be performed to identify the deficient coagulation factor³,⁴,⁵.

Currently, several coagulation factor examinations are available, including the examination of VIII factor and IX factor. Both of these tests can provide information on levels of VIII factor and IX factor; however, these tests are quite expensive and cannot be performed in standard laboratories. The substitution test is a promising alternative as a method of examining coagulation factors. To perform a substitution test, two types of plasma are
required, namely aged plasma and adsorbed plasma. The availability of these two types of plasma considerably supports the diagnosis of a coagulation disorder, even in a standard laboratory.\textsuperscript{6,7,8}

Preparation of adsorbed plasma requires a standard method so that the adsorbed plasma obtained can be used in the substitution test. In order to produce adsorbed plasma, citrated blood plasma can be mixed with barium sulphate to achieve a predetermined PT value.\textsuperscript{9} This research examines the concentrations of barium sulphate that can be used for the manufacture of adsorbed plasma with citrated blood plasma.

**Method**

This research was conducted using cross-sectional analysis. The samples of this study were collected from veins of Clinical Pathology Resident, Faculty of Medicine, Universitas Airlangga - Dr. Soetomo General Hospital, Surabaya, Indonesia. Random sampling was done until 20 samples were obtained. This research was conducted in the Clinical Pathology Laboratory, Integrated Diagnostic Center Building, Dr. Soetomo General Hospital, Surabaya, Indonesia. The inclusion criteria were samples from Dr. Soetomo General Hospital, Surabaya, Indonesia which were accommodated in citrate anticoagulant tubes with volume in accordance with the minimum limit of citrate tube filling (2.7 mL). Exclusion criteria were samples with insufficient volume, clotted blood samples, icteric, lipemic, or hemolyzed samples. Independent T-test was performed to analyze the effect of difference of barium sulphate on PPT values. p value <0.05 was considered statistically significant. Data was analyzed using MS excel and SPSS software.

**Results and Discussion**

The preparation of adsorbed plasma in this study used several variations of barium sulphate concentration. An increase in the concentration of barium sulphate can affect the PT plasma value produced. A very sharp increase in the value of PT was found in the plasma with 900 mg/mL of barium sulphate. With barium sulphate concentration of 1000 mg, the PT value that appeared on the device was no coagulation. In this case, the upper limit of the PT value that could be detected by the device was used, namely 130 seconds (Figure 1).

![Figure 1. The value of PT in the preparation of adsorbed plasma with various concentrations of barium sulphate.](image-url)
In this research, several cut-offs of barium sulphate concentrations were chosen to observe how much change in the value of PT would occur when higher concentrations of barium sulphate were used or vice versa. In Table 1, it can be seen that there are 3 cut-offs, namely 600 mg/mL of plasma, 700 mg/mL of plasma, and 800 mg/mL of plasma. A difference test was performed on each cut-off mean value of PT that could be achieved. In the use of barium sulphate <600 mg/mL of plasma, the mean value of PT was only 11.48 seconds with p value of 0.49, which was not statistically significant. When barium sulphate was used at 800 mg/mL of plasma or above, the PT value was statistically significant reaching 86.77 seconds.

Table 1. The Difference of PT Values with Barium Sulphate Concentration Grouped based on Cutoffs.

<table>
<thead>
<tr>
<th>Concentration of Barium Sulphate (mg/mL of plasma)</th>
<th>Mean Value of PT (seconds)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;600</td>
<td>11.48</td>
<td>0.49</td>
</tr>
<tr>
<td>≥600</td>
<td>61.90</td>
<td></td>
</tr>
<tr>
<td>&lt;700</td>
<td>13.54</td>
<td>0.29</td>
</tr>
<tr>
<td>≥700</td>
<td>72.52</td>
<td></td>
</tr>
<tr>
<td>&lt;800</td>
<td>15.23</td>
<td>0.03</td>
</tr>
<tr>
<td>≥800</td>
<td>86.77</td>
<td></td>
</tr>
</tbody>
</table>

The use of barium sulphate in the preparation of adsorbed plasma has been investigated for decades. Hydrophobic materials such as barium sulphate and aluminum hydroxide can absorb certain coagulation factors (especially II, VII, IX, and X factors) when added to plasma. Other studies have shown that the use of aluminum hydroxide can reduce the activity of II, VII, IX, and X factors. Increasing the concentration of aluminum hydroxide used will further reduce the activity of these coagulation factors. Barium sulphate and plasma citrate was used in this research for the preparation of adsorbed plasma. The greater the concentration of barium sulphate used, the higher the PPT value. This suggests the possibility that the coagulation factors involved in the extrinsic coagulation pathway experience decreased activity.

Vitamin K-dependent coagulation factors have one special feature, which is the fact that they have the same polypeptide structure. In Nelsestuen and Suttie’s research, it was found that there was a structure called α peptide in prothrombin. This structure disappears after adsorption process. This α peptide is a vitamin K dependent structure, and is present in all vitamin K dependent proteins.

Adsorption process caused a decrease in the activity of II, VII, IX, and X factors. This study used aluminum hydroxide in gel form as an adsorbent. Each factor showed a different decrease in activity after the adsorption process. This was also influenced by differences in temperature and pH.

A literature stated that the concentration of barium sulphate used for the preparation of adsorbed plasma is 100 mg/mL of plasma. A different literature stated that 33 grams of barium sulphate is mixed with 150 mL of plasma, to which a buffer solution has been added, then stirred at room temperature for 30 minutes. In this study, the use of barium sulphate below 600 mg/mL of plasma did not significantly increase the PT value. The use of barium sulphate at concentration of 600 mg/mL plasma...
produced plasma with PT value of 19.4 seconds (Figure 1). There are several variations in the target value of PT in the preparation of adsorbed plasma, namely 2½ times the normal PT value, or PT >60 seconds\textsuperscript{13}. If the upper limit of normal PT value is 13 seconds, then the use of barium sulphate with concentration of 800 mg/mL of plasma would succeed in achieving the minimum target value of PPT, namely 32.5 seconds.

Several cut-offs were selected to determine the appropriate minimum concentration of barium sulphate in the preparation of adsorbed plasma. Based on the PT values achieved with the use of different concentrations of barium sulphate (Figure 1), several cutoffs for barium sulphate concentrations were taken as a reference to achieve the targeted PT value. Using the most minimum concentration of barium sulphate with the most effective result is very important for the efficiency of materials and processing time.

Table 1 shows some of the cutoffs that can be considered as minimal concentrations that can be used for the preparation of barium sulphate. If a lower barium sulphate concentration could achieve the targeted PT value, it would certainly increase the efficiency and effectiveness of the process in making adsorbed plasma. Barium sulphate levels <800 mg did not reach the targeted PT value (Figure 1), and statistically also did not provide a significant difference in PT values (Table 1). Barium sulphate concentration of ≥800 mg/mL of plasma resulted in the most statistically significant p value (p 0.03) with a PPT value that reached 34.3 seconds.

**Conclusion**

The use of barium sulphate with different concentrations produce plasma with different PT values. Higher levels of barium sulphate increase the plasma PPT value, however the increase is non-linear. Barium sulphate with concentration of ≥800 mg/mL is the most optimal for the preparation of adsorbed plasma as a basic reagent for substitution tests.

**Conflict of Interest:** The author declare that they have no conflict of interest.

**Source of Funding:** None.

**Acknowledgements:** We thank Rr. Putri Amaristy Purwono and Arif Nur Muhammad Ansori for editing the manuscript.

**Ethical Approval:** The research ethic has been approved by the Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

**References**


Effects of Etanercept on Clinical and Immunological outcomes in Iraqi Patients with Rheumatoid Arthritis

Qateralnada Rifaat Altohmoschi¹, Mohammed AH Jabarah AL-Zobaidy², Nizar Abdulateef Jasim³

¹M.Sc. Student, ²Assistant Professor of Pharmacology, Department of Pharmacology, ³Professor of Rheumatology, Department of Medicine, College of Medicine/ University of Baghdad, Iraq

Abstract

Background: Rheumatoid Arthritis is a chronic autoimmune disease affects joints and connective tissues where different types of cytokines are involved in its pathogenesis and progression. Biological drugs are considered to be a more targeted treatments for rheumatoid arthritis. Therefore, the aim of current study was to explore the effects of etanercept, a biological drug that inhibits tumor necrosis-alpha, on clinical and immunological variables in Iraqi patients with RA. Methods: A prospective open-label study conducted from February to November/ 2020 at Rheumatology Unit, Baghdad Teaching Hospital/ Medical city, Iraq. The study involved 75 patients with rheumatoid arthritis who underwent a 12-week course of treatment with etanercept monotherapy or combined with methotrexate. Assessments of disease activity and immunological markers (using SDAI and ELISA technique, respectively) were performed for the patients after the course of treatment and compared with healthy controls. Results: Data from current study showed that disease activity was not affected by type of treatment and immunological markers were higher in patients with rheumatoid arthritis than in control subjects regardless of type of treatment. Conclusion: To achieve an effective control of rheumatoid arthritis disease activity, treatment with etanercept (monotherapy or combined with methotrexate) needs to be continued for more 12 weeks.

Keywords: Etanercept, Immunological markers. Methotrexate, Pharmacology, Rheumatoid arthritis, SDAI.

Introduction

Rheumatoid Arthritis (RA) is a chronic autoimmune disease that affects joints and connective tissues with associated vascular, metabolic, bone and psychological comorbidities (¹). RA can begin at any age, but the likelihood increases with age as onset of RA is highest among adults in their sixties (²). Women are 2-3 times more likely to develop RA than men, maybe because oestrogens have both stimulatory and inhibitory effects on the immune system (³). Different factors can contribute to pathogenesis of RA (Figure 1).

Figure (1): Factors contributing to development of RA (⁴).
In RA, T cells infiltrate into the synovial membrane where they induce and maintain activation of macrophages and synovial fibroblasts, transforming them into tissue-destructive effector cells (5). In addition, B lymphocytes play many important roles in the pathogenesis of RA. They are the source of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) that contribute to the formation of immune complexes and complement activation (6).

Cytokines are molecules that, in cases of inflammation, infection or injury, are secreted by immune cells and allow cell to cell communication (7). In RA, they can be categorized into four groups: pro-inflammatory cytokines (IL-1 and TNF-alpha), joint inflammatory cytokines, anti-inflammatory cytokines (IL-4, IL-10, IL-11, IL-13 and IL-20) and natural cytokine antagonists (IL-1 receptor antagonist, soluble type 2 IL-1 receptor, soluble TNF receptor and IL-18 binding protein (7).

Diagnosis of RA is achieved by reviewing symptoms, conducting a physical examination, and doing X-rays as well as biochemical tests. The latter include rheumatoid factor, anti-citrullinated protein antibodies, erythrocyte sedimentation rate and C-reactive protein (8).

The aims of treatment of RA are achieving the lowest possible level of arthritis disease activity and remission if possible, reducing joint damage and improving physical function as well as quality of life (9). Moreover, treatment options for patients with RA are presented in Figure (2).

![Figure (2): Available treatments for RA (4).](image-url)
Although they are considered to be a more “direct, defined and targeted” treatments, biological DMARDs may cause a range of unwanted effects such as increased risk of infections and neurologic symptoms similar to those of multiple sclerosis and lymphoma (10). In addition, these medications tend to be very expensive and they are often used in combination with other DMARDs, especially methotrexate.

In Iraq, biological agents are imported from international drug companies via Ministry of Health and distributed to registered RA patients in specialized centers, such as the one in Baghdad Teaching Hospital, over specific appointments (in weeks). However, this process can create time-, economic-, as well as health-, related burdens to the patient, his/her family and the health system. These burdens are related to supplementation of biologics by producers, administration bureaucracy, dispensing of the required amounts of these drugs for each patient, the cost of drugs in addition to the potential adverse effects and contraindications related to these agents.

Therefore, clinical and/or immunological evidence(s) are critical to adequately justify the use of these agents in treatment of RA.

Taken together, current study was conducted to investigate the effects of Etanercept, a biological agent that acts as an inhibitor of TNF, on clinical and immunological variables in Iraqi patients with RA.

Methods

A prospective open-label study conducted from February to November 2020 at Rheumatology Unit, Baghdad Teaching Hospital/ Medical city, Iraq.

In current study, 91 subjects (75 RA patients and 16 healthy controls), were recruited. Patients were those known to have RA as defined by the American College of Rheumatology (ACR). In addition, recruited patients were with failed response to methotrexate alone and/or another DMARD. Also, selected patients received biological treatment (Etanercept; 50mg/week subcutaneously) either alone or in combination with oral Methotrexate (MTX; 10-15mg/week) for at least 12 weeks. However, subjects who have chronic disease(s) (renal, hepatic, cardiovascular), those having Juvenile RA and/or history of TB were excluded. Moreover, women who are pregnant or breast feeding were also excluded from the study. Patients who participated in current study were interviewed to assess clinical features of the disease and to obtain medical history.

Clinical evaluation of patients was performed using Simplified Disease Activity Index (SDAI; 11) has been validated for both research and clinical uses. On the other hand, the evaluation of patients’ ratings of general health and pain was performed using 10-cm Visual Analogue Scale (VAS; 12).

Furthermore, blood samples were obtained from study participants (12 weeks after starting treatment) for measurement of serum levels of high sensitive C-reactive protein), tumour necrosis-alpha, Interleukin 1-alpha and Interleukin-6. These markers were measured using ELISA technique.

Statistical analysis was performed using the SPSS software for windows version 20.0. Categorical variables were expressed as percentages and continuous variables were expressed as mean± standard deviation. All P values less than 0.05 were considered significant. The Mann Whitney test and Kruskal Wallis test were used.

Results

Demographic characteristics of participants

Results from current study revealed that there were no significant differences between patients and controls in terms of their mean age, mean body mass index and frequency of gender distribution (P values were 0.824, 0.881 and 0.237, respectively).

Moreover, results from current study showed that the median duration of the disease in RA patients was 10 years and ranged from 1 to 30 years. Also, results showed non-significant association between existence of RA disease in recruited patients and their family history, smoking history or diabetes mellitus (P values
were 0.582, 0.633 and 0.237, respectively). However, hypertension showed significant association ($P = 0.018$).

**Assessment of disease activity**

Patients with RA who participated in current study underwent clinical assessment for their disease activity using the SDAI. The median SDAI scores obtained in current study revealed that 62 patients had moderate – high disease activity and 13 patients had low disease activity (Table 1). However, with respect to treatment strategy and regardless of disease activity, RA patients taking etanercept monotherapy and those on combination therapy (etanercept and methotrexate) were not significantly different in their median tender joint count, visual analogue scale (by patients), visual analogue scale (by physician) and SDAI score ($P > 0.05$; Table 2).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with moderate to high disease activity</th>
<th>Patients with low disease activity</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 62</td>
<td>n = 13</td>
<td></td>
</tr>
<tr>
<td>Simplified Disease Activity Index (SDAI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>22.25 (13.70)</td>
<td>10.30 (2.21)</td>
<td>&lt; 0.001 M HS</td>
</tr>
<tr>
<td>Range</td>
<td>12.20 -62.70</td>
<td>4.70 -13.80</td>
<td></td>
</tr>
</tbody>
</table>

$n$: number of cases; IQR: inter-quartile range; M: Mann Whitney U test; HS: highly significant at $p \leq 0.01$

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with low disease activity</th>
<th>Patients with moderate-high disease activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ETC n = 4</td>
<td>ETC &amp; MTX n = 9</td>
</tr>
<tr>
<td></td>
<td>ETC n = 24</td>
<td>ETC &amp; MTX n = 38</td>
</tr>
<tr>
<td>Tender Joint count</td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>0.00 (3.00)</td>
<td>1.00 (2.00)</td>
</tr>
<tr>
<td>Range</td>
<td>0.00 -4.00</td>
<td>0.00 -2.00</td>
</tr>
<tr>
<td>Swollen Joint count</td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>Range</td>
<td>0.00 -0.00</td>
<td>0.00 -1.00</td>
</tr>
<tr>
<td>Visual analogue scale patient</td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>5.00 (1.50)</td>
<td>4.00 (1.50)</td>
</tr>
<tr>
<td>Range</td>
<td>3.00 -5.00</td>
<td>2.00 -5.00</td>
</tr>
</tbody>
</table>

Table 1: Clinical assessment of disease activity in patients with RA

Table 2 Clinical assessment of patients with RA according to treatment strategy
Results from current study showed that in RA patients (those with moderate-high and those with low disease activities), serum levels of Hs-CRP, IL-1alpha and IL-6 were higher than their serum levels in control subjects. Interestingly, these findings were not affected by treatment strategy i.e. Etanercept with or without Methotrexate in the respective patients groups (Tables 3&4).

**Immunological markers**

n: number of cases; IQR: inter-quartile range; M: Mann Whitney U test; NS: not significant at p > 0.05; S: significant at p ≤ 0.05. ETC= Etanercept. MTX= Methotrexate.

**Table 3: Immunological markers of RA patients with moderate to high disease activity according to treatment strategy**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ETC subgroup n = 24</th>
<th>ETC and MTX subgroup n = 38</th>
<th>Control group n = 16</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSCRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>8.10 (12.90)</td>
<td>6.50 (5.80)</td>
<td>0.74 (0.76)</td>
<td>&lt;0.001 K HS</td>
</tr>
<tr>
<td>Range</td>
<td>0.80 -18.10</td>
<td>0.62 -18.10</td>
<td>0.04 -2.09</td>
<td></td>
</tr>
<tr>
<td>IL-1-alpha</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>624.98 (70.92)</td>
<td>616.62 (111.04)</td>
<td>355.22 (36.82)</td>
<td>&lt;0.001 K HS</td>
</tr>
<tr>
<td>Range</td>
<td>327.55 -786.46</td>
<td>326.51 -838.82</td>
<td>281.49 -408.43</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>63.06 (16.72)</td>
<td>59.81 (16.49)</td>
<td>47.50 (5.80)</td>
<td>&lt;0.001 K HS</td>
</tr>
<tr>
<td>Range</td>
<td>47.43 -197.57</td>
<td>44.98 -197.20</td>
<td>40.12 -54.52</td>
<td></td>
</tr>
<tr>
<td>TNF-alpha</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>199.00 (36.11)</td>
<td>195.28 (43.72)</td>
<td>237.47 (29.02)</td>
<td>0.002 K HS</td>
</tr>
<tr>
<td>Range</td>
<td>168.50 -777.52</td>
<td>150.13 -1016.60</td>
<td>200.60 -272.60</td>
<td></td>
</tr>
</tbody>
</table>
Table 4: Immunological markers of RA patients with low disease activity according to treatment strategy

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ETC subgroup n = 4</th>
<th>ETC and MTX subgroup n = 9</th>
<th>Control group n = 16</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSCRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>1.73 (2.90)</td>
<td>1.49 (1.09)</td>
<td>0.74 (0.76)</td>
<td>0.040 K</td>
</tr>
<tr>
<td>Range</td>
<td>4.80-1.20</td>
<td>8.60-0.40</td>
<td>0.04 -2.09</td>
<td></td>
</tr>
<tr>
<td>IL-1-alpha</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>613.30 (56.74)</td>
<td>604.13 (19.97)</td>
<td>355.22 (36.82)</td>
<td>&lt; 0.001 K</td>
</tr>
<tr>
<td>Range</td>
<td>642.00-572.09</td>
<td>651.52-582.56</td>
<td>281.49-408.43</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>53.57 (2.32)</td>
<td>51.02 (5.06)</td>
<td>47.50 (5.80)</td>
<td>&lt; 0.001 K</td>
</tr>
<tr>
<td>Range</td>
<td>55.06-51.97</td>
<td>65.11-37.81</td>
<td>40.12-54.52</td>
<td></td>
</tr>
<tr>
<td>TNF-alpha</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>217.34 (92.26)</td>
<td>230.28 (54.11)</td>
<td>237.47 (29.02)</td>
<td>0.731 K</td>
</tr>
<tr>
<td>Range</td>
<td>266.09-164.11</td>
<td>275.83-180.46</td>
<td>200.60-272.60</td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; IQR: inter-quartile range; K: Kruskal Wallis test; HS: highly significant at p ≤ 0.01 indicate significant difference at p ≤ 0.05; letter (A) takes the highest value followed by letter (B) and then by letter (C). ETC= Etanercept. MTX= Methotrexate.

Discussion

Demographic characteristics of participants

The mean age of RA patients enrolled in current study (51.23 ±10.88 years) was higher than that reported by (13), yet it was comparable to that reported by (14). These data indicated that RA can affect any age group and its prevalence increases with age.

In addition, RA patients who participated in current study were overweight (BMI> 25Kg/m²), similar data were reported by (15). These findings suggested that increased BMI could be a risk factor for development
of RA; yet it might be a strategy for prevention or control of this disease. Moreover, the higher incidence of RA in female patients reported in current study was in agreement with data reported in previous studies (3). These findings could be attributed to hormonal changes that turn females having greater immunoreactivity (3). Also, current study revealed that RA was more frequent in patients having family history of RA, but it did not reach statistical significance \( (P= 0.582) \). Previous studies (16) reported the existence of positive association between family history of RA and risk of the disease.

On the other hand, the weak association between smoking history and incidence of RA in our patients (Table 1) might be due to the small sample size and/ or the probability that most of our patients had quitted smoking in response to medical advice. The latter is supported by the findings that smoking is a risk factor for the disease and it correlates with disease severity (17,18).

The finding of current study that there was no association between diabetes mellitus and RA \( (P=0.833) \) was in agreement with that reported in a previous meta-analysis (19). However, current study showed that there was positive association between arterial hypertension and incidence of RA which agreed with previous studies (20). Moreover, the probable mechanism(s) behind this association could be the chronic inflammatory processes encountered in RA and its association with arterial rigidity, reduced arterial flexibility and subsequent increase in blood pressure.

Assessment of disease activity

In present study, the median SDAI scores were 22.25 and 10.30 in patients with moderate-high disease activity and those with low disease activity, respectively, (Table 1). These data were comparable to those reported by (21), but lower than those reported by (11). It is quite possible that use of analgesics had reduced the median score at baseline in current study.

Current study reported that treatment of RA with etanercept, with/ without methotrexate, (with regard to disease activity) did not produce significant differences regarding clinical variables (Table 2). The latter maybe because that the 12-week treatment was not enough to induce clinical improvement (22). Also, a recently-published study (23) revealed that Iraqi patients with RA who received early treatment with etanercept showed improved clinical outcomes.

Moreover, these data indicated that treatment with etanercept monotherapy was as effective as its combination with methotrexate as the latter did not produce superior effects to those of the former. Moreover, previous studies revealed that the synergistic combination of etanercept with sulfasalazine or methotrexate resulted in better outcomes than using either of the latter drugs as a monotherapy (24).

Taken together, early administration of etanercept as a monotherapy or in combination with other DMARDs is significant in treatment of RA.

Immunological markers

The reported higher serum levels of Hs-CRP, IL-1 alpha and IL-6 in RA patients enrolled in current study compared to controls might indicate that the inflammatory process underlying RA is still active in those patients despite the 12-week course of treatment. This finding is supported by previous data that reported a correlation between serum levels of these biomarkers and disease activity in RA (25). It seemed as if the 12-week course of treatment received by RA patients in current study was not enough to control disease activity and a longer course is necessary (22). Also, it is quite probable that increasing the sample size might make the results more understandable than they are.

Conclusion

SDAI formula can be used to measure RA disease activity in clinical practice. In addition, for effective control of RA disease activity, treatment, with etanercept, with/ without methotrexate, needs to be continued for more 12 weeks.

Ethical Clearance: Taken from the Research Ethics Committees at Baghdad Teaching Hospital and College
of Medicine/ University of Baghdad.

**Source of Funding:** Self-Funded study.

**Conflict of Interest:** None.

**References**


Correlation between Occupation, Stress Level and Breast Milk Production during Covid-19 Pandemic in Indonesia

R.Khairiyatul Afiyah\textsuperscript{1,5}, Chatarina Umbul Wahyuni\textsuperscript{2}, Budi Prasetyo\textsuperscript{3}, Muhammad Bagus Qomaruddin\textsuperscript{4}, Imamatul Faizah\textsuperscript{1}, RatnaYunita Sari\textsuperscript{1}, Umdatus Soleha\textsuperscript{1}

\textsuperscript{1}Lecture, Departement of Nursing, Nursing and Midwifery Faculty, Nahdlatul Ulama Surabaya UniversityJl. SMEA No. 57 Surabaya, East Java, Indonesia, \textsuperscript{2}Lecture at Department of Epidemiology, Faculty of Public Health, Universitas Airlangga, Mulyorejo,Surabaya, Indonesia, \textsuperscript{3}Lecture at Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, \textsuperscript{4}Lecture at Department of of Health Promotion and Behavioral Sciences, \textsuperscript{5}Student Doctoral Program of Public Health, Faculty of Public Health, Universitas Airlangga, Mulyorejo, Surabaya Indonesia

Abstract

Introduction: Burdens and types of occupation will cause psychological stress during Covid-19 pandemic, especially jobs in the medical field. The rapid transmission of the disease and the increasing number of people infected by Covid-19 bring anxiety and worry and decrease the level of happiness that will obstruct the secretion of oxytocin hormone that brings problems to breast milk production and breastfeeding process. This study was aimed at analyzing the correlation between occupation, stress level and breast milk production during Covid-19 pandemic in Indonesia.

Method: This correlational study was conducted by using cross sectional design. The technique used to collect the samples totaling 110 breastfeeding mothers was simple random sampling technique. The independent variables were occupation and stress level, whereas the dependent variable was breast milk production. This study used Perceived Stress Scale (PSS) questionnaire to measure the stress level and another questionnaire to measure the smoothness of breast milk production. Furthermore, data analysis was done by using Chi-square statistic test with the significance level of p < 0.05.

Result: The result of this study showed that of 110 respondents, most of them (67%) worked in the medical field; nearly all (70.9%) experienced severe stress; and nearly all (80%) had unsmooth production of breast milk. Whilst, the result of Chi-square test showed that the value of p =0.000 showing that there was a correlation between occupation, stress level and breast milk production during Covid-19 pandemic in Indonesia.

Conclusion: Occupation, stress level and breast milk production are correlated during Covid-19 pandemic in Indonesia. Therefore, the medical workers, especially nurses are expected to play their active roles to educate and teach the breastfeeding mothers how to manage stress correctly to maintain the production of breast milk in any conditions.

Key words: occupation, stress level, breast milk production, breastfeeding mothers, Covid-19

Introduction

Breastfeeding is a developmental task for postpartum mothers by way of giving breast milk (ASI). Breastfeeding is given at least 6 months (exclusive breastfeeding) after the baby is born.\textsuperscript{1}Breast milk has advantages as a nutrient compared to other nutritional sources. The macro and micro components contained in breast milk are needed at each stage of the baby’s growth. Macro components consist of carbohydrates,
proteins, and fats. Meanwhile, the micro components are vitamins and minerals. Breast milk also contains an antibody substance called IgA which acts as a defense system for the walls of the digestive tract against infection.

The World Health Organization (WHO) recommends that mothers breastfeed their babies within the first hour of delivery and continue until the first 6 months of their baby’s life for optimal growth and fulfillment of the baby’s developing nutrition. Breastfeeding mothers have concerns regarding the COVID 19 pandemic so that in Indonesia the data on breastfeeding has decreased. WHO data in 2018 globally exclusive breastfeeding is quite low, only 41%, while the RISKESDAS data for 2018 in Indonesia the number of exclusive breastfeeding only reaches 37%. The Indonesian government provides support for working mothers to continue to provide breast milk to their babies, this is supported by the enactment of government regulation Number 33 of 2012 concerning exclusive breastfeeding which requires every company or workplace to provide a space for breastfeeding mothers.

Breast milk production is greatly influenced by psychological factors. Stress due to the COVID-19 pandemic and maternal work, especially in the health sector, raises concerns for mothers to provide breast milk for babies. Breastfeeding mothers and their babies are a vulnerable group for exposure to COVID 19. Indonesia the number of positive cases of Covid-19 continues to increase. Based on data on May 5, 2020, announced by the Task Force for the Acceleration of Handling Covid-19 in Indonesia, there were 12,071. The United Nations International Children’s Emergency Fund (UNICEF) confirms that exclusive breastfeeding is safe to give to babies and still be given even though the mother is positive for COVID 19. COVID 19 is not detected in positive mother’s milk, but there is a concern of transmission through droplets while breastfeeding. Nursing mothers must apply the COVID 19 protocol, namely using a mask and washing their hands before and after touching the baby.

The psychological and emotional condition of the mother greatly affects milk production. If the mother experiences stress and an increase in workload, there will be a blockade from the oxytocin hormone release reflex/let down reflex. If the letdown reflex is not perfect, then a thirsty baby is dissatisfied. This dissatisfaction is an additional stressor for the mother. In addition, the increased workload during the COVID 19 pandemic made mothers unable to provide optimal breast milk for babies, especially mothers who work in the health sector, it is very difficult to find free time to pump breast milk. So that the result is a decrease in milk production. This study aims to analyze the relationship between work and stress levels on breastfeeding production of breastfeeding mothers during the COVID 19 pandemic in Indonesia.

Methods

The research design used was analytic with a cross-sectional approach, because there was a relationship between the independent variable and the dependent variable. The independent variable of this research is work and stress level and the dependent variable is breast milk production which is observed at the same time. The place where the research was conducted in Indonesia. The research was conducted in May. The population of this study was all breastfeeding mothers in Indonesia. The research sample was 110 respondents who were taken using probability sampling with a random sampling technique. The research instrument was a questionnaire Perceived Stress Scale (PSS) and the Smoothness of Breast Milk Production, the independent and dependent variables were nominal.

Test the validity and reliability of the questionnaire using 15 respondents. The results of the validity test of the Perceived Stress Scale questionnaire obtained the results of r count for each question between 0.580-0.922 while the questionnaire for the smoothness of breast milk production obtained the results of r count 0.615-0.901 for each question was greater than r table 0.550 so it could be stated that both questionnaires were valid to be used as questions. The results of the reliability test of the Perceived Stress Scale questionnaire showed
that the Cronbach’s Alpha value was 0.821, while the smoothness of milk production resulted in a Cronbach’s Alpha value of 0.938, which means it is very reliable or reliable so that the questionnaire is suitable for use in data collection. Data analysis was performed using the Chi-Square statistical test with a significant p <0.05.

Result and Discussion

Respondents Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Respondent n (110)</th>
<th>Characteristics</th>
<th>Respondent n (110)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td>Breastfeeding</td>
<td></td>
</tr>
<tr>
<td>Late adolescence(17-25)</td>
<td>17 f 15.5 %</td>
<td>Breast milk</td>
<td>15 f 13.6 %</td>
</tr>
<tr>
<td>Early adulthood(26-35)</td>
<td>81 f 73.6 %</td>
<td>Breast milk and Expressed breast milk</td>
<td>35 f 31.8 %</td>
</tr>
<tr>
<td>Late adulthood(36-45)</td>
<td>11 f 10.0 %</td>
<td>Breast milk and Formula milk</td>
<td>53 f 48.2 %</td>
</tr>
<tr>
<td>Early adolescence(46-55)</td>
<td>1 f 0.9 %</td>
<td>Breast milk and complementary foods breast milk</td>
<td>7 f 6.4 %</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td>Parity</td>
<td></td>
</tr>
<tr>
<td>Basic</td>
<td>14 f 12.7 %</td>
<td>Primipara</td>
<td>62 f 56.4 %</td>
</tr>
<tr>
<td>Intermediate</td>
<td>10 f 9.1 %</td>
<td>Multipara</td>
<td>48 f 43.6 %</td>
</tr>
<tr>
<td>High</td>
<td>86 f 78.2 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td>City</td>
<td>86 f 78.2 %</td>
</tr>
<tr>
<td>Village</td>
<td>24 f 21.8 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1 shows that most of the 100 respondents (73.6%) were in early adulthood. Almost all of the education levels (78.2%) are highly educated, and almost all of them (78.2%) live in cities. Breastfeeding almost half (48.2%) of respondents gave breast milk and formula milk to their babies, while the parity of most (56.4%) respondents primipara.
Table 2: Occupational Characteristics, Level of Stress, and Quality of Breastmilk Production

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Respondent n (110)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>f</td>
</tr>
<tr>
<td>Profession</td>
<td></td>
</tr>
<tr>
<td>Non-Health</td>
<td>17</td>
</tr>
<tr>
<td>Health</td>
<td>67</td>
</tr>
<tr>
<td>Does not work</td>
<td>26</td>
</tr>
<tr>
<td>Stress level</td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>8</td>
</tr>
<tr>
<td>Moderate</td>
<td>10</td>
</tr>
<tr>
<td>Weight</td>
<td>14</td>
</tr>
<tr>
<td>Very heavy</td>
<td>78</td>
</tr>
<tr>
<td>Quality of Breastmilk Production</td>
<td></td>
</tr>
<tr>
<td>Not smooth</td>
<td>88</td>
</tr>
<tr>
<td>Current</td>
<td>82</td>
</tr>
</tbody>
</table>

Table 2. It shows that most of the 110 respondents (60.9%) work in the health sector. The stress level of most respondents (70.9%) experienced very heavy stress levels and almost all of the milk production (80%) was not smooth.

3.1 Relationship Analysis

Table 3 Relationship of Employment, Level of Stress and Quality of Breastmilk Production

<table>
<thead>
<tr>
<th>Variable</th>
<th>Quality of Breastmilk Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not smooth</td>
</tr>
<tr>
<td>Profession</td>
<td>f</td>
</tr>
<tr>
<td>Non-Health</td>
<td>11</td>
</tr>
<tr>
<td>Health</td>
<td>62</td>
</tr>
<tr>
<td>Does not work</td>
<td>15</td>
</tr>
<tr>
<td>Stress level</td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>8</td>
</tr>
<tr>
<td>Weight</td>
<td>8</td>
</tr>
<tr>
<td>Very heavy</td>
<td>71</td>
</tr>
</tbody>
</table>

P value 0.000
Table 3 shows that of the 67 respondents who work in the health sector, almost all (92.5%) of the quality of breast milk production is not smooth. Meanwhile, of the 78 respondents with very heavy stress levels, almost all of them (91%) the quality of breastmilk production was not smooth. The results of cross-tabulation of the relationship between work and stress levels with the quality of breastmilk production were analyzed using the Chi-Square test, the result was $p = 0.000$ which means that there is a relationship. work and stress levels with the quality of breastfeeding production of breastfeeding mothers during the COVID 19 pandemic in Indonesia.

**Discussion**

The results showed that there was a relationship between work and the stress level of breast milk production during the COVID 19 pandemic with a value of $p = 0.000$. The age of the respondents based on table 1 is mostly (73.6%) in early adulthood, this shows that the respondents are mostly in their productive period. In general, mothers of productive age have a better ability to lactate than mothers who are more than 35 years old. In this age group, it is a good age for mothers to get pregnant and give birth because the reproductive organs are in good condition. Whereas at the age of fewer than 20 years the reproductive organs have not grown optimally and at the age of more than 35 years after experiencing a decline in the reproductive organs. In line with research conducted by Fortner (2019), healthy reproductive age is 20-35 years. Apart from the productive age, they often experience shocks which result in a lack of attention to meeting nutritional needs.

Most of the respondents in this study (56.4%) were primiparous. Parity is related to maternal knowledge and experience so that psychologically primiparous mothers are less experienced than multiparous mothers. Multiparous mothers have previous experience with children, so they are more active and diligent in breastfeeding their babies. The autocrine control system begins when milk production stabilizes. Breastfeeding is very beneficial for babies, one of which is as a source of ideal nutrition with a balanced composition and by the growing needs of a perfect baby both in quality and quantity. Sherwood stated that mothers who provide exclusive breastfeeding can stimulate the prolactin hormone to increase breast milk, the more milk that comes out, the more breast milk is produced. This is in line with a 2016 Hackman study which stated that multiparous mothers had breastfeeding resistance for 6 months compared to primiparous mothers.

Mothers who have good knowledge or a high level of education will have more positive attitudes or behaviors, but this is the opposite of the research. The results showed that almost all of them (78.2%) were highly educated and almost half (48.2%) of respondents gave breast milk and formula milk to their babies. Mothers choose to provide additional formula milk to their babies in addition to fulfilling the baby’s nutrients. Mothers are attracted to advertisements for formula milk which is attractively packaged, so there is a concern that it will damage the mother’s perspective on exclusive breastfeeding. This can affect the understanding of mothers where formula milk can meet all the nutritional needs of the baby compared to exclusive breastfeeding. Apart from the addition of formula milk in this study, a small proportion (6.4%) of mothers gave additional food for their babies. The provision of additional food is due to the community’s habit of thinking that babies will be fussy if they are only given breast milk for the first 6 months. Feeding other than breast milk at an early age can increase morbidity. These babies will be prone to gastrointestinal and respiratory infections. The infant mortality rate in Indonesia is quite high due to the high rate of gastrointestinal and respiratory infections.

Almost all of the working mothers (76.4%) of the respondents worked and most (60.9%) worked in the health sector. Working mothers devote half of their time to work so that the time with their children will be reduced. Working hours can influence exclusive breastfeeding due to differences in working hours between mothers who have shift working hours, namely in the health sector and mothers who work not on shifts. This is in line with research by Murtagh (2011) which states that mothers who work shifts tend not to
Mothers who work in the health sector have excessive stressors because they have to treat patients with positive confirmation of COVID 19, and are worried about contracting the COVID 19 virus. This condition will put pressure both physically and mentally on medical personnel. Medical personnel must wear personal protective equipment by covering all parts of their body using masks, google glasses, black clothes, and booth shoes to protect them from contracting COVID 19. Mothers who work in the health sector spend their time caring for and interacting with COVID 19 patients throughout their shifts. Most of them say that they just take a break to eat and pray, they don’t have time, let alone pumping breast milk. The activity of pumping breast milk is carried out when conditions are free and the patient is reached in the field a little so that the mother can take the time to pump breast milk. These conditions can affect the psychological condition of the mother and have an impact on decreasing milk production. This study showed that most (70.9%) experienced very heavy stress levels and almost all of their milk production (80%) was not smooth. According to Krol, if the mother is tired or stressed, the production of the hormone oxytocin will be hampered which results in the quality of breast milk production decreasing.

Stress during the pandemic was mostly experienced by breastfeeding mothers in urban areas because the urban environment was a zone for the spread of the COVID 19 virus. This is the cause of excessive stressors in breastfeeding mothers, the volume of milk produced is influenced by the psychological condition of the mother, breastfeeding mothers are not subject to stress and anxiety excessively. Stress has a close relationship with the body’s biological function. Primiparous mothers who experience stress will experience several changes in their biological functions which have an impact on milk production. During the COVID 19 pandemic, mothers experienced stress because of limited space for mothers who worked in the health sector. When working mothers other than health work at home, working mothers in the health sector must always be on standby at the hospital to care for patients. Stress activates the hypothalamus to control two neuroendocrine systems, namely the sympathetic system and the adrenal cortical system. The sympathetic nervous system responds to hypothalamic nerve impulses by activating various organs and smooth muscles. Then the adrenal cortex system stimulates the release of hormones including sex hormones, namely oxytocin, endorphins, adrenal hormones and testosterone which are carried through the bloodstream followed by the neural activity of the sympathetic branch of the autonomic nervous system such that they play a role in the fight or flight response. The release of oxytocin is followed by a let-down reflex that the mother can feel as tingling or other breast droplets that the baby is sucking in. This reflex is influenced by the psychological condition of the mother. The skin attachment between the mother and her baby is a stimulus that will be transmitted to the brain. This stimulus will trigger the release of oxytocin which will have a positive impact on milk production.

**Conclusion**

The results showed that there was a relationship between work and stress levels of breastfeeding in breastfeeding mothers during the COVID 19 pandemic. Where almost all respondents who worked their milk production were not smooth, while for mothers with high stress their milk production was not smooth. Likewise, on the other hand, the mother does not work and experiences mild stress, her milk production runs smoothly.

**Source of Fund**: Self and Ministry of High Education

**Conflict of Interest**: Nil

**Ethical Clearance**: UNUSA

**References**

1. Heird WC. Infant nutrition. Present knowledge in


Porotic Hyperostosis and Cribra Orbitalia as Indicators of Nutritional Problems in Ancient Population

Rachmadita Yoga Pratiwi
Postgraduate Student of Master Program in Forensic Science, Universitas Airlangga, Surabaya, Indonesia

Abstract

Background One of the most studied pathological conditions in human bone remains is porotic hyperostosis and cribra orbitalia, where the condition of porosity or bone tissue is porous and looks like a coral, sieve or sponge-like formation, and is often associated with a history of anemia, nutritional deficiencies, certain diseases and so on. This is often seen in the skull because the bone tries to increase the marrow space available for increased red blood cell formation. Porotic hyperostosis and cribra orbitalia (some paleopathologists put into the same category as porotic hyperostosis) occur due to conditions caused by the body’s attempts to produce more red blood cells in the marrow to compensate for iron deficiency. The longer this iron deficiency continues, the more clearly these lesions will form. Objective This study was conducted to see the picture of the lesion porotic hyperostosis and cribra orbitalia associated with anemia and nutritional deficiencies. Methods This study is a case study by observing an intact skull without a mandible, by inspecting the regions of the skull and the roof of the orbit. Results Found coral, sieve or spongy formations on the roof of the skull and the roof of the orbit. The presence of a skull lesion can be an indicator of the fragility of an individual’s bones. Conclusion Anemia and deficiency conditions can result in the formation of lesions in the skull.

Keywords: porotic hyperostosis, cribra orbitalia, iron deficiency anemia

Background

The study of human physiology that comparing of human health status in the past, aims to understand the pattern of human life so that can be evaluated in the present. A health condition of an individual or population can become a reference in physical anthropology as a new way to interpret human behavior and biocultural adaptations in the past and the present. One of the markers of human health status can appear in individual organs due to stress over a long period of time and a poor health status can affect the human condition, such as skeletal parts. So by analyzing the condition of human skeleton from the remains can be used to observe, predict, and explain the interactions between the human condition and the environment in the past.

Paleopathology is a study to understand the causes, frequency, characteristics, signs, and degrees of disease severity that existed in past populations in the prehistoric era. The study of paleopathology is often used as a measure of the health quality of a population in prehistoric era. Paleopathology can directly study a disease that has emerged and experienced by populations in the past through the discovery of prehistoric human skeletal remains, artifacts, and surviving medical records. Pathological findings of human skeletal remains are usually found in skull bones and teeth. Pathological conditions found in the skull include porotic hyperostosis and cribra orbitalia, while pathological conditions commonly found in dental findings include caries, tooth attrition, periodontitis, antemortem toothloss, and
enamel hypoplasia.  

Stress and health status that considered in human interaction with the environment can be a guide for interpreting the situation in the past, can be an anticipation for human health and condition for the present and future. The remains of human skeletons can provide very important and in-depth information about activity patterns, diet, demographics, stress and diseases that have been suffered. The human skeletons can provide information about the health status and lifestyle of humans in the past. Health status is most commonly understood as something that can be affected by the history of disease, infection, nutritional status, and psychological factors. Other factors that may affect include an individual or group’s access to good sanitation, proper nutrition, malnutrition, and resistance and resistance to stress and disease both genomically and epigenically.  

Some studies explained that the etiology of porotic hyperostosis and cribra orbitalia are the presence of pathogenic microorganisms that depend on the source of supply and the content of host’s iron. This can be exacerbated by the presence of iron deficiency conditions which are a physiological response that evolves to an increased pathogen load. Therefore iron deficiency is one of the clinical symptoms of anemia caused by nutritional deficiencies. 

The cribra orbitalia has a similar clinical characteristics of porotic hyperostosis, but the difference is that the orbital cribra is present only in the orbital roof. Many researchers treat cribra orbitalia as a symptom resulting from the same etiological and pathological processes as porotic hyperostosis. Iron deficiency anemia does not always result from malnutrition, but can also be caused by conditions of large amounts of blood loss in a short time, pregnancy, growth, menstrual conditions, chronic systemic diseases, poor personal hygiene, vitamin C deficiency, gastrointestinal ulcers, and parasites infection. This study aims to support previous theories about dietary patterns and poor environmental conditions in ancient populations that could lead to anemia and nutritional deficiencies, and to support evidence that severe anemia can result of lesions in the cranium, namely porotic hyperostosis and cribra orbitalia.

**Literature Review**

Sieve-like porous lesions, identified as porotic hyperostosis in the skull dome and cribra orbitalia on the orbital roof, are generally known having the same haematological process. The process indicates an abnormal demand for a hematopoietic marrow increases, that cause marrow hyperplasia or hypertrophy and the production of red blood cells increases. This overproduction results in an increase in the physical size of the red blood cell production centers. As the marrow space develops, the outer layer of bone becomes very thin, often exposing diploe trabecular bone. Expansion in the trabecular bone (the marrow containing space) exerts pressure on the outer table and eventually expands through it. The resulting exposure of trabecular bone on the outer surface gives a characteristic porous appearance known as porotic hyperostosis and cribra orbitalia lesions.

Porotic hyperostosis and cribra orbitalia are among the pathological lesions most frequently found in ancient human skeletal collections. Since the 1950’s, chronic iron deficiency anemia has been widely accepted as a possible cause of both conditions. Based on this proposed etiology, bioarchaeologists use the prevalence of this condition to infer the condition of individuals during their lifetime with iron deficiency, iron malabsorption, and iron loss resulting from diarrheal disease and intestinal parasitic infections in previous human populations. Some evidence suggests that the loss of acceleration and red cell overcompensation seen in hemolytic anemia and megaloblastic anemia are the most likely causes of the discovery of porotic hyperostosis.

Porotic hyperostosis lesions appear as macroscopic porosity on flat bones, especially in the frontal, parietal and occipital bones, where they produce a “coral-like” or “filter-like” shape in the solid bone of cranium. The appearance of the orbital cribra lesion is similar to that of
porotic hyperostosis, but only found on the orbital roof. Many researchers find that cribra orbitalia as a symptom resulting from the aetiology and pathological processes are the same as hyperostosis. Some etiology for both conditions are iron deficiency anemia disease, pathogen resistance, hemolytic anemia, megaloblastic anemia, or other infections.

Anemia is a pathological symptom, not a specific disease, that has literal meaning of “bloodless”. Anemia is defined as a pathological deficiency in either the red blood cells or the hemoglobin content. In a healthy homeostatic state, there is a balance between production and lysis of red blood cells in the spinal cord. There are three main causes of anemia, including massive blood loss, erythropoiesis disorders, and increased hemolysis. From an etiological perspective, anemia is divided into genetic and acquired (caused by infectious conditions, malignancy, or deficiency). The prevalence of anemia that is genetically acquired (eg, thalassemia and sickleemia) is less common than the prevalence of acquired anemia caused by excessive blood loss and nutritional deficiencies. The nutrients needed to maintain red blood cell homeostasis include essential amino acids, iron, and vitamins such as A, B12, B6, and folic acid. Iron is a key constituent of hemoglobin and thus iron deficiency is the most common cause of anemia. Although iron deficiency is frequently caused by blood loss, it can be caused by diet with iron deficiency, gastrointestinal iron malabsorption, or any combination of these factors. When the intake or absorption of iron or other essential nutrients is insufficient, red blood cell production is inhibited and can lead to anemia. The body increases its hierarchical response to anemia, shifting to cranial spinal cord expansion and remodeling only after easier measures fail to maintain homeostasis. Red blood cells usually mature over a seven day period and have a life span of about 120 days. The emergence of anemic conditions can cause hemoglobin levels to decrease and the body becomes deprived of oxygen. This hypoxic state triggers the release of erythropoietin, a hormone produced by the kidneys that accelerates the production and maturation of red blood cells.7

In chronic anemia, the cytokine response to the underlying condition (eg infection, inflammation or malignancy) occurs, via the anti-erythropoietin effect of tissue necrosis factor α (TFN-α), in suppressed mitotic activity in marrow erythroblasts. The supply of iron to the marrow for hemoglobin synthesis become impaired, resulting in the production of red blood cells with a reduced hemoglobin content (hypochromia). This is not due to iron deficiency stores, but indeed iron stores usually increase in these conditions. Thus, when iron deposits are reduced, iron supply is limited and hypochromic red blood cells are also produced. This is iron deficiency and a result of blood loss and / or a low iron diet. The functional difference between chronic anemia and iron deficiency anemia is that the erythropoietic activity is previously suppressed, whereas in iron deficiency anemia it is increased. However, since the rate of iron supply to stimulated red marrow is limited to iron deficiency, there are too many erythroblasts chasing too little iron. The result of this is that an increasing proportion will fail to make sufficient hemoglobin counts in the time available to become ready-to-function red blood cells; this is crushed in the marrow. In iron deficiency anemia there is a massive increase in intra-medullary ineffective erythropoiesis.8

The association between the incidence of porotic hyperostosis and cribra orbitalia with the incidence of iron deficiency is assumed to be the presence of known markers of skeletal conditions with clinically identified cases of hemolytic anemia, particularly sickle cell anemia and thalassemia. The premature destruction of defective red blood cells produced by this genetic condition is a contributing factor to marrow hyperplasia.5 Anemic conditions can cause disruption of hematopoiesis (production of blood cells) in the trabecular bone marrow of the skull. Porotic hyperostosis was caused by the expansion of the diploë, cranial spongy bone, either due to bone marrow hypertrophy (increased cell size) or hyperplasia (increased cell count). This process is also followed by the formation of an irregular outer cranial table, which is reabsorbed over time, making it thinner and with a visible porous lesion. In the most
severe case ever found, diploë spongy bones can also be seen. Within the cranial dome, diploe expansion occurs by gradually absorbing the outer dome of the skull. This resorption creates a porous formation which gives the hyperostotic porotic lesion that is characteristic of the “sponge” image. In extreme cases of anemia, as seen in the case of thalassemia major, diploe hypertrophy is found to be well beyond the sincipital ectocranial surface to create a palpable upper and distinct “hair-on-end” effect easily observable on radiographs.

Another explanation of iron loss that related to the body’s defense process against invading pathogens in the form of cutting iron. When faced with chronic infection and inflammatory conditions, the body’s natural response is to place iron-binding proteins at the site of entry of pathogens, reduce the absorption of iron from the gut from food, prevent the release of iron into the blood from storage sites, and bind that which is available. All of these mechanisms serve to inhibit the growth and reproduction of microorganisms, which do not store their own iron stores, and therefore require and obtain it from their hosts. This pathogen has several methods of obtaining iron from the host. They usually produce siderophores, which are iron-binding proteins that extract iron from transferrin in the blood supply, or bind directly with transferrin and other iron-carrying molecules. Some pathogens also break down red blood cells directly. In addition to inflammatory porous skeletal lesions, the porosity observed in the skeletal tissue is due to the formation of new blood vessels to remove blood accumulated along the bone surface. Because these blood vessels penetrate the surface of the cortex of the bone, they can easily be confused with the porotic lesions that are usually associated with anemia.

The general diagnosis of porotic hyperostosis is usually applied to cases of enlarged bone tissue that are porous, which can be caused by several types of systemic disease or lesions. Some diseases modify the cranial periosteum but do not affect the underlying tissue. A common type of pathology of the porotic state in the cranial, is the occurrence of hematopoietic marrow enlargement as a result of anemia, resulting in a visually porous-looking bone condition. This type of porotic hyperostosis usually results from a condition of thinning of the bone in the external lamina of the trabeculae. Enlargement of the cancellous bone can eventually create bone thickening in the form of atrophy of the hair-at-ends or comb-like structures.

In addition, pathological conditions caused by inflammatory conditions, such as periostitis, osteitis, and osteomyelitis, exhibit a different microscopic porosity structure than that observed in the appearance of hyperostotic porotic lesions. Because the disease-induced inflammatory response usually results in a combination of bone damage and reactive bone formation in areas of necrotic tissue. The resulting bone pathological condition appears as a rough surface with pitting space and new bone plaque. This is in contrast to porotic hyperostosis, where the remodeling of new bone only occurs with healed pathologies. In this early phase, porotic hyperostosis is triggered by anemia, thus showing a decrease in the structure of the bone marrow module, with the trabeculae occurring tangentially to the outside. After the outer table portion has been eroded, a thin flat squamous plate (osteophyte), in the form of an atrophic trabeculae, can be observed in its initial horizontal alignment. Since this is an early diagnosis of porotic hyperostosis (occurring before extreme hypertrophy) when the new cancellous bone appears as a radially patterned trabeculae - it cannot be mistaken for a pathological state caused by an inflammatory condition. The condition of the trabecular bone in the early stages of this pathological change has not yet formed hypertrophic bone beyond the area previously covered by the outer table, but at a more advanced stage the trabecula experiences a hypertrophic state that is expressed as generalized swelling of the cancellous bone. In addition, the marrow module exhibits a characteristic state, associated with anemia causing porotic hyperostosis, but not usually with pathologies caused by diseases related to the inflammatory response.

Inherited hemoglobinopathy conditions, such as sickle cell anemia and thalassemia, are associated with
the wide range of skeletal anatomical changes found in children. One of the changes observed, which is relevant here, is the expansion of the diploic bone and the thinning of the outer table in the frontal and parietal parts in particular; the occipital are generally spared because of their relatively lower bone marrow content. Such skeletal anatomical changes have also been observed in the previous literature with respect to the condition of iron deficiency anemia and at face value appear to support an association between iron deficiency anemia and subadult bone to skull changes. However, more research needs to be done to refute or support this theory of apparent relationships. In adult humans, at least, there is almost always room for the erythropoietic marrow to accommodate increased erythropoietic activity without disturbing the surrounding bone. Indeed, there is some evidence showing in adults that increased erythropoiesis can be accommodated without the need for physical expansion or changes in the ratio of fat cells. The wider distribution of erythropoietic marrow in children would be more likely to be, as mentioned, less accommodating, although again, more research is needed with respect to deficiency diseases in childhood (eg iron and B12 deficiency) and associated bone changes.

The apocryphal diagnosis that results from postmortem lesions, hypervascularization and osteitis is also influenced by other factors. Likewise, etiologies other than anemia, scurvy, rickets, hemangiomas and traumatic lesions can produce subperiosteal inflammation or hematoma. During the healing process, these blood clots turn into new, highly vascular, superiosteal bone plaques that on rough examination can appear identical to those of an orbital cribra lesion. Many factors aggravate chronic iron deficiency anemia and megaloblastic anemia, such as prolonged breastfeeding, weaning periods with low-iron cereals, and diarrheal diseases that have a different effect on childhood.

Methods

This research is a qualitative study with a case study approach, by observing the presence of porotic hyperostosis and cribal orbital in an intact cranium without mandible of an adult human as the study sample (Figure 1). This research was conducted in April 2019 and was carried out at the Ethnography and Anthropology Museum, Master Program of Forensic Sciences, Faculty of Social and Political Sciences, Universitas Airlangga, Surabaya, Indonesia.

Results

In the observed cranium, there were found lessions that look alike porosity, sponge, coral-like or filter-like formation in the region of the cranial dome (Figure 2) and in the orbital roof (Figure 3).
Porotic Hyperostosis and Cribra Orbitalia are the pathological conditions that most frequently found and reported in archaeological collections. This finding is important to assess the general condition or disease of human population in the past.\textsuperscript{11} The lesions are the result of pathological conditions, such as anemia, chronic metabolic disease, malignancy, chronic scalp infections, other chronic diseases, infection, or malnutrition.\textsuperscript{7} However, the only condition that is found or not present should not be considered as an indication of the health and / or nutritional status of the individual at the age of their death, because healed lesions can occur due to the condition or situation of the individual years before, and the individual can be in good shape for a long time before dying. As suggested by the paradox theory of osteology, Wood in 1992\textsuperscript{12}, investigators should be careful when interpreting the indicators of the physiological stress framework, as some may actually indicate a disease,
Hemolytic anemia conditions such as thalassemia or sickle cell anemia (which occurs when red blood cells lysis prematurely) or megaloblastic anemia (resulting from dietary deficiency and malabsorption of folic acid and vitamin B12) are etiologies of porotic hyperostosis which refers to the presence of hemolytic anemia. Chronic hypoferremic (or iron deficiency) is a physiological response to an increased pathogen load, coupled with symptoms of iron deficiency anemia caused by nutritional deficiencies. Several studies have noted that the cases observed by archaeologists of porotic hyperostosis are of increasing frequency, especially in equatorial populations, which may reflect microorganisms that thrive in hot and humid climates. When the body becomes hypoxic, it releases the hormone erythropoietin, which stimulates the acceleration and maturation of red blood cell production. Only if this initial hormonal response fails will the bone marrow go into action to produce more red blood cells. Because the erythropoiesis process is responsible for bone marrow hypertrophy which then manifests as porotic hyperostosis, and because erythropoiesis requires an adequate supply of iron, iron deficiency anemia is effectively associated with reducing the production of mature red blood cells, thereby expanding the hemopoietic marrow that paleopathologists recognize as porotic hyperostosis. and cribra orbitalia. Similar features found in the postmortem skeleton, hypervascularization and osteitis are also influenced by other factors. Likewise, etiologies apart from anemia, other factors such as scurvy, rickets, hemangiomas and traumatic lesions can also cause subperiosteal inflammation or hematoma. Study by Walker et al in 2009 reported that during the healing process, this blood clot transformed into a new, highly vascular, superiosteal bone plaque which on rough examination may appear identical to the orbital cribra. Many factors aggravate chronic iron deficiency anemia and megaloblastic anemia, such as long breastfeeding periods, weaning periods on cereals with low iron content, and diarrheal diseases that will have different effects on childhood health, especially those related to the condition.

Porotic hyperostosis in adults is most likely a lesion that heals from marrow hyperplasia that previously occurred during childhood, this is because children have bone plasticity and iron requirements are greater than adults. However, apart from that, anemia persisted in some adults, because the lesions in this case remained active and occurred until the death of the individual.

**Conclusion**

Porotic hyperostosis and cribra orbitalia are the common lesions that are identified on human bones and are result from thickening of the skull diplo in response to spinal hypertrophy, resulting in thinning and porosity of the outer part of the bone. These lesions were commonly used as indicators of the health, nutritional status, and anemia evidence of the ancient human population. Knowing the condition of an individual or a group through findings on the remains of a human skeleton can improve our understanding of the etiological, pathophysiological and physical manifestations of the skeleton with the living conditions of the past individual or group health. The result study can be a guide to overcoming the health problems for human life in the present and in the future.

**Ethical Clearance:** The academic institute declared there is no need an ethical clearance for studies that including media collections in laboratory and the action during research was a low risk/no cause a harm to the sample. The sample had been already got a prior approval by the academic institute.

**Source of Funding:** This study was self funded by the author

**Conflict of Interest:** There is no conflict interest in this study

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The Effect of COVID-19 on Blood Pressure Control in Hypertension Patients– A Literature Review

Raditya Manusakti1, Judya Sukmana2, Wachjudi Kurnia3, Liliawanti3

1Student at Faculty of Medicine, 2Lecturers and Master of Patobiologi at Faculty of Medicine, 3Lecturers at Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia

Abstract

COVID-19 is caused by Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2). First identified in Wuhan, China, in December 2019, and declared an International Public Health Emergency in January 2020, the WHO declared a pandemic in March 2020. Hypertension, a disease that has long been a problem for most people in Indonesia and around the world. COVID-19 can affect blood pressure control for people with hypertension due to obstruction of the RAAS system. This research was conducted to find out what effect COVID-19 has on blood pressure control in people with hypertension. In the condition of COVID-19 patients with comorbid hypertension, SARS-CoV-2 which attacks ACE2 can eliminate the role of ACE2 in the RAAS system. Inhibition of ACE2 can also cause buildup of angiotensin II which has a vasoconstrictive effect. This results in the absence of homeostasis in the blood pressure control system and makes blood pressure conditions that continue to be at high pressure.

Keywords: ACE2, Blood Pressure Control, Comorbidity, COVID-19, Hypertension, RAAS.

Introduction

Hypertension is a condition of systolic blood pressure ≥ 140 mmHg or diastolic ≥ 90 mmHg on three tests within 5 minutes between the three examinations. Hypertension is caused by a combination of genetic and environmental factors. Genetic factors in the pathogenesis of hypertension are played by at least 35 genetic locus, 12 of which are newly identified locus. Environmental factors that have the biggest role include hyperuricemia. Smoking, alcohol, unhealthy diet, lack of physical activity, and central obesity. This disease will arise when there is an imbalance between stroke volume and peripheral resistance due to functional / structural abnormalities of various organs that affect both chronically. Hypertension itself does not cause death directly, but can lead to various complications with high morbidity and mortality, including kidney disease, stroke, and heart disease. This condition is exacerbated by the emergence of a new disease in 2019 which is pandemic, namely Corona Virus Disease 2019 (Covid-19).

COVID-19 is a respiratory disease caused by infection with the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) virus and was identified in December 2019 in Wuhan, China. Research conducted by Fang 2020 states that 23.7% of COVID-19 patients suffer from hypertension. Another study conducted by Zheng 2020 shows that the complication with the highest prevalence in COVID-19 patients is hypertension with a proportion of 30%.

It is well known that the main transmission of SARS-CoV-2 is via droplets. However, there is a possibility of fecal-oral transmission The virus can pass through mucous membranes, especially the nasal mucosa and...
larynx, then enter the lungs through the respiratory tract. Furthermore, the virus will attack target organs that express Angiotensin Converting Enzyme 2 (ACE2), such as the heart, lungs, renal system and gastrointestinal tract\textsuperscript{10,11}. The S protein in SARS-CoV-2 facilitates the entry of the corona virus into target cells. Viral entry depends on the ability of the virus to bind to ACE2, the extracellular membrane receptor expressed on epithelial cells, and depends on the priming of protein S to the cellular protease, namely TMPRSS2\textsuperscript{12}.

S-protein SARS-CoV-2 which binds to the ACE2 receptor causes a decrease in ACE2 regulatory activity, which in turn occurs overproduction of angiotensin by the ACE enzyme due to the small amount of ACE2 capable of becoming angiotensin (1-7). SARS-CoV-2 binds to ACE2 to stimulate peptidase incorporation and the virus can apply ACE2 from the RAAS system pathway\textsuperscript{13}.

RAAS has the same endogenous counterregulation system as any other homeostatic system. The RAAS counter-regulation system has an important component, namely ACE2. ACE2 will be widely expressed in the kidney and heart, as well as in target cells especially SARS-CoV-2, pulmonary epithelial cells. ACE2 is an enzyme that determines the main regulatory activity resulting in decreased levels of angiotensin II to angiotensin (1-7). Angiotensin (1-7) has a role in the process of decreasing vasoconstrictive action and proliferation caused by angiotensin II and mediated by Angiotensin II Receptor type 1 (AT1R). An imbalance between ACE1 and ACE2 can contribute to the deregulation of the blood pressure system\textsuperscript{14}. This research was conducted to determine the effect of COVID-19 on blood pressure control in hypertension patients.

**Methodology**

Articles were collected using Pubmed and Science Direct database. The search words include ACE2, Blood Pressure Control, Comorbidity, COVID-19, Hypertension, RAAS. Articles were collected from the year 2015-2020 and indexed in Scimago and Scopus.

**The incidence of hypertension is one of the comorbidities in COVID-19**

Hypertension is a disease that causes the highest death rate in the world. Hypertension can also kill silently and can lead to various complications affecting various organs, including cardiovascular disease, hypertensive encephalopathy, cerebrovascular hypertension, and hypertensive retinopathy\textsuperscript{15}. Hypertension is one of the common comorbidities found in sufferers of COVID-19, about 15\% of cases of hypertension are found in COVID-19 patients\textsuperscript{16}.

Based on the data that has been obtained, the most common comorbidities found in COVID-19 patients are hypertension, diabetes and cardiovascular disease. Several studies have stated that hypertension is the most common comorbidity, followed by diabetes mellitus and cardiovascular disease. Several studies show the incidence of hypertension is one of the comorbidities in COVID-19 in table 1.
In a study conducted by Ye et al., 2020 on 1099 patients in China, the incidence of comorbidity for hypertension, diabetes, and CVD was 164 patients, 81 patients, and 42 patients. Likewise with the study conducted by Chen T et al., 2020 this study involved 274 patients infected with COVID-19, stating that the incidence of hypertension, diabetes and CVD was 39 patients, 23 patients, and 7 patients. With similar results, Zhang, G et al 2020 in a study involving 221 patients stated that the incidence of hypertension, diabetes and CVD was 54 patients, 22 patients, and 14 patients. Liu et al., 2020 in their study involving 137 patients stated that the incidence of hypertension, diabetes and CVD was 13 patients, 14 patients, and 10 patients. Wang D, et al., 2020 in their study also showed that hypertension was the most comorbid among the 138 patients involved, with the incidence of comorbidity for hypertension, diabetes and CVD being 43 patients, 14 patients, and 17 patients. Chen Q et al., 2020 in their study involving 145 patients stated that the incidence of hypertension, diabetes and CVD was 21 patients, 14 patients, and 1 patient. In a study conducted by Chen R et al., 2020 on 507 patients, the comorbidity for hypertension, diabetes and CVD was 28 patients, 13 patients, and 14 patients. Cao et al., 2020 in their study involving 102 patients stated that the incidence of comorbid hypertension, diabetes, and CVD was 17 patients, 5 patients, and 5 patients. Zhou et al., 2020 in his study involving 191 patients showed that the incidence of comorbidity for hypertension, diabetes and CVD was 58 patients, 36 patients, and 15 patients. Mo P, et al., 2020 in a study involving 155 patients stated that the incidence of hypertension, diabetes and CVD was 21 patients, 14 patients, and 1 patient. In a study conducted by Richardson et al., 2020 on 920 patients, the comorbidity for hypertension, diabetes and CVD was 22 patients, 15 patients, and 22 patients. Wang, Z, et al., 2020 in their study also showed that hypertension was the most comorbid among the 69 patients involved, with the incidence of comorbidity for hypertension, diabetes, and CVD being 9 patients, 7 patients and 8 patients.

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Notes: N: Number of patients in the study, HT: Hypertension, DM: Diabetes Mellitus, CVD: Cardiovascular Disorder, COPD: Chronic Obstructive Pulmonary Disease, CKD: Chronic Kidney Disease
There is research in other countries, namely in Italy, this study was conducted by Grasselli et al., 2020 with 1,591 patients showing the incidence of comorbidities for hypertension, diabetes, and CVD was 509 patients, 180 patients, and 223 patients. With similar results from Benelli et al., 2020 in their study involving 411 patients, the incidence of hypertension, diabetes and CVD was 193 patients, 67 patients, and 93 patients.

In addition, there is a study in America, this study was conducted by McMichael, et al., 2020 where 167 patients stated that the incidence of comorbid hypertension, diabetes and CVD was 67 patients, 38 patients, and 68 patients. Richardson et al., 2020 in a study involving 5,700 patients in America, showed that the incidence of comorbidity for hypertension, diabetes and CVD was 3026 patients, 1,808 patients, and 966 patients.

Table 2. The effect of COVID-19 on blood pressure control

<table>
<thead>
<tr>
<th>Blood pressure, mmHg</th>
<th>Good blood pressure control (n : 662)</th>
<th>Poor blood pressure control (n : 141)</th>
<th>p</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>Good blood pressure control (n : 662)</td>
<td>Poor blood pressure control (n : 141)</td>
<td>p</td>
<td>ref</td>
</tr>
<tr>
<td>DBP</td>
<td>Good blood pressure control (n : 662)</td>
<td>Poor blood pressure control (n : 141)</td>
<td>p</td>
<td>ref</td>
</tr>
<tr>
<td>Average SBP</td>
<td>137.0 ± 19.7</td>
<td>134.0 ± 18.0</td>
<td>153.0 ± 18.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average DBP</td>
<td>84.2 ± 12.8</td>
<td>82.7 ± 12.2</td>
<td>91.2 ± 13.0</td>
<td>0.017</td>
</tr>
<tr>
<td>SD of SBP</td>
<td>9.5 ± 4.2</td>
<td>9.2 ± 3.9</td>
<td>11.0 ± 5.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAP</td>
<td>6.5 ± 2.6</td>
<td>6.3 ± 2.5</td>
<td>7.0 ± 3.0</td>
<td>0.017</td>
</tr>
<tr>
<td>Pulse Pressure</td>
<td>95.7 ± 7.0</td>
<td>93.6 ± 5.3</td>
<td>105.0 ± 5.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>51.6 ± 8.3</td>
<td>49.9 ± 6.8</td>
<td>59.8 ± 9.8</td>
<td>0.017</td>
</tr>
<tr>
<td>Female</td>
<td>394 (49.1%)</td>
<td>320 (48.3%)</td>
<td>74 (52.5%)</td>
<td>0.017</td>
</tr>
<tr>
<td>Male</td>
<td>409 (50.9%)</td>
<td>342 (51.7%)</td>
<td>67 (47.5%)</td>
<td>0.017</td>
</tr>
<tr>
<td>Age</td>
<td>338 (42.1%)</td>
<td>282 (42.6%)</td>
<td>56 (39.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;65 years</td>
<td>465 (57.9%)</td>
<td>380 (57.4%)</td>
<td>85 (60.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥65 years</td>
<td>6 (0.8%)</td>
<td>216 (0.5%)</td>
<td>3 (2.1%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Status</td>
<td>Good blood pressure (n : 1.437)</td>
<td>Poor blood pressure (n : 1.391)</td>
<td>p</td>
<td>ref</td>
</tr>
<tr>
<td>Severity of COVID-19</td>
<td>Good blood pressure (n : 1.437)</td>
<td>Poor blood pressure (n : 1.391)</td>
<td>p</td>
<td>ref</td>
</tr>
<tr>
<td>Mild</td>
<td>135.0±18.5</td>
<td>132.0±16.8</td>
<td>162.0±17.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Moderate</td>
<td>82.5±11.2</td>
<td>80.7±10.8</td>
<td>98.5±11.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Severe</td>
<td>9.2±4.0</td>
<td>8.9±3.7</td>
<td>10.3±4.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Critical</td>
<td>6.2±2.4</td>
<td>5.8±2.2</td>
<td>6.7±2.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>n : 2828</td>
<td>Good blood pressure (n : 1.437)</td>
<td>Poor blood pressure (n : 1.391)</td>
<td>p</td>
<td>ref</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>Good blood pressure (n : 1.437)</td>
<td>Poor blood pressure (n : 1.391)</td>
<td>p</td>
<td>ref</td>
</tr>
<tr>
<td>SBP</td>
<td>135.0±18.5</td>
<td>132.0±16.8</td>
<td>162.0±17.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average DBP</td>
<td>82.5±11.2</td>
<td>80.7±10.8</td>
<td>98.5±11.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SD of SBP</td>
<td>9.2±4.0</td>
<td>8.9±3.7</td>
<td>10.3±4.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average DBP</td>
<td>6.2±2.4</td>
<td>5.8±2.2</td>
<td>6.7±2.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SD of DBP</td>
<td>1.386(48.8%)</td>
<td>672(47.8%)</td>
<td>714(52.2%)</td>
<td>0.0016</td>
</tr>
<tr>
<td>Female</td>
<td>1.442 (51.2%)</td>
<td>698(48.2%)</td>
<td>745(51.8%)</td>
<td>0.424</td>
</tr>
<tr>
<td>Male</td>
<td>1.148(44.3%)</td>
<td>526(46.5%)</td>
<td>622(53.5%)</td>
<td>0.0016</td>
</tr>
<tr>
<td>Age</td>
<td>1.680(55.7%)</td>
<td>689(41.6%)</td>
<td>991(58.4%)</td>
<td>0.593</td>
</tr>
</tbody>
</table>

Notes : n : Number of patients in the study, SBP: systolic blood pressure, DBP : diastolic blood pressure, MAP : mean arterial pressure, SD : standard deviation, RR :Respiratory rate
Zhuang, Z et al. in his study of 803 patients in the above study, 609 had control of blood pressure at admission, and 295 (48.4%) had normal blood pressure at admission (SBP / DBP <140/90 mmHg). The mean systolic blood pressure and diastolic blood pressure at admission were 137.0 mmHg (± 19.7) and 84.2 mmHg (± 12.8), respectively. 82.4% (662/803) of COVID-19 patients had good blood pressure control, and 17.6% (141/803) had poor blood pressure control during hospitalization. Compared to those with good blood pressure control, patients with poor blood pressure control had higher mean systolic and diastolic blood pressure, higher SD SBP and DBP, and higher MAP and PP during this period. Patients with poor blood pressure control are more likely to have COPD and chronic kidney disease. Zhuang Z, et al. in his research also stated that the severity status of COVID-19 was also an influence on good and bad blood pressure control. Zhuang Z, et al in their study also stated that age ≥65 years had more poor blood pressure control results than those aged <65 years. Zhuang Z, et al. in his research also stated that the incidence of COVID-19 with comorbid hypertension in men was more than in women. In a retrospective study of 803 coexisting COVID-19 patients with hypertension, it was found that a high mean SBP and high SBP / DBP variability during hospitalization were independently associated with death in hospital, ICU admission, and heart failure. The findings suggest that low and stable blood pressure control is optimal to achieve a favorable prognosis for coexisting hypertensive COVID-19 patients.

Gao X, et al. in his study of 2828 patients in the above study, 2828 patients had blood pressure control and 1437 (50.4%) had normal blood pressure at admission (SBP / DBP <140/90 mmHg). As many as 50.4% (1497/2828) COVID-19 patients had good blood pressure control and 49.6% (1391/2828) had poor blood pressure control.

**Discussion**

Hypertension is a disease that causes the highest death rate in the world. Hypertension can also kill silently and can lead to various complications affecting various organs, including cardiovascular disease, hypertensive encephalopathy, cerebrovascular hypertension, and hypertensive retinopathy. Hypertension is one of the common comorbidities found in sufferers of COVID-19, about 15% of cases of hypertension are found in COVID-19 patients. Based on the data that has been obtained, the most common comorbidities found in COVID-19 patients are hypertension, diabetes and cardiovascular disorders, with an incidence of 5.6% to 53%. In the study of Richardson et al, the co-morbid incidence rates of hypertension, diabetes and cardiovascular disorders were 56%, 31.8%, and 17%.

SARS-CoV-2 is the newest β-coronavirus type. There are seven species of β-coronavirus that have been identified to cause infection in humans, with four species causing the flu effect, and three of them (SARS, MERS and COVID-19) causing illness that results in death. SARS-CoV-2 contains S-protein which has the ability to push viruses into host cells. The SARS-CoV-2 virus envelope consists of glycoproteins that bind to ACE2 receptors. SARS-CoV-2 enters cells through the process of fusion of the viral membrane with the plasma membrane. After the virus enters the cell, the RNA genome from the virus will be released into the cytoplasm and the genome will be translated into two polyproteins and structural proteins. Then the viral genome that has been translated will begin to replicate. The newly formed glycoprotein envelope will enter the membrane of the endoplasmic reticulum or golgi body, and the combination of genomic RNA and nucleocapsid proteins will form the nucleocapsid. Then there is the growth of viral particles in the endoplasmic reticulum-golgi compartment (ERGIC). Finally, the virus particles contained in the vesicles will combine with the plasma membrane to release the virus.
binds to ACE2 to stimulate peptidase incorporation and the virus can remove ACE2 from the RAAS system pathway\textsuperscript{13}.

Renin-Angiotensin-Aldosterone System (RAAS) is an important regulator that regulates blood volume and systemic vascular resistance. Meanwhile, when the baroreceptor reflex reacts to a short-term drop in arterial pressure, this RAAS has a role in systemic adjustment. There are three essential compounds in the RAAS system consisting of renin, angiotensin II and aldosterone. Of the three essential compounds, they function to increase the pressure of arterial blood flow to the distal tubule and increase beta-agonism as a reaction to increased renal blood flow. From this mechanism, the body can increase blood pressure for a long time. The binding of Angiotensin II which is the main active substrate of RAAS with Angiotensin II Receptor Type 1 (AT1R) causes vasoconstriction, salt retention and fibrosis\textsuperscript{14}.

RAAS has the same endogenous counterregulation system as any other homeostatic system. The RAAS counter-regulation system has an important component, namely ACE2. ACE2 will be expressed extensively in the kidney and heart, as well as in target cells especially SARSCoV-2, pulmonary epithelial cells. ACE2 is an enzyme that determines the main regulatory activity resulting in decreased levels of angiotensin II to angiotensin (1-7). Angiotensin (1-7) has a role in the process of decreasing vasoconstrictive action and proliferation caused by angiotensin II and mediated by Angiotensin II Receptor type 1 (AT1R). An imbalance between ACE1 and ACE2 can contribute to the deregulation of the blood pressure system\textsuperscript{18}.

Increased activity or expression of these compounds can occur in ACE2. In elderly people and male sex has the potential to have a higher ACE2 expression. Research conducted on mice shows that administration of ACE inhibitors and Angiotensin II Receptor blockers (ARBs) can increase mRNA expression for ACE2 in the heart, kidneys, aorta and various organs and tissues of the body. In a study conducted on ACEi-treated healthy humans and controls, the results showed that the duodenal ACE2 mRNA expression level increased by an average of 1.9 times compared to the untreated controls. Apart from gender and age, arterial hypertension and diabetes mellitus can be a factor in increasing ACE2. On the other hand, ACE2 levels decrease when there is inflammation and acute respiratory distress syndrome\textsuperscript{19}.

Consumption of ACEi or ARB class drugs causes an increase in ACE2 activity and expression in the heart of patients with COVID-19 with hypertensive comorbidities, so that they can play a protective role in the cardiovascular system. It is currently unknown whether ACE inhibitors or ARBs present in ACE2 in other organs can affect the level of ACE2 expression and activity in the lungs or not. If ACEi and ARB have a role in increasing the activity and expression of ACE2 in the lungs, then these two components play a dual role in handling COVID-19. On the other hand, if there is a higher level of ACE2 it can increase the susceptibility of cells to SARS-CoV-2 and activation of ACE2 can repair acute lung injury caused by SARS-CoV-2\textsuperscript{20}.

Therefore, ACE2 expression in patients infected with SARS-CoV-2 who are accompanied by comorbidities such as chronic hypertension who are treated with ARB-class drugs may protect against acute lung injury rather than put them at a higher risk of developing SARS. This can occur because of two complementary mechanisms, namely: preventing the activation of AT1R which is mediated by excessive angiotensin due to viral infection and increasing ACE2 so that angiotensin production by ACE decreases and increases the production of angiotensin vasodilators (1-7)\textsuperscript{21}.

ACE 2 receptors attacked by SARS-CoV-2 can decrease the activity of ACE2 in the RAAS system. ACE2 which has decreased its effectiveness can inhibit the process of angiotensin formation (1-7). In the RAAS feedback system, Angiotensin (1-7) is one of the compounds that plays a role. Inhibited ACE2 can also be a contributing factor to the buildup of angiotensin II which has a vasoconstrictive effect. This causes no homeostasis or imbalance in the blood pressure control system and results in blood pressure that continues
to be in a high pressure state. So that SARS-CoV-2 infection indirectly worsens the condition of patients with hypertension. Compared to those with good blood pressure control, patients with poor blood pressure control had higher mean systolic and diastolic blood pressure, higher SD SBP and DBP, and higher MAP and PP during this period. Patients with poor blood pressure control are more likely to experience COPD and chronic kidney disease. In this study coexisting co-morbid co-patients with co-morbid hypertension, it was found that a high mean SBP and high SBP / DBP variability during hospitalization were independently associated with heart failure. Findings suggest that low and stable blood pressure is optimal for achieving a favorable prognosis for coexisting hypertensive COVID-19 patients.

**Conclusion**

Based on the data that has been obtained, the most common comorbidities found in COVID-19 patients are hypertension, diabetes and cardiovascular disorders, with an incidence of 5.6% to 53%. In the study of Richardson et al, the co-morbid incidence rates of hypertension, diabetes and cardiovascular disorders were 56%, 31.8%, and 17%.

SARS-CoV-2 infection that attacks ACE2 can eliminate the role of ACE2 in the RAAS system. Inhibition of ACE2 can also cause buildup of angiotensin II, which has a vasoconstrictive effect. This results in the absence of homeostasis in the blood pressure control system and makes blood pressure conditions that continue to be at high pressure.

Ethical Clearance – Not required since it is a literature review

Source of Funding – Nil

Conflict of Interest – Nil

References


Facebook Ads for Health Promotion Against Covid-19: An Environmental Medicine Perspective

Rahmad Agus Dwianto¹, Nur Wening², Elita Dwi Hapsari³

¹Doctoral Student, ²Associate Professor, Post Graduate Program of Management, University of Technology Yogyakarta, ³Doctor, Dlingo II Community Health Center, Ministry of Health of the Republic of Indonesia

Abstract

This research aims to provide innovative ideas to disseminate information on environmental medicine during the Covid-19 pandemic via Facebook. The socialization of the environmental medicine information to the public was hampered during the Covid-19 pandemic. This study provides innovative information dissemination strategies and to increase public awareness and knowledge about environmental medicine. The research method uses literature reviews from various recent studies on the use of social media Facebook for the dissemination of information. The study found that Facebook advertising as a digital platform for the dissemination of environmental medicine during a pandemic to the public is more efficient in terms of time, energy, costs, and a wider target market. This study found innovations, namely the use of Facebook ads as a digital marketing platform for the disseminating of environmental medicine programs to the public during a pandemic. Further studies could combine Facebook Ads with other platforms such as Google Ads, SEO, and TikTok.

Keywords: Dissemination Strategy, Environmental Medicine, Facebook Ads

Introduction

Environmental degradation can cause several problems, such as natural disasters, drastic climate change, the emergence of disease, and environmental pollution (1). The causes of disease can be in the form of unhealthy environmental factors and the entry of pollutants into the human body. Pollutants are substances that are harmful to the environment and when they enter the human body they will accumulate in the body and cause short and long-term effects depending on the type of substance. Diseases that arise are not instantaneous but can occur over a long period.

During a pandemic like this, disease cases due to the influence of environmental conditions also have a great potential to occur. The quality of the environment (water, air, and land) is decreasing, the management of B3 waste and waste that is carried out improperly is outside of potentially bad procedures such as health problems for the people who live in the environment. The situation is getting worse with the current COVID-19 pandemic because medical waste is increasing. The Indonesian Institute of Sciences (LIPI) (2) noted that in the range of March 2020 to September 2020, it was recorded that the number of piles of medical waste including masks and Personal Protection Equipment (PPE) was estimated to be 1,662.75 tonnes.

Although the problem of environmental hazard is a serious threat and challenge in the health sector, public attention and awareness of environmental health are still low. From the results of research data from the Ministry of Health, it is known that only 20% of the total Indonesian people care about hygiene and health. This means that of the 262 million people in Indonesia, only around 52 million people have a concern for the cleanliness of the surrounding environment and its impact on health (3).

Corresponding author:
Rahmad Agus Dwianto
Email: Rahmadagusdwiantouty@gmail.com
Various socialization programs to increase environmental and health awareness have not run optimally. Previously, socialization was carried out directly through meetings, appeals, as well as regular community habituation movements to care for the environment. However, socialization activities during the Covid-19 period were hampered due to the number of government policies stipulating to minimize activities outside the home to prevent the spread of the virus.

To increase public awareness of the importance of environmental and health, as well as to socialize environmental hazards and environmental medicine, other, more effective ways are needed. One way that can be used is through a strategy through advertising or promotion with the social media Facebook. The tendency of people today like to access information through social media and follow trending habits. Therefore, Facebook can be a good alternative for the promotion of this field with a broad target because Facebook itself has users from various groups ranging from adolescents to adults, besides that the costs incurred are low, the reach is wide and the results can be monitored and evaluated periodically.

Facebook Ads has many advantages that can be obtained in this study, including the main attraction of Facebook as an advertiser is that they collect information about interests, clear and manageable demographic characteristics including age, gender, reach of location, sociography, networking, social, and online behavior that can be used in developing the right profile for targeting (4). The low cost offered to create and distribute advertisements online and the ability to target advertisements online more precisely can broaden the range of candidates advertising and allow candidates to create messages to a narrower audience than on television (5). Facebook provides as an advertising interface has enabled a wide range of individuals to commission advertising, ranging from high-budget advertising, national advertising, with small budgets, as well as local initiatives (6). Facebook data also has the advantage of being up to date and available in real-time (7).

Materials and Methods

The research method used in this research itself is using qualitative research methods. Qualitative method is research that uses a scientific background, meaning that it interprets a phenomenon that occurs and is carried out by implementing existing methods. Qualitative research is usually used in “exploration” and generally qualitative research is used in “measuring” (8). In using qualitative research methods, these methods are usually used for interviews, observation or observation, and documentation (9).

Results and Discussion

The World Health Organization defines health as a state of complete physical, mental and social well-being and not simply the absence of illness or weakness. The living environment where all the inhabitants of the earth have a very big influence on the health and disease burden of the existing inhabitants. All diseases that exist theoretically can be caused by two factors, namely in the form of genetic factors or environmental factors. The causes of the disease depend on two things, firstly on inheritance and secondly on environmental pollution.

The reciprocity between humans and the environment raises several negative things that are accepted by the environment and have a direct impact on humans, namely in the form of water pollution, food poisoning, occupational hazards, and surrounding air pollution. While the effects of negative things that arise for humans themselves are in the form of high doses, namely acute health effects, and low doses, namely chronic health effects. High doses are acute health effects in the form of accidents, systemic poisoning, and respiratory diseases. While low doses are chronic health effects in the form of respiratory diseases, cancer (carcinogens), genetic diseases (mutagens), cardiovascular disease, neurological diseases.

An environmental problem can be assessed as a whole, with at least four steps involved namely determining the source and nature of any environmental contaminant or stress, assessing how and in what form
it relates to people, measuring effects, and applying controls when and where appropriate. Environmental health contains two important things, namely the danger to the environment, the effect on health, and the development of effective ways to protect from hazards in the environment. The goal of environmental health is to improve health by identifying, preventing, and reducing hazards in the environment and by assessing and managing the risks involved.

Environmental medicine is part of the broadest discipline - environmental health - a subfield of public health that is concerned with assessing and controlling the impact of people on their environment and the impact on them of the environment. All of these are interactions between risk genes and human health, including various risk factors, various diseases, and various genetic predispositions to disease. The goal of environmental medicine is to prevent disease. Environmental medicine focuses only on explaining how disease causes in an environmental context focusing on the effects that occur on the general population in the outdoor and indoor environment through air, water, food, consumer products, or soil. Besides, occupational medicine is a part of environmental medicine that deals with understanding the hazards of the work environment and their impact on the health of workers.

**Environmental Medicine Ads Targeting**

Facebook ads targeting is an advertising service by adjusting the audience according to the target Facebook has. Indonesia has a number of Facebook users around 130 million per month. With Facebook ads targeting, you can choose the types of audience according to the criteria that match the ad\(^{(10)}\).

**Audience Demographic Characteristic**

**Gender.** Based on ads targeting result, the target audience for the “Environmental Medicine” ads is 759,153,510 Facebook users. From the data, the proportion of gender in the audience, both male and female, is the same.

**Age.** The target audience for these ads is 18-65 years of age and over (Figure 1). With a percentage of 19% of the audience is 18-24 years old, 34% of the audience is 25-34 years old, 20% of the audience is 35-44 years old, 13% of the audience is 45-54 years old, and 7% of the audience is over 65 years old. From the data above, it can be seen that most Facebook users are with a percentage of 34% of the audience aged 25-34 years. Meanwhile, the smallest percentage was 7% of the audience aged 65 years and over.

![Figure 1. Age of Target Audience Environmental Medicine](image-url)
Marriage Status. Not only based on gender and age which are used for the advertising target of “Environmental Medicine” but also based on marital status. In the data above (Figure 2), marital status can be divided into 4 statuses such as single status which has a percentage of 34.5% of the audience, relationship status with a percentage of 14% of the audience, engaged status has 3.7% of the audience, and married with a percentage of 47.8% of the audience. With this data, it can be seen that the most marital status is married with a percentage of 47.8% of the audience.

Figure 2. Marital Status of Target Audience Environmental Medicine

Education Level. Quite some audiences who are the target of the “Environmental Medicine” advertisement include the world of education starting from high school, college, to graduate school. The highest percentage of the audience is at the tertiary level. As many as 23.6% of the audience is from high school education, 68.8% of the audience is from tertiary education and the lowest percentage of the audience is 7.5% of the audience is from postgraduate education (Figure 3).

Figure 3. Education Level of Target Audience Environmental Medicine
Job. It is not only the audience from the world of education that is the target of advertisements but also from the world of work. There are 10 occupations included in the ad target, namely Administrative Service with a percentage of 25.2% of the audience, Sales with 20.1% of the audience, Management with 17% of the audience, Healthcare and Medical Service with 16.7% of the audience, Production of 14.9% of the audience, Education and Libraries 13.6% audience, Art, Entertainment, Sport and Media 12% audience, Business and Finance 10.7% audience, Community and Social Service 9.6% audience, and the last one is Installation and Repair Service work as much as 8.2% of the audience (Figure 4).

![Figure 4. Job of Target Audience Environmental Medicine](image)

In the field of work, the Administrative Service is the target of “Environmental Medicine” advertisements because it can retrieve information from advertisements that are seen for additional knowledge purposes and as material in making decisions or policies related to administrative or public policy fields. Then in the field of work Sales can sell health products so that the information available in advertisements can be useful in attracting consumers and can increase the number of requests for a product being offered. Besides, management work in the environmental or even health sector can be useful as an ingredient in managing and taking actions that will have a major effect on decisions taken. This kind of advertisement is certainly very relevant and can be very enthusiastic for the field of work of Healthcare and Medical Service because it can be used as a medium in educating the general public regarding health. Not to forget, in the field of work, medical device production can consider what health products have a high chance of being produced and sold, especially during the current COVID-19 pandemic.

If the ad is targeted to a potential audience and has a work field that is relevant to the information displayed, then the ad has the opportunity to get high engagement, the information is conveyed right on target, and the purpose of the ad will be achieved.

### Devices Used to Access Facebook

Device. The highest number of devices used to access Facebook in the “Environment Medicine” advertisement were Android devices, followed by iPhone / iPod, Mobile web, Computer and finally iPad. The use of Android devices in accessing these ads reached 75.9%. The use of iPhone / iPod devices is ranked second in accessing Facebook ads, reaching 20.4%. 2.1% of mobile web
devices are used to access Facebook ads. Furthermore, 1.2% of computer devices are used to access Facebook ads. Finally, 0.5% of the devices used to access Facebook ads are held by the iPad (Figure 5).

![Figure 5. Device Use by Target Audience Environmental Medicine](image1)

![Figure 6. Desktop and Mobile Use of Target Audience Environmental Medicine](image2)

The number of percentages of the devices described on the ads targeting results (Figure 6). Where the result of the conclusion is that Desktop & Mobile are the most frequently used devices in accessing Facebook ads, then the next device for accessing Facebook ads is Mobile Only and the last device that is rarely used to access ads is Desktop Only. This data is also supported by a report from We Are School, where mobile phones now account for 53% of the time spent online, but the data clearly show that other devices still play an important role in life (11).
Countries. The country that most frequently accessed Facebook ads in terms of “Environment Medicine” was occupied by the US (United States) at 22.7%, followed by India which reached 19.9%, the Philippines at 8%, Pakistan at 3.8%, Bangladesh at 3.6%, UK (United Kingdom) 3.4%, Malaysia 2.8%, Nigeria at 2% and the last at 1.8%, namely Australia and Canada. This data is taken from all human populations in the world who are over 13 years of age. The data above is also supported by a report from We Are School, wherefrom the previous year to the present year there is an increase in the audience with ages between 13 years and 17 years all over the world who access Facebook ads (11).

Keywords Suggestion

Keyword Suggestion is a keyword that appears or is most often suggested automatically to internet users when typing a keyword in the search field (Table 1). This keyword suggestion is especially useful for website optimizers. The main benefit of keyword suggestion is getting the most searched keywords in the search field. Many applications provide services for keyword suggestions. The image below is an example of a keywords suggestion.

<table>
<thead>
<tr>
<th>#</th>
<th>Keyword</th>
<th>Search Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Environmental Medicine</td>
<td>10,000</td>
</tr>
<tr>
<td>2</td>
<td>Occupational and Environmental Medicine</td>
<td>1300</td>
</tr>
<tr>
<td>3</td>
<td>Wilderness &amp; Environmental Medicine</td>
<td>260</td>
</tr>
<tr>
<td>4</td>
<td>Occupational Environmental Medicine</td>
<td>260</td>
</tr>
<tr>
<td>5</td>
<td>Center for Environmental Medicine</td>
<td>90</td>
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</table>

Conclusion and Acknowledgement

Facebook Ads as a platform for advertising the dissemination of environmental medicine information in by optimizing various superior features of Facebook. Optimization of environmental medicine dissemination by targeting advertising audiences with the right demographic, psychographic and geographic characteristics. Facebook ads enhance public brand awareness about environmental hazard with a wide advertising reach, high engagement, and low advertising costs.

Ethical Clearance - We have no ethical clearance to disclose. We have coordinated with the ethical clearance committee of the Research and Development Institute, Ministry of Health of the Republic of Indonesia. This research paper does not require ethical clearance. This research does not use human subjects or other living things. The data presented in this study is not in the form of data on living things, but in the form of modeling to develop science and promote environmental medicine.

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Conflict of Interest - We have no conflicts of interest to disclose.
References


Study of Causes of Death, Histopathological and Microbiological Changes in Cases of Burns Brought for Autopsy at Gandhi Medical College, Bhopal

Rajendra Kumar Mourya¹, Priyamvada Kurveti Verma², Garima³, Pawan Rathor⁴, Dheeraj Singh Verma⁵

¹Assistant Professor, Department of Forensic Medicine and Toxicology, Government Medical College, Shivpuri, Madhya Pradesh, ²Ex-Resident, ³Associate Professor, ⁴Demonstrator; Department of Forensic Medicine and Toxicology, Gandhi Medical College, Bhopal, ⁵Associate Professor, Department of Microbiology, Gandhi Medical College, Bhopal, Presently ⁴Medical Officer, District Hospital, Shivpuri, Madhya Pradesh

Abstract

Death may occur immediately after burns or may get delayed for days & weeks, where burns may not be the actual cause of death; but its sequels & its complications leads to death. In such cases, determination of exact cause of death may be difficult. Hence, this study was aimed to find out the causes of death in burn cases during autopsy utilizing histopathology and microbiology & its comparison with clinical causes of death. All cases of burns autopsied at Gandhi Medical College & associated Hamidia Hospital Bhopal, for a period of 1.6 years were included in the study.

Out of 100 cases included, 45 (45.00%) showed the cause of death as septicemia, followed by hypovolemic shock in 30 (30.00%) cases, hypovolemic shock with acute renal failure seen in 4% cases. Bronchopneumonia was seen in 29% cases, out of which 12% cases were associated with septicemia. Multiple Organ Failure with septicemia was seen in 11% cases and in 2 (2.00%) cases of spot death; cause was neurogenic shock with asphyxia.

On histopathological examination, lungs revealed congestion and alveolar edema in 48% cases; Liver showed sinusoidal congestion in (39 %) cases; Kidneys showed hydropic degeneration of tubules in 62 % cases; being the most common findings. Pseudomonas aeruginosa was the commonest isolate, 61% in blood and 47% in pus culture. So, there is need of strict and 100% implementation of aseptic measures in burn wards, along with adequate supply of appropriate drugs specially antibiotics to the all hospitals.

Keywords: burns, cause of death, septicemia, histopathology, culture.

Introduction

A severe burn injury is the most devastating injury a person can sustain and yet hope to survive. Burns are the fourth most common type of trauma worldwide, following traffic accidents, falls and interpersonal violence.

Death may occur immediately after burns or may get delayed for days & weeks, where burns may not be the actual cause of death; but its sequels & its complications may result in death. The patient may survive in the initial post burn period, progressing satisfactorily and may even apparently become normal; even then sometimes death may occur suddenly as a result of sequel, complication
or some remote intervening cause.

Determination of exact cause of death may be difficult as external appearance may show the effect of burn as a cause, but internal findings may point to some other causes of death; for example, shock, electrolyte imbalance, septicemia, renal failure, hepatic failure, lung dysfunction and so many other causes.

So, to ascertain the exact cause, it needs meticulous autopsy to be done by autopsy surgeon along with gross and microscopic examination of relevant organs by pathologist, blood and pus culture by microbiologist & toxicological analysis in forensic science lab.

Hence, this study was designed to find out the causes of death in burn cases during autopsy; utilizing histopathology and microbiology & its comparison with clinical causes of death.

**Material and Methods**

Present study was carried out in the Department of Forensic Medicine and Toxicology in collaboration with Department of Pathology and Department of Microbiology at Gandhi Medical College & associated Hamidia Hospital Bhopal, for a period of 1.6 years.

All the burn cases either admitted or directly brought dead to the Hamidia Hospital Bhopal, cases with proper hospital records and the cases of spot death were included. Out of those, 100 cases were selected for the study by simple random sampling. Burn cases without proper hospital records, unknown, mutilated, decomposed bodies were excluded.

The relevant data was collected using a questionnaire that included demographic profile of deceased, history taken from the relatives and police, inquest papers, hospital records and autopsy findings. After gross examination, sections were taken from lungs, liver and kidneys (with due consent obtained from relatives). Histopathological examination of the sections was performed at department of Pathology; and blood and pus culture was done at department of Microbiology, Gandhi Medical College, Bhopal.

**Results and Discussion**

On analyzing the data, following results were obtained:

<table>
<thead>
<tr>
<th>Table-1: Causes of Death in Burn cases revealed after autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cause of Death</td>
</tr>
<tr>
<td>Asphyxia</td>
</tr>
<tr>
<td>Neurogenic Shock</td>
</tr>
<tr>
<td>Asphyxia with Neurogenic Shock</td>
</tr>
<tr>
<td>Hypovolemic Shock</td>
</tr>
<tr>
<td>Hypovolemic Shock with Acute Renal Failure</td>
</tr>
<tr>
<td>Septicaemia</td>
</tr>
<tr>
<td>Septicaemia with Acute Renal Failure</td>
</tr>
<tr>
<td>Bronchopneumonia</td>
</tr>
<tr>
<td>Bronchopneumonia with Acute Renal Failure</td>
</tr>
<tr>
<td>Bronchopneumonia with Septicaemia</td>
</tr>
<tr>
<td>Bronchopneumonia with Acute Renal Failure with Septicaemia</td>
</tr>
<tr>
<td>Multi Organ Failure</td>
</tr>
<tr>
<td>Multi Organ Failure with Septicaemia</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
In majority of cases i.e. 45 (45.00%) the cause of death was septicemia, followed by hypovolemic shock in 30 (30.00%) cases and hypovolemic shock with acute renal failure in 4% cases. Bronchopneumonia was seen in 29% cases in which 12% cases were associated with septicemia. Multiple Organ Failure with septicemia was seen in 11% cases and in 2 (2.00%) cases of spot death; the cause was neurogenic shock with asphyxia.

Burn shock is rapidly developing hypovolemic circulatory failure seen in the first 72 hours after burn injury. Infectious Complications are currently the principal cause of in-hospital death in burn victims. The infection usually is airborne and less often hematogenous from wound infection. Septicemia is another common cause of death and has a high mortality in burns patients. It usually is secondary to infection of the burn site.

Septicemia was common in victims who survived for more than 3 days. Similar observation were made by Harish D et al (2013) in their study on 381 burn cases, where cause of death was given as Burns shock in 31 (08%) cases, Toxemia & shock in 67 (18%) cases and Septicemia in 269 (71%) cases. Chawla R et al (2011) and Ande JD et al (2013) also observed the major cause of death as septicemic shock in 56% cases and septicaemia as the leading cause of death in 52.38% cases; respectively.

Khandare SV and Pawale DA (2014) also observed cause of death as septicaemia in 76.7% cases and shock due to burns in 20% cases. Pawar V et al (2014) studied 348 admitted burn cases out of which 196 patients died during treatment. Commonest cause of death was septicemia alone or in combination with other causes constituting (84.69%) cases.

On the contrary, Mazumdar A and Patowary A (2013) observed shock as the most common cause of death (122 cases), followed by exhaustion (62 cases) and septicemia (58 cases). Mangal HM et al (2007) observed maximum percentage of victims died within first 24 hours due to hypovolemic shock (burns- shock). Only 16 victims (5.33 %) were found dead on spot due to neurogenic shock, while 19 victims (6.33%) died within a duration of 24-36 hours, followed by 46 victims (15.33%) in 36-72 hours, 31 (10.33%) in 3-7 days and only 22 victims (7.33%) could survive more than a week.

In this study, Bronchopneumonia was seen in 29% cases; which is similar to the study of Mostafa M. Afifya et al (2006-2010) who observed that the majority of deaths occurred within a week (82%) and most of the victims died from neurogenic shock (54.7%) followed by Septicemia and pneumonia (23.5%). Kumar and Tripathi (2004) in their study reported Bronchopneumonia in 22 (20%) cases.

### Table-2: Clinical Causes of Death given in case files

<table>
<thead>
<tr>
<th>Clinical Cause</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asphyxia with Inhalational Injury</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Shock (Neurogenic/Hypovolemic)</td>
<td>9</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>Multiple Organ Failure</td>
<td>22</td>
<td>39</td>
<td>61</td>
</tr>
<tr>
<td>Multiple Organ Failure with Septicaemia</td>
<td>06</td>
<td>09</td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soot Particles</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present (yes)</td>
<td>7</td>
<td>7.00%</td>
</tr>
<tr>
<td>Absent (no)</td>
<td>93</td>
<td>93%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>
On studying the case files of admitted deceased, clinical causes of death mentioned were multiple organs failure in 61% cases and burn shock (neurogenic/hypovolemic shock) in 24% cases and multiple organ failure with septicemia in 15% cases. This was also seen in the study of Kallinen O, Maisniemi K et al (2012) who reported that, out of 71 burn deaths, 40% were caused by multiple organ failure (MOF). All 28 patients with multiple organs failure had acute renal failure, followed by liver damage, of which four patients had acute or chronic liver failure.

Table-3: Soot particles in Trachea

Soot particles were found in trachea in only 7 (7.00%) cases, out of 100, the rest 93% cases were devoid of the soot particles in the trachea. This observation was different from the findings of Mazumdar A and Patowary A (2013) who found soot particles in trachea in 19% of cases, Das. K.C.(1998) who observed soot particles in trachea in 18.05% cases, Nath D (2007) who reported soot particles in trachea in 34.07% cases and Kumar and Tripathi (2004) who noted soot particles in 26% of cases studied.

Most of the victims in the present study died in the hospital after receiving treatment, which included intravenous fluid and some oral medication. Many of the cases might have occurred in some open spaces. These two may be the reason for absence of soot particles in the trachea in most of the victims.

Table-3: Gross Pathological Findings in Major Vital Organs

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Liver</th>
<th>Lungs</th>
<th>Kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congestion</td>
<td>95%</td>
<td>48%</td>
<td>48%</td>
</tr>
<tr>
<td>Edema</td>
<td>-</td>
<td>31%</td>
<td>-</td>
</tr>
<tr>
<td>Petechial Hemorrhages</td>
<td>-</td>
<td>-</td>
<td>03%</td>
</tr>
<tr>
<td>Consolidation</td>
<td>-</td>
<td>58%</td>
<td>-</td>
</tr>
<tr>
<td>Multiple Abscesses</td>
<td>05%</td>
<td>52%</td>
<td>-</td>
</tr>
<tr>
<td>Fatty change</td>
<td>08%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Loss of Cortico-medullary Demarcation</td>
<td></td>
<td></td>
<td>29%</td>
</tr>
</tbody>
</table>

Table-4: Histopathological Findings in Liver, Lungs, Kidneys

<table>
<thead>
<tr>
<th>Findings</th>
<th>Liver</th>
<th>Lungs</th>
<th>Kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congestion &amp; Enema</td>
<td>39%</td>
<td>48%</td>
<td>15% (congestion only)</td>
</tr>
<tr>
<td>Inflammatory Cell Infiltration</td>
<td>21%</td>
<td>43%</td>
<td>-</td>
</tr>
<tr>
<td>Necrotic Zone</td>
<td>15%</td>
<td>21%</td>
<td>-</td>
</tr>
<tr>
<td>Cloudy Degeneration</td>
<td>-</td>
<td>-</td>
<td>62%</td>
</tr>
<tr>
<td>Tubular Necrosis</td>
<td>-</td>
<td>-</td>
<td>48%</td>
</tr>
<tr>
<td>Tubular Casts</td>
<td>-</td>
<td>-</td>
<td>27%</td>
</tr>
</tbody>
</table>
Lungs revealed congestion and in alveolar edema in 48% cases, inflammatory cell infiltrate in 43% and necrotic zone in 21%, macrophages in 16% cases. In most cases, these changes were observed more frequently after 72 hours of survival, alveolar edema had been increasing with increasing duration of survival whereas macrophages were more frequent after 7 days of survival.

Liver showed sinusoidal congestion in 39% cases, inflammatory cell infiltrate in 21%, necrotic changes in 15% and fatty change in 8% cases. Sinusoidal congestion was prevalent throughout, irrespective of the duration of survival. In Kidneys, hydropic degeneration of tubules was seen in 62% cases, tubular necrosis in 48%, tubular casts seen in 27% cases.

Similarly, Shinde AB and Keoliya AN (2013) studied 110 burn cases and observed similar findings in major vital organs. In lungs, bronchopneumonia was seen in 22 (20%) cases, pulmonary oedema in 61 (55.45%) cases, atelectasis in 16 (14.54%), emphysema in 19 (17.27%), interstitial haemorrhage in 42 (38.18%), intra alveolar hemorrhage in 39 (35.45%), interstitial pneumonitis in 30 (27.27%), macrophages in 51 (46.36%), congestion in 81 (73.63%), hemorrhagic necrosis in 5 (4.54%). These changes more frequently after 72 hours of survival.

In liver, they found congestion in 64 (58.18%) cases, fatty change in 17 (15.45%), centrlobular necrosis in 39 (35.45%), dilated and congested sinusoids in 42 (38.18%), infarction in 16 (14.54%), degenerative changes in 21 (19.09%) focal necrosis in 21 (19.09%), focal hemorrhage in 21 (19.09%), portal inflammation in 11 (10%), fibrin deposition in 2 (1.81%) and periportal necrosis in 6 (5.45%) cases.

In kidneys, they reported acute tubular necrosis in 18 cases and in 12 cases it was observed in death within first 5 days. Tubular casts in 35 and in 21 cases it was observed in death within first 5 days. Cloudy degeneration was present in 17 of 110 cases and in 12 cases it was observed in death within first 5 days.

<table>
<thead>
<tr>
<th>Findings</th>
<th>Blood culture</th>
<th>Pus culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>61%</td>
<td>47%</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>30%</td>
<td>8%</td>
</tr>
<tr>
<td>E.coli</td>
<td>9%</td>
<td>-</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>04%</td>
<td>1%</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>-</td>
<td>12%</td>
</tr>
</tbody>
</table>

Psedomonas aeruginosa was the commonest isolate 61% in blood and 47% in pus culture, followed by Klebsiella pneumoniae 30% in blood and 8% in pus; Staphylococcus aureus seen in 12% cases. Similarly, Rajbahak S et al (2014) identified total of 215 bacterial isolates from 168 pus swabs. P. aeruginosa accounted for the highest percentage 98 (45.6%) from the burn wounds followed by S. aureus 41 (19.1%) and Acinetobacter spp. 38 (17.7%).

Skin burning causes general immunosuppression. The denatured protein in burn injured tissue provides a good substrate for microbial growth. The relative avascularity as a consequence of thermal thrombosis further promotes infection. Not surprisingly, the risk of burn infection is proportional to the area burned. Full-thickness skin burns usually are colonized by bacteria within a few days, with sparse Gram-positive organisms in the first week and dense Gram-negative organisms
thereafter.\textsuperscript{11}

Most episodes of septicemia occur between 6 and 10 days after the burn. Wound infection by specific organisms such as Streptococcus pyogenes or Pseudomonas aeruginosa or heavy colonization of a burn wound predispose to invasive sepsis with organisms invading living tissue adjacent to the wound.\textsuperscript{18}

**Conclusion**

The commonest cause of death revealed at autopsy was septicemia (45.00%), followed by hypovolemic shock (30.00%); while most frequent clinical cause of death was multiple organ failure in 61% and burn shock (neurogenic/hypovolemic shock) in 24% cases. Lungs revealed congestion and alveolar edema in 48% cases, liver showed sinusoidal congestion in 39 % cases, kidneys showed hydropic degeneration of tubules in 62 % cases; which were the most common histopathological changes observed. Pseudomonas aeruginosa was the commonest isolate 61% in blood and 47% in pus culture.

Similar findings have been observed by Harish D et al (2013)\textsuperscript{5} and Khandare SV and Pawale DA (2014)\textsuperscript{7} who reported septicemia as cause of death at autopsy in majority of cases; while Kallinen O, Maisniemi K. et al (2012)\textsuperscript{6} found Multiple organ failure as commonest clinical cause of death.

Shinde AB and Keoliya AN (2013)\textsuperscript{17} reported pulmonary edema, observed sinusoidal congestion in liver, found acute tubular necrosis and cloudy degeneration in kidneys; as commonest histopathological changes, which supports the findings of present study. Similarly, Rajbahak S et al (2014) found Pseudomonas aeruginosa accounting for the highest percentage (45.6%) of the burn wound flora.

Major causes of death found were septicemia, bronchopneumonia and hypovolemic shock. So, there is need of strict and 100% implementation of aseptic measures in burn wards following the guidelines given by WHO. Government should ascertain the supply of sufficient and appropriate drugs specially antibiotics to the all hospitals without interruption.

Study of gross and histopathological findings in visceral organs in autopsy of burn cases, as well as microbiological analysis of blood and pus in cases of spot death/brought dead and other burn cases, may help in planning an efficient treatment protocol for burn cases in future.

**Conflict of Interest**: None

**Source of Funding**: self with assistance from the institute.

**Ethical Clearance**: The study protocol was approved by the Institutional Ethics Committee of Gandhi Medical College, Bhopal.

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Determination of Capoten by Analytical Methods

Rana.S.A.L-Shemary

Lecture, Babylon University, College of Pharma, Science Dept., Hilla, Iraq

Abstract

The present analysis article points out numerous analytical methods for the quantitative identification of ACE inhibitor (ACE Inhibitor) by one in all the spectroscopical techniques (UV-spectrophotometry) and separation techniques, like superior liquid natural action (HPLC). Additionally, we tend to examine the contribution of optical biosensors to the clinical and pharmaceutical study of ACE inhibitor, which has applicable analytical procedures for internal control, pharmaceutical drug formulations and human.

In this report, a comprehensive sample of scientific papers published in different journals relating to medicinal, clinical and analytical chemistry was collected. Captopril was chosen because it is a popular medication in the pharmacy, a first-line antihypertensive agent, with a fast release, limited course of operation

Key words :- Captopril, ACE inhibitor, HPLC, UV, biosensor

Introduction

Captopril is the first oral angiotensin converting enzyme inhibitor to become available. It was used mainly for hypertension. In mild to severe hypertension, captopril is nearly as effective as normal doses of hydrochlorothiazide or propranolol, nearly one-half of these patients need the use of a diuretic to gain satisfactory regulation of blood pressure. (1)

In extreme hypertension captopril plus a diuretic (and in some cases a beta-blocker) blood pressure has typically lowered considerably better than could be done with ‘normal combination treatment’ in cases whom have not responded sufficiently to this protocol, and this also resulted in an increased sense of well-being in highly hypertensive patients that have previously undergone comprehensive multiple drug therapy. Indeed, at the present stage of drug production, patients who do not respond to or accommodate ‘ordinary’ antihypertensive treatment are the most effective candidates for captopril therapy (2).

Ultimately, the final role of captopril in the treatment of hypertension may depend on further confirmation of its profile of adverse effects. Captopril has shown promising changes in a limited number of patients with serious congestive heart failure resistant to traditional treatment, in addition to research on hypertension (3).

Captopril must be considered an interesting addition to the clinical armamentarium; it will continue to create a lot of excitement as its final role in therapy is best described by additional well-designed experiments to evaluate captopril, such as HPLC, UV-visible spectroscopy, GLC, optical biosensor, etc (4). Captopril tablets are shown in Fig.1.

Figure 1: A picture of captopril tablets.

Discovery and synthesis of captopril
One of the primary medications marketed for lowering blood pressure. Three researchers at the U.S. drug maker Squibb (now Bristol-Myers Squibb) made-up angiotensin converting enzyme inhibitor in 1975: Miguel Ondetti, Claude Bernard Rubin, and David Cushman. In February 1976, Squibb applied for U.S. patent rights for the drug and the U.S. In September 1977, a patent was issued. Captopril’s development was one of the early achievements of the groundbreaking idea of ligand-based drug design.

In the mid-20th century, the renin-angiotensin-aldosterone pathway was thoroughly researched and this mechanism provided many important goals in the production of new therapies for hypertension. The first 2 goals that were tried were proteinase associate degree an angiotensin converting enzyme inhibitor. ACE inhibitor was the results of attempts by Squibb’s labs to supply an angiotensin converting enzyme inhibitor. Ondetti, Cushman, and colleagues distended on work undertaken among the 19 Sixties by a team of researchers semiconductor unit by John Vane at the Royal college of Surgeons of European nation. Kevin K.F. created the first breakthrough. In 1967, the transfer of angiotensin I to angiotonin was blocked throughout its passage through the pulmonary circulation mistreatment bradykinin potentiating factor (BPF) equipped by Sergio Ferreira metric Ng and Vane. Later, BPF was found to be a peptide in the lancehead viper venom (Bothrops jararaca), which was the transforming enzyme’s “collected-product inhibitor.” A sting from this snake triggers an immediate decrease in its prey’s blood pressure, rendering it difficult to run. After it absolutely was found by QSAR-based modification that the terminal sulfhydryl moiety of the amide created a high efficiency of ACE inhibition, Captopril was formed from this peptide.

A non-peptide with a proline residue at the C-terminal is found in the venom. A effective regulator of the so-called angiotensin-converting enzyme (ACE) was found to be this non-peptide. It is a zinc-containing enzyme that catalyzes the synthesis of the angiotensin I peptide into the hormone angiotensin II active peptide that raises blood pressure. While the venom peptide’s very quick action can be useful for a starving snake, it is not helpful for humans.

Medicinal chemists have produced a sequence of proline-containing zinc-binding compounds using the catalytic mechanism of similar zinc-containing enzymes as the starting point. Succinyl-(S)-proline was the first ACE inhibitor to be synthesized. Medicinal chemists were able to improve the production of the ACE inhibitor more than 1000-fold by replacing the succinyl group with a structurally similar sulfhydryl group. In 1980, Captopril entered the market as the first inhibitor of ACE, as seen in Fig.2.
It was proprietary in 1976 and approved in 1980 for medicative use. attributable to its mechanism of action and conjointly attributable to the event method, it absolutely was the primary angiotensin converting enzyme inhibitor developed and was thought-about a breakthrough. At E, Captopril was found and grown. Centered on ideas pioneered by Nobel laureate Sir John Vane, R. Squibb & Sons Pharmaceuticals is now sold by Bristol-Myers Squibb.

On April 6, 1981, Captopril received FDA approval. In Feb 1996, once the business exclusivity maintained by Bristol-Myers Squibb for Capoten all over, the medication became a generic medication within the U.S. The production of Capoten was claimed as an indication of ‘biopiracy’ (marketing standard medicines), as no benefits flowed back to the associate estral Brazilian community WHO 1st used viper venom as an point poison (10).

Indication

1. Hypertension

Adult /Initially twelve.5-25 mg doubly daily, then augmented to a hundred and fifty mg daily in a pair of separated doses if necessary, doses to be augmented at periods of a minimum of a pair of weeks, once daily dose are often decent if any concomitant medicine medications square measure taken.

Initially 6.25 mg doubly daily, then augmented to a hundred and fifty mg daily if needed in a pair of split doses, doses to be augmented at intervals of a minimum of a pair of weeks, once daily dose are often decent if any concomitant medicine medicines square measure taken.

2- Prophylaxis of symptomatic internal organ failure in clinically healthy patients with symptomless left chamber malfunction (beginning 3-16 days when infarction) following infarction (under close to surgical supervision)

Adult / at first 6.25 mg daily, then hyperbolic to twelve.5 mg three times daily for two days, then hyperbolic to twenty five mg three times daily if tolerated, then hyperbolic to 75-150 mg daily if tolerated in 2-3 separated doses, rising steady to doses larger than seventy five mg daily.

3-Diabetic nephropathy in type 1 DM

Adult/75-100 mg in split doses daily.
4-Heart failure

Adult/(under shut medical supervision) at first 6.25-12.5 mg 2-3 times every day, then hyperbolic more and more at periods of a minimum of a pair of weeks, if tolerated up to a hundred and fifty mg daily in divided doses.

5-Short-term treatment of clinically competent patients with myocardial infarction within 24 hours of onset.

Captopril analytical measurement

Several assay strategies, like coulometric (13), conductometric, and colorimetric, are developed for the quantitative determination of Capoten (14). Infrared spectroscopy (15), mass spectroscopy and nuclear magnetic resonance spectroscopy (16) are used to determine Captopril. Captopril’s UV spectrum with one band at two hundred nm was obtained, whereas the CD spectrum consists of one peak at 210 nm (17).

Captopril by spectrophotometric method was calculated by Alberto (18) and iron and copper complexation with ACE inhibitor was additionally assayed (19) by ultraviolet illumination photometer. the amount of chromatographical strategies for the determination of ACE inhibitor has been defined as gas chromatography-mass spectrometry (20) Ahmed et al. (21) also published HPLC stability-indicating methods for its determination.

This approach is employed for the assessment of angiotensin converting enzyme inhibitor in pure type within the presence of the disulphide chemical compound and in pharmaceutical preparations, an answer containing 0.025 C and w / w Pd(II) chloride was used as a mobile step in a very mixture of acetonitrile-methanol-water comprising ten millimetre Britton-Robinson [BRb] pH scale 4.00 and 0.25 M KCl solution [1:4:5 v / v / v].

Another HPLC methodology determined by Stulzer et al. angiotensin converting enzyme inhibitor in controlled unleash tablets and analyzes were performed at temperature at the inverted part Phenomenex Luna column C18 (250 millimetre × 4.6 mm), mobile part water: methyl alcohol (45:55; v / v) and pH scale 2.5 at 1.0 mL.min⁻¹, and also the response was linear at zero.3–1.5 mg.mL⁻¹ (r² = 0.9983) (22).

Ivanovic et al. have revealed a valid RP-HPLC method for analyzing hydrochlorothiazide and Capoten in tablets. Jankowski et al., calculated by HPLC in blood with captopril (23). The captopril adduct recovery achieved 93.1% and the detection limit was 15 ng.mL⁻¹, while the conceptual limit was 30 ng.mL⁻¹. Inter and intra-assay RSD were below 9%, but precision was found to be below 8%. Captopril was calculated by Saleem et al. and Amini et al. (24) in plasma.

For the quantitative determination of angiotensin converting enzyme inhibitor by the quantity of scientists victimisation HPLC (25), variety of assay strategies are developed. a range of examinations are recorded victimisation HPLC for the determination of angiotensin converting enzyme inhibitor in bulk drug substances and their formulations (26). Direct determination of the four ACE-inhibitors Zestril, Enalapril, angiotensin converting enzyme inhibitor and Fosinopril in prescription drugs and blood serum by HPLC analyst separation was accomplished by RP-HPLC gradient with a mobile step consisting of acetonitrile: water (60:40 v / v) with ortho oxyacid changed to pH 3.0 (26), Fig.3 demonstrates captopril’s chemical composition.
Monitoring of in vitro trials of ACE inhibitor association with LC-UV symptom agents and parallel LC determination of rosvastatin, lisinopril, captopril, and angiotensin converting enzyme inhibitor in API, medicament formulations, and human blood serum (27).

Another Facile and Manifest Liquid chromatographical method for coinciding Determination of ACE inhibitor and NSAIDs in API and Pharmaceutical Formulations has been according and CAP has been isolated from NSAIDs by means that of a column of Purospher STAR C18 (250.4.6 mm, five μm) and a mobile section consisting of alcohol, water (80:20,v / v) (28).

Therefore, ACE inhibitor with water pill HydroDIURIL and diuretic in active pharmaceutical ingredients, medicative indefinite quantity formulations and human body fluid area unit used as combination indefinite quantity forms for alternative distinctive forms of drug substances like metal channel block medicinal drug, diuretics, etc. (29).

Other hypoglycaemic Capoten, medicinal drug and H2 receptor antagonist ways ar recorded in bulk, formulations, and human humor by RP-HPLC (30-32).

Biosensors have become important bioanalytical instruments in the last few years for environmental testing, biotechnology, pharmaceuticals, food safety and other consumer industries. Due to their high sensitivity, high precision, low expense, compact size and simple activity, the use of biosensors to test chemical species is an exciting opportunity.

High sensitivity and fast detection are key criteria for a sensor for detecting biomaterials. There has been a great deal of study over many decades to develop quick and responsive biosensors for various applications. In particular, owing to the probability of fast and direct (unlabelled) detection, optical methods have been reported to have a high potential.

A variety of biosensor instruments, including several interferometers, surface plasmon resonance sensors and micro ring resonator sensors, have been used to detect various biomaterials. While all these sensors offer sensitive and quick detection, they are only suitable for items up to 100 nm in size (33).

A light detector resistance (LDR) is AN optical device mounted in an exceedingly black PTFE cell and paired to a cheap multimeter (Ohmmeter). The chemical analysis is based on the reduction of ammonium ion molybdate by Capoten, producing a green-yellow compound in the presence of sulphuric acid (λmax 407 nm). By plotting the electrical resistance of the LDR against the CPT concentration, the standardization curves were obtained within the vary of 4.60 x 10^{-4} to 1.84 x 10^{-3} mol.L^{-1} with an affordable constant of determination (r^2 = 0.9962) (34).

Moreover, as a result of their uncommon optical properties and catalytic ability, Molybdenum compound nanomaterials have recently attracted widespread interest. There is, however, no literature up to now on the utilization of photoluminescent nanomaterials of Molybdenum compound in biological and pharmaceutical sensing.

Via Associate in Nursing easy method, photoluminescent Molybdenum oxide compound quantum dots (MoOx QDs) were synthesized and so the synthesized MoOx QDs were more additional as a
A replacement sort of photoluminescent probe to create a replacement off-on angiotensin converting enzyme inhibitor (Cap) detector supported the idea that the quenched photoluminescence of MoOx QDs by Cu$_2^+$ was fixed up to Cap by precise interaction between thiol cluster of Cap and Cu$_2^+$. The rebuilt photoluminescence strength showed a powerful linear relationship with the Cap material, variable from 1.0 to 150.0 μM, with a 0.51 μM (3σ / k) detection most, beneath ideal conditions. additionally, with the recently designed off-on device, the content of Cap was with success known in pharmaceutical samples, and therefore the recoveries were 99.4-101.7 percent, indicating that the newest off-on device includes a high accuracy.

Replicate (n = 10) measurements were created on an equivalent answer comprising the equivalent of 1.15 x 10$^{-3}$ mol.L$^{-1}$ of CPT to check the repeatability and preciseness of the measure methodology. Determinations (n = 10) were created with an equivalent answer containing CPT adequate to 1.15 x 10$^{-3}$ mol. L$^{-1}$.

This resolution was tested in conjunction with the prescribed approach for measurement the CPT content of pharmaceutical formulations. In terms of share relative variance (% RSD) (36), the accuracy was measured.

**The Biosensors Specifications**

The essential specifications for biosensors are the following:

Ø Ability to provide real-time data during a project at each development stage or at multiple time points. This would help in improved tracking and regulation of blood sugar levels, for example.

Ø It is feasible to customise several biosensor technologies to permit continuous flow analysis that’s terribly helpful in food process, observance of installation, and air quality.

Ø Through miniaturization, biosensors can reduce usage costs and can be incorporated into efficient, highly capable lab-on-a-chip tools.

Ø Biosensors is used for point-of - care or on-the-spot analysis wherever progressive molecular analysis is conducted while not the requirement for a progressive laboratory (37).

**Biosensor system**

In general, a biosensor uses a facet of biological recognition that detects the presence of an analyte (the species to be detected) and produces a physical or chemical change that’s remodeled into a symptom by an electrical device (detector). The general block diagram of the framework of biosensors is defined in Fig. 4. Specific system for biosensing. An analyte is inserted into the detector by the sampling machine. The identification issue attaches or responds to a specific analyte, giving a biodetection specificity. Enzymes, antibodies, proteins, DNA or additionally cells like yeast or bacterium are used as parts in biorecognition (38). In general, stimulus may be generated by optical, electrical, or other kinds of force fields that, as a result of biorecognition, extract a reaction. The transduction mechanism converts the physical or chemical process of biorecognition into an optical, electrical or another variety of signal within the presence of external stimuli that’s then detected by the detector device. For identification of the analyte, the detection device may provide pattern recognition.

A broad variety of real life applications are defined by biosensors (39). In essence, future uses are medicinal and nonclinical (40). The use of biosensors to track toxins (41), micro-organisms, microbes, viruses (42) and chemical and biological defense against terrorism is of more recent concern. In agricultural and environmental applications (43), it is also common. A few days now, applications based on nanotechnology (44) are also being created.
The Immobilization Techniques (Mechanism)

The biological part has to be correctly attached to the transducer to make a biosensor. Immobilization is called this method. On a solid support, the biorecognition components are typically immobilized. A membrane, rubber, copolymer, or semiconductor material are typically the solid supports. A biorecognition component, either by a physical methodology (such as adsorption) or by chemical association, is immobilized on the solid support. In sure ways, the issue of biorecognition is cursed with controlled porosity within the volume of the matrix (solid support), wherever solid support typically provides property against associate analyte of a precise size in step with its pore dimension (44).

Conclusion

A significant variety of drugs for effective treatment are administered to people diagnosed with hypertension, raising the risk of adverse effects and drug reactions. But Gupta et al. (37-44) have documented some electro analytical approaches.

UV and HPLC strategies and optical biosensors for the determination of ACE inhibitor in active ingredients, pharmaceutical formulations and biological specimens square measure investigated alone or in conjunction with different medication during this study.

In general, pricey instrumentation, provision for the utilization and disposal of solvents, labour-intensive sample preparation procedure and private skilled chromatographical techniques were needed for HPLC strategies.

Furthermore, several of the examined HPLC approaches have the ability to apply drug combination, multi-drug pharmacokinetics trials and association studies to clinical science.

Thus, optical biosensors are one of the most interesting alternatives in this situation, since they play an important role in drug quantification and are one of the most specific, sensitive, low-cost and easy-to-use options available at the moment. This has provided a niche for them as new clinical instruments that allow more precision medicine to be controlled therapeutically by minimizing symptoms or secondary effects as much as possible and contributing to safer patient treatment and prescription.

In addition, the use of biosensors offers doctors a benefit when making the most precise choices for
real-time dosing at the bedside of a patient, since biosensors are compact instruments that are easy to use and miniaturized. When formulating medicines, they have stricter and more effective regulation. There is an immediate need for more research based on the quantification of blood medications and including all pharmacological classes to ensure that a greater range of medications have personalized doses in accordance with the premise of usage.

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**Conflict of Interest** there has been no conflict of interest of any kind with the authors of this work

**Ethical Standard:** The study was formally approved the research plan by the ethical committee board at the Babylon health directorate.

**Informed Consent** was taken from all the participant patients before being enrolled in the study

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Immunohistochemical Characterization of Hepatic Nuclear Factor 4 Alpha Expression in the Choroid Plexus of the lateral and 4th Ventricles of Adult Male Rat Brain

Rasha A. Salman¹, Taghreed Abdulrsool Ali², Duaa AL musawi³

¹Assistant Lecturer, Dept. of Biology, Madenat Alelem University College, Baghdad, Iraq, ²Assistant Lecturer, Dept. of Dentist, AlTurath University College, ³Lecturer Baghdad, Iraq, Dept. of Biology, Madenat Alelem University College.

Abstract

The choroid plexus (CP) is present in brain ventricles. It is responsible for cerebrospinal fluid (CSF) secretion and various vital functions. Special proteins present in choroidal epithelium play important roles in CSF production and energy metabolism.

This study aims to compare between the lateral and fourth ventricles CPs using hepatocyte nuclear factor 4 alpha (HNF4α), metabolism marker, to evaluate the functional activity of this tissue in the two regions.

Ten adult male albino rats were used to study the histological features of the CPs and to study the functional activity by quantitative immunohistochemical labeling with HNF4α marker.

The CP of the fourth ventricle had more functional activity than the CP of the lateral ventricle. A quantitative assessment of HNF4α using Aperio ImageScope Software Analysis showed that the lateral ventricle CP mean positivity 0.264 ± 0.083 pixel/micron² while the fourth ventricle CP have mean positivity 0.297 ± 0.043 pixel/micron². The immunohistochemical expression of marker in the fourth ventricle CP were significantly, P ≤ 0.05 higher than those in the lateral ventricle at P ≤ 0.05. Immunohistochemical detection of metabolism marker went along with findings of other histological and biochemical studies to define the CP as a highly dynamic structure with regional variations forming a continuum of one entity tissue capable of functional adaptation according to body needs.

Keywords: Choroid Plexus, Lateral Ventricles, Fourth Ventricle, HNF4α

Introduction

The choroid plexuses (CPs) are leaf-like highly vascular structures (1). Four CPs floating inside the ventricular cavities of the brain: one in each of the two lateral ventricles, one in the third, and one in the fourth ventricle (2).

Corresponding author:
Rasha A. Salman
Assistant lecturer at Madenat Alelem University College, Al-kadhmiya, Baghdad, Iraq
E-mail: Rashaabjad@gmail.com

The CPs are extensions of the ependymal lining of the ventricular walls and consist of a fenestrated vasculature core surrounded by a single layer of polarized cuboidal epithelium with an interstitial stromal layer of connective tissue rich in fibroblasts and cells of the immune system in between (3). Adjacent CP epithelial cells are joined together by tight junctions to form the blood-cerebrospinal fluid (CSF) barrier. Together with adherens junctions, the tight junctions also ensure the apico-basal polarity of membrane proteins (for example, transporters) that are critical for normal epithelial cell function (4).
The main known function of the CP epithelium is to produce CSF via passive filtration of peripheral blood across the choroidal capillary endothelium in the vasculature core followed by regulated active secretion across the single-layered epithelium (5).

Hepatocyte nuclear factor 4 alpha (HNF4α) may play a role in the transcriptional control of drug transporters. It is a member of the nuclear receptor superfamily that regulates a broad range of xenobiotic–metabolizing enzymes and thus regulating the metabolism in the CP (6). The HNF4α gene can also be found in the liver, pancreas, intestine, brain and recently in epithelial cell of CPs (7).

**Materials and Methods**

**Animals and tissue preparation:**

A sample of 10 adult male rats (*Rattus norvegicus albinus*). The animals aged 3-6 months, with 300 ± 50 g body weight, and were fed with standard pellet diet. Animals were euthanized with chloroform soaked cotton in an air tight chamber for 5 minutes, then the brains were removed from the skulls and fixed for 18 hours in 4% paraformaldehyde at room temperature (22°C).

The brains were cut in coronal planes rostral to the optic chiasma and caudal to the midbrain in order to obtain lateral and third ventricles specimens, while fourth ventricle samples were made by trimming the remaining caudal part of the brain (cerebellum and brainstem). The specimens were then left in the fixative for another 18 hours and finally transferred into commercial 70% methanol where they were kept until further processing. Paraffin blocks were made and 5 µm thickness sections were cut for immunohistochemical labelling (8).

**Immunohistochemistry Labeling:**

The Super Sensitive IHC for Detection Kit HNF4α antibody was found in CP by following all subsequent steps, which carried out at room temperature in a humidified chamber. Super Sensitive IHC Detection Kit was used. Sectioning at 5 µm were used and deparafinization, Incubate tissue in appropriate pretreatment or digestive enzyme for primary antibody and PBS/TBS wash 3 times for 2 minutes. Then incubate slide in Hydrogen Peroxide Blocking Reagent for 10 minutes, PBS/TBS wash 3 times for 2 minutes. Apply Blocking Reagent and incubate for 5 minutes, PBS/TBS wash 3 times for 2 minutes. Apply primary antibody and incubate according to manufacturer’s recommended protocol (overnight) incubation, PBS/TBS wash 3 times for 2 minutes. Apply HRP Polymer and incubate for 10 minutes, PBS/TBS wash 3 times for 2 minutes. Add 20µl of DAB Chromogen to 1 ml of DAB Substrate, mix by swirling and apply to tissue. Incubate for about 3-5 minutes, PBS/TBS wash 3 times for 2 minutes. Finally counter stain and cover slip using a permanent mounting media (9).

**Controls**

For positive controls, adult male rat kidney sections were labelled for HNF4α in the same procedure, while for negative controls adult male rat brain and kidney sections were labelled in the same procedure except that primary antibodies of HNF4α were replaced by PBS.

**Immunohistochemical Reaction Assessment**

For HNF4α marker, forty field images of immunohistochemically labelled slides were captured from the lateral ventricle CP, and a similar number of fields were captured from the 4th ventricle CP. A LEICA DM 750 light microscope equipped with Digital Microscopic Camera 5 Mega pixel digital camera were used to capture the fields. Images were processed with Aperio ImageScope v.11 program for total positivity. Microsoft office Excel® 2013 program was used to describe the collected data by calculating the Descriptive Statistics and t-Test were used to compare between means in this study.

**Results**

**Immunohistochemical Labeling of the Choroid Plexus**

**Hepatocyte Nuclear Factor 4 Alpha (HNF4α)**
Light microscopic examination of sections labeled with anti-HNF4α showed high reactivity in choroidal epithelium compared with other cells of brain tissue. There was no detectable difference between reactivity of lateral and fourth ventricles CPs. Ependyma showed weaker reactivity to HNF4α marker than the choroidal epithelium (Figures 1-2). Endothelium of choroidal vessels were highly reactive to HNF4α marker whereas blood cells inside these vessels were non-reactive with this marker (Figure 2).

Controls

External positive and negative controls, and internal negative controls are seen in (Figure 3).

Aperio ImageScope Software and Statistical Analyses

Assessment of Anti-HNF4α Reactivity

Statistical analysis of anti-HNF4α reactivity in the lateral and fourth ventricles CPs gave mean values of 0.264 ± 0.083 pixel/micron² and 0.297 ± 0.043 pixel/micron², respectively, with a wider range of reaction intensity in the lateral ventricle CP than that in the fourth ventricle CP (Figure 4). Two-sample assuming equal variances t-Test revealed a statistically significant difference between these values (p<0.05) (Tables 1-2).

Table 1 Descriptive statistics of HNF4α marker labeling in the lateral and fourth ventricles CPs.

<table>
<thead>
<tr>
<th>Descriptive Statistics</th>
<th>Lateral ventricle CP HNF4α</th>
<th>Fourth ventricle CP HNF4α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.264</td>
<td>0.297</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.013</td>
<td>0.006</td>
</tr>
<tr>
<td>Median</td>
<td>0.258</td>
<td>0.292</td>
</tr>
<tr>
<td>Mode</td>
<td>#N/A</td>
<td>#N/A</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.083</td>
<td>0.043</td>
</tr>
<tr>
<td>Range</td>
<td>0.428</td>
<td>0.185</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.102</td>
<td>0.208</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.531</td>
<td>0.394</td>
</tr>
<tr>
<td>Count</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 2 Comparison of the total positivity of HNF4α marker in the lateral and fourth ventricle CP.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lateral ventricle CP HNF4α</th>
<th>Fourth ventricle CP HNF4α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.264</td>
<td>0.297</td>
</tr>
<tr>
<td>Variance</td>
<td>0.007</td>
<td>0.001</td>
</tr>
<tr>
<td>Observations</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Df</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>t Stat</td>
<td>-2.211</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>1.990</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Previous studies on the CPs of the lateral, third and fourth ventricles considered them as one entity but some authors reported differences in activities of certain metabolic enzymes of the various CPs (10). The immunohistochemical reactivity of HNF4α in the CPs of both lateral and fourth ventricles were estimated with Aperio ImageScope software that could detect the cells labeled with the specified marker and categorized them into three groups: strongly positive, positive, and weakly positive, while negative areas were those without any reactivity. That was applicable for choroidal epithelium, ventricular ependyma, and endothelial cells of choroidal vessels, but not blood cells within (Figures 1(B)-3).

Positively labeled cells were marked up with Aperio ImageScope software as brown, light brown and yellow colored, indicating strongly positive, positive and weakly positive, respectively (Figure 4). In this study, it was not possible to localize, with precision, HNF4α receptors in cells which were previously localized in the basolateral side of choroidal epithelium plasma membrane, though it was clear to identify HNF4α labeling as a granular stain occur in the cytoplasm. This might be due to the presence of aggregations of transporter proteins across the B-CSF-B like ABCC, ABCB1, ABCB4 and transthyretin. In addition, HNF4α was observed in the endothelium preventing backflow of metabolites to the blood as ABCC proteins play a protective role in choroidal epithelium and mediate basolateral efflux of conjugates resulting from CSF drugs metabolism into the blood while ABCB1 proteins are distributed in the apical side of endothelium (11, 12).

Analysis of HNF4α reactivity in the CPs showed statistically significant higher readings in the fourth ventricle CP compared to that of the lateral ventricle (Tables 1-2), indicating higher activity in the choroidal epithelium of the fourth ventricle. However, cells of the CP of the lateral ventricle showed wider range of HNF4α expression, possibly reflecting a diverse state of activity in that CP since it is spread over wide regions in the brain’s ventricles when compared to the smaller size CP impacted in the fourth ventricle.

Expression of HNF4α regulates many proteins and metabolizing enzymes like the ATP binding cassette ABCC4 and ABCC1 in human and rat (12), and transthyretine which is one of the proteins present in the cytoplasm of CP cells (13) at the BCSFB. Demonstration of intracellular reaction of HNF4α by binding, for example, with transthyretin in choroidal cells cytoplasm reflects its role in regulation of this protein activity. The presence of well-developed endoplasmic reticulum and Golgi apparatus in CPs makes their ability to secret this protein high (14). Transthyretin is secreted specifically by the CP and not in other parts of brain and it binds with HNF4α to control drug transportation (15). All the above mentioned proteins can be labeled with anti- HNF4α to give a cytoplasmic reaction which may highlight an assumption of drugs metabolizing and transporting enzymes to be more concentrated in the fourth ventricle CP than that of the lateral ventricle, with clinical and pharmacological implications (16).

In this study, expression of HNF4α was significantly higher in the fourth ventricle CP compared to that of the lateral ventricle suggesting that protein regulation and metabolic activity are more in fourth ventricle CP, which is in contrast to that reported by Al-Kafagi et al. (16) who suggested the regulation of drug transporters is more in lateral ventricle CP. This disagreement might be due to the lack of use of controls in their work, or it might be caused by the different experimental setting when their conclusions were drawn on a different species. In addition, this study contradicts other findings on certain drugs metabolism where the CPs of lateral and fourth ventricles were found to be of similar activity (17), however, it is understood that the different methodology applied might explain this discrepancy.

Ependymal cells lining the lateral and fourth ventricles showed reactivity to HNF4α marker (Figures 1(B) and 2(B)), albeit at lesser extent on qualitative assessment. The mere observation of the ependymal cells expressing less HNF4α marker than the choroidal epithelium, but higher than the adjacent white matter of
the brain, needs to be analyzed quantitatively in a further extension from this study.

In addition to the ependymal lining, HNF4α labelling was also seen in endothelial lining of choroidal vessels (Figures 2(A)), however, assuming equal vascular density of both the lateral and fourth ventricles CPs, this labelling would not bias the results in this study, but further quantitative analysis of the vascular profile of the CPs is indicated.

In this study, the expression of HNF4α in choroidal cells of the fourth ventricle was higher than that of the lateral ventricle. Therefore, it might be expected to have abundant amounts of secreted proteins in the cytoplasm of choroidal cells, suggesting that the endoplasmic reticulum content of the fourth ventricle CP is higher compared with that of the lateral ventricle and consequently the metabolic rate is higher in the fourth ventricle CP, which agrees with previous studies (10).

Conclusion

While carrying the same name as a CP, that part in the fourth ventricle proved distinct functional characteristics from that in the lateral ventricle despite the structural similarities of their cells. In terms of transport system, this study showed preponderance in favour of the fourth ventricle CP, as well as in terms of metabolic activity no matter whether this is related to internal protein synthesis and fluid secretion, or is related to external substance metabolism.

These findings might add to previous works that showed higher functional activity in the CP of the fourth ventricle compared to that of the lateral ventricle, however short of addressing the two regions as distinct entities. Rather, they form a continuum of tissue capable of functional adaptation according to the body needs.

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Conflict of Interest: Authors declare no conflict of interests.

Funding: No external funding sources.

Ethical Clearance: This study had been approved by the University of AL-Nahrain, College of Medicine, Baghdad, Iraq.

Abbreviation: Choroid plexus: CP, Cerebrospinal Fluid: CSF, Hepatocyte Nuclear Factor 4 Alpha: HNF4α

References


AgO Nanowires: Synthesis, Characterization and Bioactivity

Rawaa A. Faris¹, Layla M.H. Al-ameri¹
Lecturer, Institute of Laser for Postgraduate Studies, The University of Baghdad

Abstract

This study is focused on Nanostructured AgO due to its novel properties. Here AgO-NWs manufactured by low-cost, fast green synthesis using curcumin extract. AgO-NWs structures were characterized by FESEM field emission electron microscope scanning. Uniform distribution can be seen for the AgO -NWs. The AgO -NWs thin film exhibits a promising giant third-order nonlinearity and ultrafast character. It behaves as a tunable refraction and absorption character the exciting energy is varied in the range of 80–120 mJ. Silver is a wide-spectrum natural antimicrobial agent that applies to patented wound dressings with broad applications. Today, AgO -NWs have attracted attention in Biophotonic Sensor. Here, AgO-NWs used to as antimicrobial agent against common infecting pathogenic microbes E-coli. The results demonstrated that the AgO-NWs against pathogenic bacterial organisms, they have possible antimicrobial activity and could be regarded as an alternative antibacterial agent.

Keywords: green synthesis;FESEM; Z-scan; third-order, E-coli

Introduction

Nanowire research (NWs) is one of the nanomaterial studies that has drawn interest in recent years. NWs are one-dimensional (1D) nanostructures that range from a few hundred nanometers to micrometers with a diameter of less than 100 nm and a length [¹,²]. Silver Oxide nanowires (AgO-NWs) are one of the materials that have been developed as a result of a fascinating analysis of mechanical, magnetic, electrical, optical properties and their use as catalysts, scanners, nanoelectronics, and photonic devices [³-⁵]. Besides, extremely high electrical conductivity, silver nanowire (Ag NW) is a highly attractive metal [⁶,⁷]. Different Ag NW applications are available, like photocatalysis, biosensors, nanobiosensors, photonic crystals, and surface-enhanced Raman scattering [⁸-¹⁰]. Previous methods to prepare AgO- NWs have also attracted scientists. A variety of synthetic methods, including template free and template-directed, have been successfully demonstrated up to now. Specifically shaped nanomaterials such as carbon nanotubes, or micelles with rod shaped are prepared with controlled measurements through the use of templates [¹¹]. The manufacturing of these templates, however, is often complicated and expensive. Researchers synthesized very long AgO-NWs with polyol-process multi-step growth. Other scientists have investigated the effect of poly(vinyl pyrrolidone) (PVP) on the time of reaction, temperature and molecular weight on the morphology of silver nanowires [¹²,¹³]. The effects of using mediated agents on the morphology and growth of AgNWs have been considered by a few studies. Also, researchers used EG and glycerol as humorous agents and investigated the mechanisms of self-seeding and development of AgNWs. Here, a natural reducing agent, curcumin, a natural polyphenolic substance well established as a wound-healing agent, has been used in recent studies to achieve the green synthesis of silver oxide nanowires.

Materials and Procedures

AgO-NWs preparness

40ml solution of 1M AgNO₃ was prepared and stirred for 30 minutes. The freshly prepared Curcomin extract was added to the solution and heated to 25 Celsius with continuous stirring for 4 hours. It was noted that the colour of the solution changed to nearly
brown indicating the formation of Ag nanostructure. The solution was centrifuged for 30 minutes at 5000rpm to separate the obtained powder from the solution. The obtained powder was incubated at 50 degree Celsius overnight to remove the moisture from the samples.

**Characterization of the AgO-NWs mesurement**

**Fied emission scanning electronic microscopy**

The AgO nanowires casting on glass substrate surface was examined using MIRA3 (Czech Republic), a high-performance FE-SEM device with a high-brightness emitter from Schottky for high-resolution and low-noise imagery.

**Optical properties**

The surface plasmon resonance of the samples was reported in the wavelength range of 300-1000nm using a UV-VIS (SHIMADZU 1800) spectrophotometer in addition to the substance structure and chemical characterization.

The nonlinear optical features of the thin film of the as-prepared AgO nanowires have been studied. Using a Z-scan technique, this characterization was carried out. In Fig.1, the Z-scan configuration is shown. A ns-532nm Nd: YAG laser with an energy of 120 mJ, a beam diameter of <1.5 mm, and a beam divergence of 0.82 mrad is included in the system.

To focus the laser beam on the specimen, a lens with a 20 cm focal length was used.

To investigate the variance of the pulsed laser strength, AgO-NWs was placed on a linear translation step that can move around the focus. To test the difference in the signal, a photo-detector (RJ-7610) was used.

![Fig.1. Z-scan experimental setup.](image)

To calculate the nonlinear refractive index ($n_2$) and the nonlinear absorption coefficient ($\beta$), respectively, the open and closed aperture methods of the Z-scan technique have been used \[14-16\]. The normalized open-aperture signal is estimated as the following relationship.

$$ T(Z) = \sum_{m=1}^{\infty} \left( \frac{\beta \omega_0 L_{eff}}{l_0} \right)^m \left( \frac{1}{(m+1)^{2/2}} \right) $$

It refers to the intensity of the laser beam at the focusing point and describes the effective duration of propagation within the sample. Although the fitting equation was determined using\[17\]:

$$ n_2 = \frac{\Delta\phi_0}{kL_{eff}} $$

$k$ is wavenumber which equals to ($k=2\pi\lambda$) and, $\Delta\phi$ is the phase shift estimated by the equation:

$$ |\Delta\phi_0| = \frac{\Delta T_{p-v}}{0.406(1-S_p)^{0.25}} $$

The third-order nonlinear susceptibility $\chi^{(3)}$ can be calculated by below equation\[18-20]\:

$$ \chi^{(3)} = \sqrt{(\text{Re}\chi^{(3)})^2 + (\text{Im}\chi^{(3)})^2} $$

$$ \text{Re}\chi^{(3)} = 10^{-4} \frac{\varepsilon_0 c^2 n_2^2}{\pi} n_2 $$

$$ \text{Im}\chi^{(3)} = 10^{-2} \frac{\varepsilon_0 c^2 n_2^2 \lambda}{4\pi^2} \beta $$

**Bioactivity test**

Green synthesis antibacterials for AgO-NWs were tested using a well-diffusion system against E-coli. Microorganisms in the medium of Uti Chrom Agar (UCA) were grown at 37 °C. 18 to 24 hours of single colonies on agar plates were used for the preparation of bacterial suspension with a turbidity of 0.6 McFarland (equal to 1.6 X10^8) colony-forming units (CFU)/ml. With 90 µl impregnated with synthesized AgO-NWs, holes of 10 mm in diameter were filled and held at 37 °C. After the 24-hour incubation period, the diameter of growth inhibition zones was measured in millimeters.
Results and Discussion

FESEM results

FE-SEM images in Figure (2) showed a sparse and uneven distribution of AgO-nanowires in solution, and few clusters were observed or nanoclusters had a uniform distributional substratum surface with an average diameter of thirty nanometers.

Figure 2. FESEM images AgO nanowires (The magnification for images are 200,000 times and the bar of scale is 200 nm)

Optical properties results

Surface plasmon resonance property of our silver nanowires was characterized using UV-Vis spectroscopy. The spectrum (fig.3), shows two relatively sharp surface plasmon resonance peaks at 352 and 392 nm positions, which correspond to quadrupole resonance excitation and the transverse plasmon resonance of the nanowires, respectively. It is also observed that the plasmon resonance associated with the long and short axes are completely decoupled, confirming the high aspect ratio of our pentagonal nanowires.

Figure 3. Surface plasmon resonance spectrum

For both closed aperture and open aperture, the Z-scan profile of AgO-NWs at 120 mJ is shown in Fig. 4. AgO nanowires display a broad variety of nonlinear properties from this figure, such as self-defocusing, saturable absorption activity with massive absorption behavior where $\beta\beta = \frac{-0.06593 \ cm^2/GW}{-4834.28 \ cm/GW}$, and a strong $\chi^{(3)}$ of about $4.5*10^{-8}$ esu.

Figure 4. Normalized transmission of (a) closed aperture, and (b) open aperture.
Antibacterial activity of AgO-NWs against E-coli

The antibacterial activity of AgO-NWs showed positive results. The plant extract (curcumin) solution was no effect against E. coli, while AgNO₃ was showed little effect against E. coli with a zone of inhibition=11mm, but AgO-NWs showed a greater effect against E. coli with a zone of inhibition =18 mm compared to Trimethoprim-Sulfamethoxazole was effective against E. coli with a zone of inhibition=12mm as shown in figure (5).

Conclusions

Silver Oxide nanowires were successfully obtained from the green synthesis method thus paving the way for further investigation and synthesis of AgO-NWs structures using the green synthesis. The obtained AgO-NWs were characterized with FESEM. Synthesized AgO-NWs has shown the ability to serve as a great antioxidant agent and have anti-bacterial activity against E-coli.

References

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Determinant of Primary Preventive Behaviour Cervical Cancer in an Adolescent Girl

Muh Zul Azhri Rustam1, Dwi Helynarti Syurandhari2, Deni Susyanti3, Irma Handayani3, Fitriani Pramita Gurning4, Muchti Yuda Pratama5

1Researcher, STIKES Hang Tuah Surabaya, Indonesia ; 2Researcher, Sekolah Tinggi Ilmu Kesehatan Majapahit, Indonesia; 3Researcher, Nursing Study Program, Akademi Keperawatan Kesdam I/Bukit Barisan Medan, Indonesia, 4Researcher, Faculty of Public Health, Universitas Islam Negeri Sumatera Utara, Medan, Indonesia, 5Researcher, Faculty of Health Sciences, Universitas Sumatera Utara, Medan, Indonesia

Abstract

Cervical cancer is caused by infection with the human papilloma virus which can infect the genitals, anus, throat, mouth and cells on the surface of the skin. Cervical cancer is often late so that it can cause death, so efforts are needed to reduce the risk factors for cervical cancer, namely through primary prevention efforts. The purpose of this study was to determine the relationship between primary factors and cervical cancer prevention behavior in adolescent girls. The sample in this study were 77 young women who were taken by technique purposive sampling. The research design used was an observational analysis using a cross sectional approach and analyzed using chi-square. The results of this study illustrate that there is a relationship between knowledge, attitudes and social support with cervical cancer prevention behavior in adolescent girls. So we need efforts to reduce cervical cancer risk factors, namely through primary prevention efforts, by increasing outreach activities in the community to carry out a healthy lifestyle.

Keywords: knowledge, attitudes, social support, cervical cancer prevention behavior.

Introduction

Reproductive health is a health sector that is closely related to female reproduction, one of which is cervical cancer. Cervical cancer ranks second in prevalence after breast cancer. Cervical cancer is a disease that is difficult to detect, if it can be detected, it is often at an advanced stage so it is difficult to treat. Cervical cancer is also known as the ladies silent killer because it is the second killer after breast cancer (1,2).

Developing countries such as Indonesia, women who suffer from cervical cancer at this time still rank second after breast cancer, where the incidence of cervical cancer in Indonesia is estimated at 100 / 1000.000 thousand per year. Cervical cancer is a frightening disease, this is because cervical cancer is very malignant and can even cause death. There has been an increase in cervical cancer in Indonesia and one of the causes of death at productive age (3,4).

Cervical cancer can be cured if detected and treated early on, but due to the lack of symptoms, the treatment for cervical cancer is often too late so that it can cause death. The most important examination in early detection of cervical cancer is the Papanicolaou Smear (Pap Smear), especially for women who are already sexually active (5,6).

All women are at risk for cervical cancer, factors that have a high risk of cervical cancer such as use of oral contraceptives, smoking and casual sex. Adolescence is a period of transition from children to adulthood and at this time the teenager’s soul is still full of turmoil. Many of the teenagers behave deviantly such as smoking, free
sex. Adolescent smoking behavior is not only a gateway for all types of substance abuse but also causes various health problems \(^{(7,8)}\).

Cervical cancer is caused by infection with the human papilloma virus which can infect the genitals, anus, throat, mouth and cells on the surface of the skin. Women who do not use condoms during sex and frequently change sexual partners from adolescence have a higher risk of cervical cancer. Heredity is also a risk factor for cervical cancer because it has a tendency to be unable to fight infection with the human papilloma virus which can cause cervical cancer in women \(^{(9,10)}\).

So efforts are needed to reduce cervical cancer risk factors, namely through primary prevention efforts, by increasing outreach activities in the community to carry out a healthy lifestyle such as delaying sexual activity, using contraception and using vaccines \(^{(1,6)}\). This research is aimed at developing prevention efforts against cervical cancer problems in adolescent girls, planning strategies or activities health promotion programs in reducing the incidence of cervical cancer in Indonesia.

**Material and Method**

This study used an observational analysis design using a cross-sectional approach. The independent variables in this study were knowledge, attitudes, social support and the dependent variable was cervical cancer prevention behavior. The population used in this study were all students. Sampling using a technique purposive Sampling is a way of taking subjects not based on random, regional or strata-based, but based on the existence of certain goals. In this study, a sample of 77 respondents was obtained. The data analysis method used in this study is chi-square to see the relationship between the independent variable and the dependent variable.

**Findings**

*Table 1. Relationship between knowledge and cervical cancer prevention in adolescent girls.*

<table>
<thead>
<tr>
<th>Knowledge</th>
<th>Cervical Cancer Prevention Behavior</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Bad</td>
</tr>
<tr>
<td>Good</td>
<td>24 (57.1%)</td>
<td>18 (42.8%)</td>
</tr>
<tr>
<td>Bad</td>
<td>20 (57.1%)</td>
<td>15 (42.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>44 (57.1%)</td>
<td>33 (42.8%)</td>
</tr>
</tbody>
</table>

Test Chi-Square 0.000

Based on the results of table 1, it is stated that as many as 57.1% or 24 young women who have good knowledge with good cervical cancer prevention behavior and as many as 20 young women or 57.1% who have bad knowledge with good preventive behavior. The results of the analysis said the p-value was 0.000, which means that there is a relationship between knowledge and cervical cancer prevention behavior in adolescent girls.
Table 2: Relationship between attitudes and cervical cancer prevention in adolescent girls.

<table>
<thead>
<tr>
<th>Attitude</th>
<th>Cervical Cancer Prevention Behavior</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Supporting</td>
<td>27 (56.2)</td>
<td>21 (43.7)</td>
</tr>
<tr>
<td>Unsupportive</td>
<td>17 (58.6)</td>
<td>12 (41.3)</td>
</tr>
<tr>
<td>Total</td>
<td>44 (57.1%)</td>
<td>33 (42.8%)</td>
</tr>
</tbody>
</table>

Test Chi-Square 0.001

Based on the results of table 2, it is stated that 56.2% or 27 young women have a supportive attitude with good cervical cancer prevention behavior and as many as 17 girls or 58.6 % who have an unsupportive attitude with good preventive behavior. The results of the analysis said the p-value was 0.001, which means that there is a relationship between attitudes and cervical cancer prevention behavior in adolescent girls.

Table 3: Relationship between social support and cervical cancer prevention in adolescent girls.

<table>
<thead>
<tr>
<th>Social support</th>
<th>Health Status</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Supporting</td>
<td>32 (71.1)</td>
<td>13 (28.8)</td>
</tr>
<tr>
<td>Unsupportive</td>
<td>20 (62.5)</td>
<td>12 (37.5)</td>
</tr>
<tr>
<td>Total</td>
<td>52 (67.5%)</td>
<td>25 (32.4%)</td>
</tr>
</tbody>
</table>

Test Chi-square 0.001

Based on the results of table 3, it is stated that as many as 71.1% or 32 young women who get social support with good cervical cancer prevention behavior and as many as 20 young women or 62.5% who do not get social support with good preventive behavior. The results of the analysis said the p-value was 0.001, which means that there is a relationship between knowledge and cervical cancer prevention behavior in adolescent girls.

Discussion

Knowledge

Increased knowledge in society, especially young women can change negative behavior into positive behavior, knowledge can also form trust. Cervical cancer prevention behavior can prevent young women from risk factors that can trigger disease, namely cancer that can be caused by exposure to cigarette smoke, improve health, especially endurance by consuming a balanced nutritious diet and contain lots of vitamins such as folic acid, vitamin C and A. In implementing cervical cancer
prevention behavior, knowledge of risk factors that must be avoided and conducting early detection checks and increasing nutritional intake is needed. A person’s behavior which is based on knowledge will be more lasting than those that are not based on knowledge (5,11).

Information is one way to strengthen knowledge and change behavior. Knowledge is the impression in the minds of men as a result of the senses, which is quite different from the beliefs (beliefs), superstitions (superstitious), and caption information wrong (misinformations). This theory statement emphasizes that the meaning of correct information can be positive (12–14).

Knowledge is a person’s way of determining steps, choices, and attitudes in decision making, to determine steps for cervical cancer prevention behavior so that someone makes a behavior change, a fundamental stimulus through health education is needed so that information can reach the community, especially young girls to get their rights to it. reproductive health (15,16).

Attitude

A person’s attitude is influenced by several factors, namely culture, personal experience, mass media, emotional level, other people who are considered important, religion or educational institutions. Attitudes that shape cervical cancer prevention behavior support are influenced by the personal experiences of young women in the learning process at educational institutions, peers or family, the mass media as a media for scientific information about cervical cancer prevention and educational institutions that provide learning about reproductive health. These factors can provide a stimulus for young women in responding to cervical cancer prevention behavior (17–19).

From the description above it can be concluded that the attitudes of young women towards cervical cancer prevention behavior are formed through personal experiences, experiences of other people who are considered important such as parents, emotional level, educational institutions and thus stimulating the emergence of intentions to prevent cervical cancer behavior (20,21).

Social Support

Forms of social support such as reward support, emotional support, group support, instrumental support and information support. Support from other people such as family is a determining factor for this because support from family will provide reinforcement of motivation to carry out cervical cancer prevention behavior. families who have good knowledge can provide understanding and support for young women to carry out healthy behaviors (22,23).

The continuity and success of young women with healthy behavior really needs support from family members. Support from families, especially parents, is very important to improve the health status of young women. Family support can provide emotional benefits or can affect behavior in cervical cancer prevention behavior (22,23).

The culture of Indonesian society is still being carried out today where the family is the determinant of decisions that affect the behavior of young women in cervical cancer prevention behavior. This makes family support very meaningful in the continuation of preventive behavior considering that the family often acts as a decision maker for efforts to maintain the health of their family (22,24).

Conclusion

Analysis of this study illustrates that there is a relationship between independent variables such as knowledge, attitudes and social support with cervical cancer prevention behavior in adolescent girls.

Conflicts of Interest: All authors have no conflicts of interest to declare.

Source of Funding: The source of this research costs from self.

Ethical Clearance: The study was approved by the institutional Ethical Board of Ibn Khaldun University.
References


Significant Association of Adam 33 Polymorphism with COPD in Javanese Population of Indonesia

Retno Ariza Soeprihatini Soemarwoto¹, Jamsari², Yanwirasti³, Andika Chandra Putra⁴, Syazili Mustofa⁵, Wawan Abdullah Setiawan⁶, Ifan Aulia Candra⁷

¹Associate Professor, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Lampung, Bandar Lampung, Indonesia, ²Professor, Department of Plant Breeding, Faculty of Agriculture, Universitas Andalas, 25136, Padang, Indonesia, ³Professor, Department of Anatomy, Faculty of Medicine, Universitas Andalas, Padang, Indonesia, ⁴Medical Staff, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Indonesia, National Respiratory Referral Persahabatan Hospital, Jakarta, Indonesia, ⁵Medical Staff, Department of Biochemistry, Molecular Biology and Physiology, Faculty of Medicine, Universitas Lampung, Bandar Lampung, Indonesia, ⁶Lecturer, Department of Biology Faculty of Mathematics and Natural Science, Universitas Lampung, Bandar Lampung, Indonesia, ⁷Staff, Department of Biotechnology, Post-Graduate Program, Universitas Andalas, Padang, Indonesia

Abstract

Background: Chronic Obstructive Pulmonary Disease (COPD) is one of World health cases that is commonly known, which is triggered by the combination of environmental factors especially cigarette smoking and genetic factors. The association between A disintegrin and metalloprotease 33 (ADAM33) polymorphisms and COPD has been investigated and reported by other researchers. Objective: The main aim of this study is to identify the association between single nucleotide polymorphisms (SNPs) in ADAM 33 gene with COPD in the Javanese population in Lampung, Indonesia. Methods: A randomized cross-sectional study was used in this research. PCR-Sequencing method was involved to analyze the polymorphic for three SNPs (T1, T2, and Q-1) of the ADAM33 gene. Statistical analysis data was performed in descriptive and comparative as well as it was measured by parametric/non-parametric tests. Results: The results showed that the T2 GG, and T1AG genotypes in COPD group were significantly more frequent rather than in control group (p < 0.05). In case of allele, it was found that the T1G and T2G was higher in COPD group rather than in the control group (p = 0.440 and 0.131, respectively). Conclusion: The results clearly conclude that there was significant association between T1 and T2 polymorphisms of ADAM33 gene and COPD in the Javanese population of Lampung, Indonesia.

Key Words: ADAM 33 gene polymorphism, COPD, smoking, Javanese, SNP

Introduction

A major disease which can cause morbidity and mortality in the world is chronic obstructive pulmonary disease (COPD). In addition, persistent airflow obstruction is identified as one of its characteristics. The COPD is correlated to an enhanced chronic inflammatory response in airways and lungs to noxious particles and gases, and it is also progressive(1). The global burden of COPD estimates that affects 300 million people worldwide resulting in approximately 3 million deaths annually (2). The prevalence of COPD kept increasing globally. In a recent survey the estimated prevalence of COPD was 4.5% in Indonesia(3). This is highly regarded in low tobacco control policies and large of smokers population(4). In a recent health cost study suggested estimation of the smoking treatment cost in Indonesia attained 2.5% of the gross domestic product.

Corresponding author:
Syazili Mustofa
syazilimustofa.dr@gmail.com
in 2015. Moreover, it became the biggest contributors of Indonesian state burden\(^5\).

There are great numbers of genes which have been associated to the pathogenesis of COPD. The protease–antiprotease pathways, inflammatory response to cigarette smoke pathways, and oxidant–antioxidant pathways have been explored by case–control genetic association\(^6\). Nevertheless, COPD genetic profiling use in the clinic is current limited to find out Alpha-1 antitrypsin deficiency\(^7, 8\). Furthermore, the relationship between A disintegrin and metalloprotease 33 (\textit{ADAM33}) polymorphisms and COPD has been reported by other researchers\(^9\)\(^-\)\(^11\).

\textit{ADAM} 33 is a complex molecule. It comes from a zinc-dependent metalloproteases \textit{ADAM} family and is produced by mesenchymal cells, involving smooth muscle and fibroblast. It is important for cell adhesion, fusion, signaling and proteolysis by releasing various of growth factors as activator and Th2 cytokines\(^12\). \textit{Its} gene, can be found in human chromosome 20\textit{p}13. It was investigated as COPD susceptibility gene\(^13\). The first genetic study reported on the association \textit{ADAM} 33 genetic polymorphisms and susceptibility done by Van-Dieman in a cohort of 1390 subjects in the general population. The research results highlighted that there was significant correlation between four SNPs (F+1, S1, S2, T2) and accelerated lung function decline\(^14\). Moreover, the reported research was strengthened by several similar studies in European and Asian regions such as India, China and Mongolia. Polymorphisms in \textit{ADAM33} to airway hyper-responsiveness have been correlated to airway inflammation in COPD, and accelerated lung function decline by other research\(^11\)\(^,\)\(^13\)\(^,\)\(^15\). As association between low lung function and high mortality risk, particularly on account of COPD, it is important to explore the genetic components that escalate susceptibility to lung function decrease and COPD. Later on, other researches, across different country studies were clearly explained the share of this gene in as COPD causation. COPD has become one of the common problems in Indonesia, especially for Javanese population. By considering smoking as their habit, they have high probability to get this disease\(^4\).

Therefore, the current study was conducted to explore \textit{ADAM 33} gene polymorphisms and COPD association in the Javanese population of Indonesia, which attained a high prevalence of this disease. This study might give an insight into the genetic basis of this disease and help in finding predictors for preventing COPD.

\textbf{Method and Material}

\textbf{Setting}

A randomized cross-sectional study was undertaken in this research. It included COPD male smoker patients who were randomly selected from Harum Melati Pulmonology Clinic and male healthy smokers who lived around Pringsewu District, Lampung Province, Indonesia. All researchers don’t have any conflict of interest. The research permission was acquired from Ethics Committee of Medical Faculty, University of Lampung. Participants who were involved attained at least 40 years old, they had smoked (The Brinkman Index, number of years of smoking multiplied by the number of cigarettes smoked per day, more than 0) and they also had no symptoms of lung cancer, infections, and other lung diseases. All of them fulfilled admission of spirometry and a baseline which were standardized questionnaire involving smoking history and social and demographic information. Thirty-two healthy age-matched male smokers (control group) who had no family history of COPD were already involved. In addition, The Institutional Ethics Committee of Medical Faculty, Universitas Lampung approved this research by number 1914/UN26.18/PP.05.02.00/2018. All the participants were taken part to make written informed consent.

Furthermore, Pulmonary Function Tests (PFT) were performed by using spirometer according to the Pneumobile Indonesian Lung Health Survey utilizing a flow spirometer which was made by CHEST MI, INC, in Tokyo, Japan its brand namely CHESTGRAPH HI 101\(^16\). All of measurement values, (forced vital capacity
(FVC) values and Forced expiratory volume in the first second (FEV1), were calculated and standardized in percent based on reference. The participants who had post bronchodilator FEV1/FVC attained airway obstruction if its ratio was less than 70%. Moreover, classification of airway limitation severity in COPD was categorized taken from the Global Initiative for Chronic Obstructive Lung Disease as source.

**Genetic analysis**

SNPS (T1, T2, and Q1) of the ADAM 33 gene were undertaken as genetic analysis (table 1). The genomic DNA extraction process was originated from 500 µL of blood using material from Promega’s DNA Wizard® made in Madison, USA following the manufacturer’s procedure. The results of DNA extraction were examined by using implant, a digital DNA nanophotometer, made in Germany. The DNA extracts were frozen at –20°C until they were analyzed.

Amplification of ADAM33 was performed using kit NEXpro™ e PCR 2x Master Mix solution (Nex diagnostics, South Korea). Two primer pairs were used thereby designed in nearly located on SNPs. The first pair amplified the SNP at the locus rs2280090 (T2) and rs2280091 (T1), it was caused by the distance between SNP rs2280090 and rs2280091 only 29 nitrogen bases. The second pair amplified rs612179 (Q-1). We had confirmed the results of PCR using QIAxcel DNA High Resolution Kit digital electrophoresis equipment (Singapore). Next, the amplicons were sent to Indonesian Bioneer Ltd for two directional sequencing. It was analyzed by BioEdit software developed by Tom Hall. Thereafter, the researchers constructed a phylogenetic tree using Mega X which was developed by Kumar et al\(^{(17)}\). It was involved from several representative samples using maximum likelihood, bootstrap method and 1000 times number of replications (Figure 1).

**Statistical Analysis**

The experimental data was analyzed by using parametric and non-parametric statistical tests. The data is showed as mean ± SD. The researchers performed data through SPSS type 11.0 which involved students't-test and chi square test. A significant different was indicated as p value of less than 0.05. Data analysis used IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA).

**Result**

There were three position of SNPs ADAM 33 gene that researchers focused on. They were T1, T2, and Q-1. The amplifications of gene sequence that contained of them were attained by using the primers in table 1. Primers were designed by using Gene Bank code access DQ995342.1 from the website https://www.ncbi.nlm.nih.gov. The research involved 42 COPD subjects who were matched for age and sex with 32 healthy smoker controls. All participants were Javanese and they had smoking history. The COPD subjects were in clinically stable condition. On the other hand, the healthy smokers did not have lung disease. Their characteristics were listed in Table 2. Related to COPD patient included in this study were generally similar to the control but slightly older and had lower Brinkman Index. Interestingly, there were significant pulmonary functions identified in the male COPD patients compare to the male healthy smokers as a control. The result found that there was more significant frequency T2GG genotype and T1AG genotype in COPD group if the researchers compared them with in the control group \((p < 0.05)\). But, in the case of Q-1, there was no significant difference of genotype frequencies in the two groups (Table 3). Furthermore, there were no significant different allele frequencies in the two groups (Table 4). Based on phylogenetic tree the sequence of ADAM33 gene from Javanese ethnic in Lampung province was closely related to several populations especially Li and Han in China (Accession No: AF466288.1; DQ995342.1) with 99% and 86% respectively (Figure 1).
Table 1. The location of investigated ADAM33 SNPs and primer sequence

<table>
<thead>
<tr>
<th>Chromosome position</th>
<th>Reference SNP ID</th>
<th>SNP Name</th>
<th>Alel</th>
<th>Primer Sequence</th>
</tr>
</thead>
</table>
| 3590205             | 2280090          | T2      | A/G  | F: 5’-TTCTCAGGGTCTGGAGAAA-3’
|                     |                  |         |      | R: 5’-GCAACCTCCTGGACTCTTA-3’ |
| 3590234             | 2280091          | T1      | A/G  | F: 5’-TTCTCAGGGTCTGGAGAAA-3’
|                     |                  |         |      | R: 5’-GCAACCTCCTGGACTCTTA-3’ |
| 3592207             | 612709           | Q1      | A/G  | F: 5’-GGATTCAAGGCAAGGAG-3’
|                     |                  |         |      | R: 5’-GTTCACCTAGATGGCCAGGA-3’ |

Table 2. The clinical information of patient recruited

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case (n = 42)</th>
<th>Control (n = 32)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>64.5 ± 8.6</td>
<td>61.6 ± 9.5</td>
<td>0.79</td>
</tr>
<tr>
<td>Brikman Index</td>
<td>409.6 ± 199.22</td>
<td>426.14 ± 243.14</td>
<td>0.632</td>
</tr>
<tr>
<td>FEV1</td>
<td>45.40 ± 20.47</td>
<td>103.81 ± 20.07</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>FVC</td>
<td>74.50 ± 19.49</td>
<td>94.64 ± 16.18</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>60.89 ± 14.51</td>
<td>110.64 ± 18.64</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

Note: *significant p < 0.05.

Table 3. Association between ADAM 33 genotypes and COPD

<table>
<thead>
<tr>
<th>SNP</th>
<th>Case (n = 42)</th>
<th>Control (n = 32)</th>
<th>OR (CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2280090 (T2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>28 (66.66)</td>
<td>15 (46.87)</td>
<td>2.29 (0.35-0.81)</td>
<td>0.041*</td>
</tr>
<tr>
<td>AG</td>
<td>12 (28.57)</td>
<td>15 (46.87)</td>
<td>0.46 (0.15-0.58)</td>
<td>0.052</td>
</tr>
<tr>
<td>AA</td>
<td>2 (4.76)</td>
<td>2 (6.25)</td>
<td>0.82 (0.05-0.19)</td>
<td>0.420</td>
</tr>
<tr>
<td>rs2280091 (T1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>14 (33.33)</td>
<td>16 (50.00)</td>
<td>0.5 (0.18-0.62)</td>
<td>0.058</td>
</tr>
<tr>
<td>AG</td>
<td>26 (61.90)</td>
<td>13 (40.62)</td>
<td>2.45 (0.40-0.86)</td>
<td>0.024*</td>
</tr>
<tr>
<td>GG</td>
<td>2 (4.65)</td>
<td>3 (9.37)</td>
<td>0.53 (0.05-0.15)</td>
<td>0.236</td>
</tr>
<tr>
<td>rs612709 (Q-1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>33 (78.57)</td>
<td>27 (84.37)</td>
<td>0.82 (0.67-0.95)</td>
<td>0.329</td>
</tr>
<tr>
<td>AG</td>
<td>8 (19.04)</td>
<td>5 (15.62)</td>
<td>1.07 (0.02-0.30)</td>
<td>0.444</td>
</tr>
<tr>
<td>AA</td>
<td>1 (2.38)</td>
<td>0 (0.00)</td>
<td>0.1 (0.02-0.06)</td>
<td>0.142</td>
</tr>
</tbody>
</table>

Note: *significant p < 0.05; SNP: Single Nucleotide Polymorphism; OR: Odds ratio; CI: Confidence Interval.
Table 4. Association between Allele frequency and COPD

<table>
<thead>
<tr>
<th>SNP</th>
<th>Group</th>
<th>Genotype</th>
<th>Allele Frequency</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
</tr>
<tr>
<td>T1</td>
<td>Case</td>
<td>14</td>
<td>26</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>16</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>T2</td>
<td>Case</td>
<td>2</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Q1</td>
<td>Case</td>
<td>1</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>5</td>
<td>27</td>
</tr>
</tbody>
</table>

Figure 1. The phylogenetic tree of the ADAM33 gene sequence in Javanese smokers of Lampung. There are two big clades that are produced by phylogenetic analyzing ADAM33 gene. We can find ADAM33 gene sequence form Javanese which are located in same clade with ADAM33 gene sequence form Caucasian and China from them. It indicates that Javanese ADAM33 gene is similar with Caucasian and Chinese. ADAM33 Javanese sequence shown by the following arrow (by using code number start with 22).

Discussion

COPD is identified as a complex genetic disease. It also involves environmental risk factor such as tobacco smoking. COPD pathophysiology aspect is an imbalanced proteolysis theory. In line with this case, ADAMs is proteinases which strikingly associated with Matrix Metalloproteinases (MMPs). ADAMs proteins contain of metalloproteinase and disintegrin domains providing them with aspects of proteinases and adhesion
molecules. In addition, all kinds of physiological, pathological processes and display a wide spectrum of biological impacts comprising cell adhesion, cell fusion, cleavage of various substrates from the extracellular matrix, “shedding process”, cytokines or growth factors are correlated to proteinases of the ADAM family\(^{(18)}\).

*The structure of ADAM33* consists of a signal sequence, pre-domain, catalytic domain, disintegrin domain, cysteine-rich domain, EGF domain, transmembrane domain, and cytoplasmic domain with a long 3′-untranslated region (UTR). The structures of ADAM 33 that has been explained above indicate that it is involved in many cellular activities, including cell activation, adhesion, fusion, proteolysis and intracellular signaling. Angiogenesis is promoted by soluble ADAM33. It is explained that as a gene of remodeling tissue which has potential to influence lung functions single-handedly of inflammation and airflow obstruction\(^{(19)}\). It can stimulate the release of growth factors and cytokines. It can trigger inflammatory cells to infiltrate into the airway\(^{(18)}\). Furthermore, there is an implication of soluble ADAM33 in forming of new vessels and angiogenesis. It may stimulate cytokines and inflammatory cells or growth factors in the airway.

In the case of ADAM33, it has contribution in airway remodeling in consequence of its high expression in epithelium, fibroblasts, as well as the airway smooth muscle cells (ASMCs). Declining of proliferation and increasing the apoptosis of ASMCs in a rat model of allergic asthma can be caused by silencing of ADAM33\(^{(20)}\). The genetic susceptibility to patients who get chronic bronchitis caused by smoking or aerosols and industrial dust can be enhanced by ADAM33 gene polymorphisms\(^{(21)}\).

In addition, this current research demonstrated that there was an association between the polymorphisms of ADAM 33 and COPD in Javanese. To the best of our knowledge, it is the first research report which conducted in Indonesia context. In this association study, we genotyped 42 well-characterized COPD cases and 32 healthy smoker controls, who were long-term tobacco smokers for ADAM33 gene of three SNPs (T2, T1 and Q-1). The results revealed that SNPs T2 and T1 of this gene were significantly associated with COPD. Consistent with our result, Wang’s research indicated the T2 was significantly associated with total cells in COPD patient’s phlegm and SNP T1 was significantly associated with macrophage\(^{(9)}\). Our findings will give great contribution related to COPD genetic marker in Indonesia. It is caused by number of Javanese that attains 40% from of all Indonesian population and it is as the biggest tribe in Indonesia.

Furthermore, in East Asian population, the changes of pulmonary function and components of cells in sputum of COPD were obviously associated with T2, T1, and Q-1. Moreover, cytokines and mediators of inflammation in airway of COPD in recessive models were meaningfully associated with T1 and Q-1\(^{(9)}\). In the Kashmiri population of India, ADAM33 gene polymorphism in three SNPs (T1, T2, and Q-1) were obviously associated with COPD\(^{(11)}\). The results of present study showed that the similarities of findings in T1 and T2, but Q-1 was different. It was also supported from the study reported by Li et al found in a Chinese individual the polymorphism of T1 in ADAM33 would escalate patients COPD chance\(^{(10)}\).

In other studies, another ADAM33 SNPs was also associated with COPD. Vijaya Laxmi et al reported that the polymorphism of ADAM33 gene which had SNPs name S1 and S2 become the main genetic factors and risk for COPD\(^{(22)}\). Aierken et al also reported that the inclined risk of COPD in Chinese but not in Caucasians is correlated to S1 (rs3918396) polymorphism of ADAM33\(^{(13)}\). In addition, Tan et al, found COPD in the Mongolian of China was associated with Seven SNPs in ADAM33\(^{(15)}\). Study of Reijmerink et al, who investigated the polymorphism V4 of ADAM33 is associated with COPD in Venezuelan patients. Interaction of *in utero* cigarette smoke exposure with *ADAM33* results in reduced lung function\(^{(23)}\). It was also supported by meta-analysis study of Zhou et al which clearly revealed that the ADAM33 polymorphisms which had SNPs name F+1, T2, T1, S1, ST+5 and Q-1, correlation to the risk of COPD. Moreover, T2, T1, S1, ST+5 and Q-1 pointed
of the risk of COPD in the Asian populations. T2, ST+5
and Q-1showed the risk of COPD in the European populations(24).

In this present study, the results clearly showed that
when compared to the wild allele, heterozygous AG
genotypes of T1 SNP, and homozygous GG genotype
of T2 SNP were distributed significantly in higher (p
< 0.05) frequency among the populations; suggesting
that the increased susceptibility among carriers of these
genotypes to develop COPD.

Genetically, the Javanese is closer to the ethnic
groups in the Southeast Asia region, such as Thailand
and Vietnam while tribes in eastern Indonesia are
closer to people in the Pacific Ocean region. The Nias
and Mentawai tribes are closer to the native tribes of
Taiwan(25). The phylogenetic analysis of this study has
given the clear picture of the associations and the genetic
similarity of the 33 ADAM gene sequences in this study
with various populations in the world, thereby revealed
that the Javanese tribe ADAM33 gene sequence showed
similarities with Caucasian and Chinese populations.

Conclusions

The results demonstrated that T1 and T2
polymorphisms of ADAM33 gene have shown an
association of COPD. The results revealed that it might
be risk factors for COPD susceptibility. The contribution
of the disease-connected to SNPs must be clarified yet,
specifically in the COPD pathophysiology context. The
future studies are warranted to extend this preliminary
research to conduct with large number of sample sizes,
more SNP numbers to fully elucidate the potential
candidate genes implicated in the genesis of COPD
in Javanese population, and also further to develop
screening procedures to identify patients at risk of
developing COPD.

Abbreviation

ADAM33: A Disintegrin And Metalloprotease
33; ASMCs: Airway Smooth Muscle Cells; COPD:
Chronic Obstructive Pulmonary Disease; FEV1: Forced
Expiratory Volume in the First Second; FVC: Forced
Vital Capacity; MMPs: Matrix Metalloproteinases; PFT:
Pulmonary Function Tests; SNPs: Single Nucleotide
Polymorphisms; UTR: Untranslated Region.

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Scores in a Chronic Obstructive Pulmonary Disease


A 56 Year Old Man with Parkinson’s Disease and Depression: A Case Report on Treatment Management

Risza Subiantoro¹, Agustina Konginan², Erikavitri Yulianti²

¹Resident, ²Lecturer, Department of Psychiatry, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

Abstract

Background: Depression is the strongest predictor of quality of life for Parkinson’s disease (PD).

Presentation of Case: A 56 years old man came with the main complaint of difficulty sleeping due to thinking about tremors in his left hand since 6 months. Complaints are accompanied by feelings of sadness, lack of enthusiasm, and decreased appetite accompanied by weight loss. The patient had been diagnosed with PD 8 years previously and was on medication. One month before the visit, the patient underwent a thalamotomy operation. After surgery, the patient received therapy in the form of a combination tablet of levodopa/carbidopa/entacapone (100/25/200 mg) 4×1 tablet and 1×2 mg of ropirinole. On the Beck Depression Inventory (BDI) examination, a score of 23 was obtained and on the Montgomery Asberg Depression Rating Scale (MADRS) examination a score of 31 was obtained, both of which correspond to major depression. The patient was diagnosed with a major depressive episode without psychotic symptoms and was given escitalopram therapy 1×10 mg and lorazepam 1×2 mg. Patients also received biofeedback therapy with family psychoeducation and supportive psychotherapy. At the time of control, the patient admitted to feeling calmer and able to sleep after receiving therapy. Conclusion: Escitalopram has good efficacy for the management of depression in PD patients.

Keyword: Depression, antidepressants, parkinson’s disease

Introduction

Parkinson’s disease (PD) is the second most common neurodegenerative disease occurring in 0.5-5% of the population over 65 years of age. Depression is one of the most common psychiatric complications of PD with a prevalence of 20-35%. The prevalence of major depressive disorder was 17%, while the prevalence of dysthymia and mild depression were 13% and 22% respectively(1, 2). Depression is also the strongest predictor of quality of life in PD(3).

Depression is caused by changes in the dopaminergic, noradrenergic, and serotonergic systems. Depression is associated with reduced dopamine transporters in the striatum and limbic regions, reduced forebrain serotonin innervation, reduced dopaminergic and noradrenergic innervation at the locus ceruleus, thalamus, and limbic region, increased loss of neurons and gliosis at the locus ceruleus, and loss of cortical cholinergic neurons. Psychological reactions to a diagnosis of PD or a disability related to PD can also trigger depression(2). Depression in PD patients is quite difficult to diagnose because of the overlap between depressive symptoms and PD symptoms and the side effects of PD treatment(1). Based on the description above we are interested in reporting the management of PD patients with depression.

Corresponding author:
Agustina Konginan
Department of Psychiatry, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Jalan Mayjend Prof. Dr. Moestopo No. 6-8, Airlangga, Gubeng, Surabaya, East Java 60286, Indonesia
E-Mail: agustinakonginan@yahoo.co.id
Case Presentation

A 56 years old man came to the hospital complaining of difficulty sleeping because of thinking about tremors in his left hand since 6 months. Complaints are accompanied by feelings of sadness, lack of enthusiasm, and decreased appetite accompanied by weight loss.

Tremors were first felt 8 years earlier on the right thumb. At that time, the patient was diagnosed with PD and received combination tablet therapy of entacapaone and levodopa. The patient is routinely treated, but after the tremor is reduced, the patient is no longer in control. At that time, there was no change in behavior. Two years later the tremors returned so that the patient returned to treatment and was given 2×1 tablets of a combination of levodopa and benzerazide HCl (100 mg/25 mg). Since 2 years ago, the patient began to experience walking problems so that the dose of the combination tablet of levodopa and benzerazide HCl was increased to 3×1 tablet and the patient was also given 2×2 mg of trihexyphenidil. One month before the visit, the patient was hospitalized because of complaints of severe tremor in the right hand, so the patient was decided to undergo thalamotomy surgery. After surgery, the patient received therapy in the form of a combination tablet of levodopa/carbidopa/entacapaone (100/25/200 mg) 4×1 tablet and 1×2 mg of ropirinole. The patient felt that the complaints of tremor in the right hand had decreased after surgery, but the tremors in the left hand were still felt. The patient had no previous medical history or history of smoking, coffee/alcohol consumption, and psychotropic use.

In the psychiatric examination, the patient’s awareness of comos mentis, sad mood, depressive effects, realistic thought processes, coherent flow, preoccupation thought content on complaints of tremor on the left, perceptions within normal limits, decreased willpower. Based on auto and heteroanamnesis, prior to experiencing PD, the patient had anancastic personality traits. The results of Mini Mental State Examination (MMSE)\(^4, \, 5\) obtained a score of 23 (normal). On the Beck Depression Inventory-II (BDI-II)\(^6\) examination, a score of 23 was obtained and the Montgomery Asberg Depression Rating Scale (MADRS)\(^7\) was obtained a score of 31, both of which correspond to major depression. The results of Hamilton Anxiety Rating Scale (HAM-A)\(^8, \, 9\) showed a score of 23 (moderate anxiety).

The patient was diagnosed with a major depressive episode without psychotic symptoms and was given escitalopram therapy 1x10 mg and lorazepam 1x2 mg. Patients also received biofeedback therapy with family psychoeducation and supportive psychotherapy. At the time of control, the patient admitted to feeling calmer and able to sleep after receiving therapy.

Discussion

Depression is one of the most common psychiatric complications of PD with a prevalence of 20-35%, where the prevalence of major depressive disorders is 17%. The risk factors for depression in this patient were the severity of motor symptoms (tremor) which was quite severe, the presence of anxiety which was confirmed by the HAM-A examination, and sleep disturbances\(^1, \, 2\). Apart from the pathophysiology associated with changes in the dopaminergic, noradrenergic, and serotonergic systems, in this patient depression was mainly due to a reaction to PD symptoms in the form of tremors\(^2\). Symptoms in these patients are consistent with depressive symptoms often found in PD patients with depression, namely loss of energy, anhedonia, changes in appetite and sleep, fatigue, and impaired concentration\(^3\).

In these patients, the management of depression was carried out with antidepressants as well as non-pharmacological therapy. The antidepressant used in this patient was escitalopram, a selective serotonin reuptake inhibitor (SSRI) class. A multicentre study in 2019 reported that SSRIs are the standard therapy often given to PD patients with depression. However, several previous studies reported that SSRIs were associated with higher apathy than other antidepressants. The study reported a side effect rate of 36% with SSRIs. Side effects found included nausea, vomiting, headache, somnolence, diarrhea, abdominal discomfort, and tremors. SSRIs have been shown to significantly reduce
depressive symptoms\(^{10}\). Apart from being able to improve symptoms of depression, SSRIs are also able to improve daily life and motor function\(^{2}\). Escitalopram, citalopram, or sertralin are also options in elderly patients in advanced PD. SSRIs are also an option in patients with comorbid anxiety disorders. This is consistent with this patient who has entered old age and has preoccupation/anxiety related to tremor symptoms. The ability of SSRIs to improve motor symptoms can also reduce tremor complaints that patients complain about. Another option that can be given to patients is antidepressants that have a sedative effect such as amitriptyline, mirtazapine, and trazodone given the difficulty of sleeping\(^{11}\). However, the patient has been given lorazepam which has a similar function and has succeeded in improving the symptoms experienced by the patient.

Patients also received biofeedback therapy with family psychoeducation and supportive psychotherapy. Previous case reports demonstrated the ability of psychotherapy to improve depression scores in PD patients. Studies comparing CBT with doxepine and placebo in PD patients have also shown that CBT is able to better improve the sleep quality index\(^{12}\). Other non-pharmacological therapies that can be applied to patients include physical activity\(^{13}\), acupuncture therapy\(^{14}\), or dancing\(^{15, 16}\), while the efficacy of repetitive transcranial magnetic stimulation and electroconvulsive therapy (ECT) is unclear\(^{2, 3}\). Deep brain stimulation (DS) also has inconsistent results in the management of depression in PD\(^{17}\).

**Conclusion**

Management of depression in PD patients includes non-pharmacological and pharmacological therapy. Escitalopram has good efficacy for the management of depression in PD patients.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** None.

**Ethical Approval**

All procedures performed in studies involving human participants were in accordance declaration of helsinki the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

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Legal Protection for Nurses in Pharmaceutical Services Where there are No Pharmaceutical Staffs at the Community Health Center

Rizka Dianita¹, Retno Ariani¹, Swabawa Wicaksana¹, Mokhamad Khoirul Huda²
¹Master of Law Student, Hang Tuah University, Surabaya, Indonesia, ²Professor, Faculty of Law, Hang Tuah University, Surabaya, Indonesia

Abstract

Puskesmas is a first-level healthcare service facility that organizes health efforts prioritized promotive and preventive actions. In executing its health efforts, puskesmas must take actions according to standards, including in pharmaceutical services. It will be different when the pharmacy service is provided by nurses instead of provided by pharmacy staffs. Following the prevailing law in Indonesia today, nurses’ authority in carrying out their pharmacy duties in practice is only limited. To provide assurance and legal protection for nurses in performing pharmaceutical services in health centres where there are no pharmacists/pharmaceutical technical staffs, laws and regulations related to nursing protection have an essential function. It also protects nurses to avoid criminalization of nurses by law enforcement officials.

Keywords: the community health center, Nurses, Pharmacy, Law, Protection

Introduction

In the National Health Insurance System era, healthcare services are no longer concentrated in hospitals or advanced healthcare facilities, but every healthcare facility level must provide it based on the patient’s medical needs. This condition aims to improve the community’s healthcare services, mainly focused on healthcare services at Authorized Primary Care Facilities. One of which is the community health center.¹

The community health center, as a healthcare service facility, organizes public health efforts and first-level individual health efforts by prioritizing promotive and preventive actions in its working area. The working area of the puskesmas covers based on the administrative working area; it is one sub-district or part of a sub-district. It has regional responsibility as one basic principle where Puskemas must empower and be responsible for health development in its working area.

One of the efforts to strengthen primary healthcare facilities requires health staff, including pharmacists and the person in charge of pharmacy at puskesmas, to provide standardized healthcare services. It needs sufficient human resources as the critical factor and also standardized facilities and infrastructures. Pharmacists as the person in charge of pharmaceutical services at the puskesmas are expected to be able to carry out standardized pharmaceutical services to improve service quality and patient safety.¹

A situation becomes different when there are no pharmaceutical staffs at the puskesmas. Other health staffs will take this duty so that pharmaceutical services can continue to run, and health staffs who usually replace pharmaceutical services at puskesmas are nurses. As stated in Decree of the Minister of Health
No. 74 of 2016 on Standard Pharmaceutical Services in Community Health Centers, Article 12 (paragraph 1), “At the time this Ministerial Regulation came into force, for Puskesmas with no pharmacists as the person in charge, the implementation of limited pharmaceutical services was carried out by pharmaceutical technical staffs or other health staffs assigned by the head of the district/city health office.”

According to the Law No. 38 of 2014 on Nursing (from now on referred to as Law of Nursing) article 29 paragraph (1).

In organizing nursing practices, nurses serve as:

a. nursing care provider;
b. counsellors for patients;
c. manager of the nursing service;
d. nursing researchers;
e. executing tasks based on the delegation of authority; and
f. task holder under certain limitations.

Article 33 paragraph (1): Implementation of duties in certain circumstances of limitations as referred to in Article 29 paragraph (1) letter f is an assignment of government carried out in the absence of medical staff and pharmacy staff in an area where nurses work.

Article 34 paragraph (3): In implementing the duties in certain circumstances of limitations as referred to in paragraph (1), the Nurse is authorized to:

a. giving treatment for common diseases in the absence of medical staffs;
b. refer patients according to the provisions of the referral system; and

c. perform limited pharmaceutical services in the absence of pharmaceutical staffs.

Based on the articles above, the nurse can carry out limited pharmaceutical services, include a. management of pharmaceutical preparations and medical consumables; and b. prescription services in the form of drug compounding, drug delivery, and drug information provision.

In line with science and technology development, pharmacy has shifted pharmaceutical services from managing medicines as a commodity to comprehensive services (pharmaceutical care). This orientation means that they are not only managing drugs but, in a broader sense, including the implementation of providing information to support the correct and rational use of drugs, monitoring of drug use to determine the ultimate goal, and the possibility of medication errors.

Pharmaceutical services are also required to directly interact with patients, such as providing drug information and counselling to patients in need. Pharmacists must understand and be aware of the possibility of medication errors in the service process and identify, prevent, and overcome drug-related problems, pharmaco-economic problems, and social pharmacy (socio-pharmacoeconomic). In order to avoid errors, pharmaceutical services must be based on service standards.

According to the enactment of Law no. 8 of 1999 on Consumer Protection provide opportunities for users of service or goods to file lawsuits against the business actors if there is a conflict between the customers and the business actors deemed to have violated their rights, being late in doing/not doing/being late in making something that causes losses to the users of service/goods, property loss or injury, or it could be death. In this case, the patients as consumers of healthcare services can sue hospitals, doctors or other health staffs if there is a conflict.

The current positive law applied in Indonesia only gives nurses authority in the pharmacy practice to have a limited pharmaceutical service. To guarantee legal certainty and protection for nurses who carry out pharmaceutical services at health centers where there are no pharmacists/pharmaceutical technical staff, laws and regulations related to nursing protection have an
essential function and effect to avoid criminalization of nurses by law enforcement officials (police). 8

Discussion

Standard Operational Procedure for Medical Services at The Community Health Center with the shortage of pharmaceutical staffs

Pharmaceutical services in puskesmas are an integral part of implementing health efforts, which play an essential role in improving the community’s healthcare services quality. It aims to identify, prevent and resolve drug problems and health-related problems. Patients and the community’s demands for a better quality of pharmaceutical services require a transformation to the new paradigm from the product-oriented (drug-oriented) into the patient-oriented paradigm. 9

The regulations on pharmaceutical practices are listed in Law No.36 of 2009 on Health and Government Regulation No.51 of 2009 on Pharmaceutical Works. As listed as follows:

1. Law No.36 of 2009 concerning Health - Article 108: Pharmaceutical practice covers manufacturing, including quality control of pharmaceutical preparations, security, procurement, storage and distribution of drugs, drug services for doctor’s prescription, drug information services and development of drugs, medicinal ingredients and traditional medicines carried out by authorized health staffs following statutory provisions.10

2. PP No.51 of 2009 on Pharmaceutical Works as follows:11

Article 2 paragraph 1: The Government Regulation regulates pharmaceutical works that include the procurement, production, distribution, distribution, and pharmaceutical preparations services.

Article 2 paragraph 2: Pharmaceutical works as intended in paragraph (1) must be carried out by a pharmaceutical staff with expertise and authority. The expertise and authority of the pharmaceutical staffs are proven by a license to practice. The pharmaceutical staffs referred to in this article are pharmacists and pharmaceutical technical personnel. A pharmacist is a bachelor of pharmacy who has graduated as a pharmacist and has taken the oath of office of a pharmacist. Meanwhile, what is meant by pharmaceutical technical staffs are personnel who assist pharmacists in carrying out their pharmaceutical work, consisting of bachelor of pharmacy, pharmacy intermediates, pharmaceutical analysts, and pharmacy intermediate staff/pharmacist assistants.

Article 21 article 3: In remote areas where there are no pharmacists, the Minister can place pharmaceutical technical staff whose Registration Certificate for Pharmaceutical Technical Officers at basic healthcare service facilities is given the authority to mix and deliver medicines to patients. The Registration Certificate for Pharmaceutical Technical Officers is written evidence provided by the Minister to registered pharmaceutical technical staffs.

Article 22: In remote areas where there are no pharmacy, doctor or dentist who already has a Registration Certificate has the authority to mix and deliver medicines to patients, which is carried out following the provisions of laws and regulations. The above laws and regulations are made as a legal basis for the public to obtain professional pharmaceutical care and protect the pharmacist profession in carrying out their pharmaceutical practices.

Legal protection for nurses in pharmaceutical services at the Puskesmas where there are no pharmaceutical staffs

Enforcement of regulations in the pharmaceutical sector creates contradictions when applied in areas with a shortage of health staff. Nurses as unauthorized health staffs to practice pharmacy are confused by the conditions in which both pharmacist and doctors are difficult to reach in the area. It causes a dilemma and is vulnerable to be blamed by law enforcement officials. On one side, there are limitations to the authority granted by law, and on the other hand, there is a shortage of authorized and having-expertise health staffs.12
Pharmaceutical practice by nurses where pharmaceutical practice regulation is enforced amid limited pharmacy in various places, nurses who practice pharmacy should not be legally blamed. It is usual for nurses, as health staffs have a bigger number than doctors and pharmacy staffs. They commonly play a double role in carrying out medical procedures and treatment in areas with no other authorized health staff.\textsuperscript{13}

As stated in article 108, paragraph (2) of Law No. 36 of 2009 on Health, pharmaceutical practices can only be carried out by health staffs who have the authority and expertise following statutory provisions; it called pharmaceutical staffs. In the absence of pharmaceutical staffs, sure health staffs can carry out limited pharmaceutical practices; it is the provision of free drugs and limited over-the-counter drugs, while hard drugs such as antibiotics and analgesics are prohibited from giving by nurses.\textsuperscript{14}

Ethical considerations are the justification basis of pharmaceutical practice by nursing staffs.

Based on the ethical decision-making framework above, the violations of pharmaceutical practices committed by nursing staffs where there are no other authorized and having-expertise health staffs in pharmacy can be described as follows:\textsuperscript{15}

\textbf{Value of Personal Belief}

The value of personal belief is influenced by religious principles and primary education (science). Religious teachings can provide a foundation of belief for humans in determining their attitudes and behaviour. Although it is rather challenging to be used as a guide in solving concrete problems, the principles in religious teachings can become the basis for a person to understand what is good and evil, the obligation to help others, and the threat of sanctions for violations. Primary education can influence a person’s perspective too. The fewer references to someone’s knowledge, the less their ability to study a problem based on scientific thinking.

\textbf{Code of Ethics.}

A code of ethics is a statement of professional standards used as a code of conduct and provides a framework for making decisions. In carrying out the duties and functions, a nurse in Indonesia is bound by the Indonesian national nurse code of ethics rules to avoid ethical violations. In article 9 of the nurse code of ethics, it is stated that “Nurses always prioritize the protection and safety of patients in performing nursing duties and are mature in considering abilities if they accept or transfer responsibilities related to nursing.”\textsuperscript{16} Likewise, in the Indonesian medical code of ethics, Article 10 states, “Every doctor must always remember his obligation to protect human life”. The health professional oath always prioritizes humanity and high respect for life. It is one aspect of health science, called a nurturer, where all aspects of life are respected and strived to be maintained and a balancing aspect that views disease and death as a cycle that is also respected naturally.

\textbf{Law}

Referring to Article 108 of Law No.36 of 2009 on Health and Article 2 and Article 22 of Government Regulation No.51 of 2009 on Pharmaceutical Works, the actions of nurses who give hard drugs to their patients are indeed against the law. However, as a guideline, the rule of law, in general, is applied to anyone, anywhere. Indonesia has a geographical condition consisting of thousands of islands and heterogeneous communities. Based on this background, law enforcement should adapt to the local wisdom conditions to achieve the values of justice and order in society. If it is applied without paying attention to society’s condition, the law loses its sociological effect. On the other hand, health science also has its legal principles. Although the law principle is not a concrete legal rule, it can be used as a general basis or a guide for applicable law. The principle in health science is Agroti Salus Lex Suprema (patient safety is the highest law) and Sa science et sa conscience (both knowledge and conscience are good). The point is that the intelligence of a health professional should not conflict with his conscience and humanity. Deminis
noncurat lex (the law does not interfere in trivial matters). Meaning is negligence committed by health staff; as long as it does not adversely affect the patient, the law will not prosecute.16

The moral concept determines whether a behaviour is good or bad from an ethical point of view. Therefore, the moral principle is the highest and cannot be conquered by other rules. This principle is formulated in positive and negative forms into a concept of action (moral concept). According to this view, every health profession in directing action is not based on choice but on what should be done for patients’ safety.

**Ethical principles and theories**

In classical ethical theory, there is something called teleology which means goal. This theory provides the basis that whether an action is correct or not depends on the results. It means if the action has a good result, it is permissible to do so. On the other hand, if the action has a bad result, it is prohibited. Second, still in classical theory, there is also a view of utilitarianism, which views an action as considered good if it benefits many people. One of the approaches is the intuitionism approach, which is the human view of knowing right and wrong regardless of the rational or irrational thinking of a situation. For example, a nurse in a remote area treats a patient, where no doctor can treat the patient immediately because of the long-distance and challenging terrain. Although professionally nurses are incompetent in providing diagnosis and treatment, their actions can be justified in the absence of a doctor. These actions result in more benefits for many people.

From the various considerations in ethical decision-making, giving hard drugs to patients by nurses is acceptable as long as they aim to save the patient and emergency. The emergency itself is defined as there is no pharmaceutical staffs or doctors in the area. This justification also applies legally because the nurses are put in a condition to escape from punishment even though they act against the law. The justification used includes the first because the nurses are in an emergency where there is no authorized health personnel, but the patient needs help. Second, orders and oaths as a nurse can be subject to criminal sanctions if they do not intentionally assist patients in an emergency. It is precisely the act of leaving patients in need of help that should not be justified because the community’s right to get healthcare services is not fulfilled.

Nurses who practise pharmacy where there are no authorized health staffs assigned to the area concerned is not an act of violation of the law. Such action is justified based on various considerations in making ethical decisions in health. However, this condition cannot be allowed to continue. Pharmaceutical practice is an essential element in the health sector, so that its implementation must be provided by authorized staffs and have expertise. Besides, nurses assigned to remote areas need legal certainty to improve their community’s health level but are confused about legal violations if they act outside their authority. Therefore, the distribution of health staffs, especially pharmacy staffs and doctors, must be considered evenly. The realization of Article 21 Government Regulation No.51 of 2009 on Pharmaceutical Works, which contains provisions for the placement of authorized pharmaceutical technical staffs at basic healthcare service facilities in remote areas, needs to be carried out immediately. Furthermore, the preparation of legal products, especially those concerning fundamental rights and the community’s welfare, must pay more attention to various realities in society so that the legal products produced are not viewed solely based on urban conditions. Regulations on authorized health staffs in pharmaceutical practices, which is contained in the Health Law and Government Regulations on Pharmaceutical Works, need to reconsider their application in emergencies involving the patient’s life.

**Conclusions**

One of the efforts to strengthen primary healthcare facilities requires health staff, including pharmacists and the person in charge of pharmacy at the community health center, to provide standardized healthcare services. It will be different when there are no pharmaceutical
staffs at the puskesmas. Pharmaceutical services at puskesmas will be carried out by other health staffs so that pharmaceutical services can continue to run. The current positive law applied in Indonesia only gives authority to nurses to practice limited pharmaceutical services. Nurses who practice pharmacy where there are no authorized health staff assigned to the area concerned are not acting against the law. Such action is justified based on various considerations in making ethical decisions in health. However, this condition cannot be allowed to continue. Pharmaceutical practice is an essential element in the health sector, so that its implementation must be provided by authorized staffs and have expertise.

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Health Risk Behavior Related to Stroke in Indonesia

Rizmi Novishia Wijaya1, Djazuly Chalidyanto2, Agung Dwi Laksono3
1Magister Student, Faculty of Public Health, 2Lecturer, Faculty of Public Health, Airlangga University, Surabaya, Indonesia, 3Researcher, National Institute of Health Research and Development, the Ministry of Health of the Republic of Indonesia, Jakarta, Indonesia

Abstract
Stroke is a condition with clinical signs that develop rapidly in focal and global neurologic deficits, which can be severe and lasts ≥24 hours and cause death, without other apparent causes besides vascular. The study aims to analyze ecologically the health risk behavior related to the prevalence of stroke in Indonesia. The research conducted an analysis using secondary data from the 2018 Indonesia Basic Health Survey. The study takes all provinces as samples. Apart from the prevalence of stroke, four other variables analyzed as independent variables were the prevalence of obesity, the percentage of the population with less physical activity, the percentage of the people with fatty/cholesterol/ fried food consumption habits ≥1 per day, and the percentage of daily smokers. Data were analyzed using a scatter plot. The study results found that the higher the prevalence of obesity in a province, the higher the prevalence of stroke. The higher the percentage of the population with less physical activity in an area, the higher the stroke prevalence. The higher the rate of people with fatty/cholesterol/ fried food consumption habits ≥1 per day, the higher the prevalence of stroke in that province. The higher the percentage of daily smokers in a region, the higher the prevalence of stroke. The study concluded that the four health risk behavior analyzed ecologically were positively related to most stroke in Indonesia.

Keywords: stroke, ecological analysis, physical activity, healthy behavior

Background
Stroke is a non-communicable disease. Globally, stroke is the second leading cause of death globally after heart disease and the third leading cause of disability. World Stroke Organization data shows 13.7 million new stroke cases each year, and around 5.5 million die from stroke1. According to data from the South East Asian Medical Information Center (SEAMIC), Indonesia is a country in Southeast Asia with the largest stroke mortality rate2.

According to the World Health Organization (WHO), stroke is a condition that has clinical symptoms that develop rapidly in the form of focal and global neurological deficits. It can be severe and last ≥24 hours and can cause death, with no other apparent cause other than vascular. A stroke occurs when the brain’s blood vessels become blocked or burst, which will result in part of the brain not getting the blood supply that carries the necessary oxygen, resulting in cell/tissue death1.

The WHO states that Indonesia is ranked 97th globally for the highest number of stroke sufferers, with the death rate reaching 138,268 people or 9.7% of the total deaths in 20113. According to the 2018 Indonesia Basic Health Survey results, the prevalence of stroke based on doctor’s diagnosis in the population aged ≥15 years increased compared to 2013, namely from 7‰ to 10.9‰ or an estimated 2,120,362 people. The highest prevalence was in East Kalimantan province, namely 14.7‰ and DI Yogyakarta 14.6 ‰. The lowest prevalence was in Papua (4.1‰) and North Maluku provinces (4.6‰)4.

Corresponding Author:
Agung Dwi Laksono
Email: agung.dwi.laksono-2016@fkm.unair.ac.id
In terms of financing, *BPJS Kesehatan* noted that the cost of health services for stroke has increased from 2016 to 2018. If in 2016 it reached 1.43 trillion IDR, the figure would raise the following year to 2.18 trillion IDR and get 2.56 trillion IDR in 2018. The best prevention of stroke is to know the risk factors for stroke. It can control the risk factors more quickly, and it shows to be effective at reducing mortality from stroke even in some low-income people. For this reason, it is necessary to have an adequate understanding of what factors are related to stroke in Indonesia. Based on this background, this study aims to analyze ecologically health risk behavior related to the prevalence of stroke in Indonesia.

**Materials and Methods**

**Study Design**

The author designed the study using an ecological analysis approach. Ecological studies focus on comparisons between groups, not individuals. The data analyzed is aggregate data at a particular group or level, which in this study is the provincial level. Variables in ecological analysis can be in the form of aggregate measurement, environmental measurement, or global measurement.

**Data Source**

The study conducted the analysis using secondary data from the 2018 Indonesia Basic Health Survey report. The 2018 Indonesia Basic Health Survey reports is an official publication from the Ministry of Health of the Republic of Indonesia. The unit of analysis in this study is the province. The study analyzed all regions in Indonesia as a sample (34 provinces).

**Data Analysis**

The dependent variable in this study was the prevalence of stroke. Stroke was recorded based on the doctor’s diagnosis history. There were four independent variables analyzed in this study: prevalence of obesity, percentage of the population with less physical activity, percentage of the population with fatty/cholesterol/fried food consumption habits ≥1 per day, and percentage of daily smokers.

Data were analyzed bivariately using a scatter plot. The study used the linear fit line to determine the tendency of the relationship between the prevalence of stroke and the independent variable. The entire analysis process utilizes SPSS 26 software.

**Results and Discussion**

Table 1 shows the descriptive statistics of the prevalence of stroke and other variables analyzed in this study. The information presented informs that the lowest prevalence is 4.1%, while the highest prevalence is 14.7%. The range of prevalence of stroke among provinces in Indonesia is quite wide. Meanwhile, the prevalence range or the percentage of other variables also appears to be relatively high. For example, in the variable percentage of the population with fatty/cholesterol/fried food consumption habits 1 per day, the range is between 10.3%-58.4%.

<table>
<thead>
<tr>
<th>Descriptive Statistics</th>
<th>Prevalence of Stroke</th>
<th>Prevalence of Obesity</th>
<th>Percentage of Population with Less Physical Activity</th>
<th>Percentage of Population with Fatty/Cholesterol/Fried Food Consumption Habits ≥1 per day</th>
<th>Percentage of Daily Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
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<tr>
<td>Mean</td>
<td>10.082</td>
<td>21.703</td>
<td>34.879</td>
<td>33.326</td>
<td>23.494</td>
</tr>
<tr>
<td>Median</td>
<td>10.500</td>
<td>21.050</td>
<td>33.950</td>
<td>33.200</td>
<td>23.350</td>
</tr>
</tbody>
</table>
Cont... Table 1. Descriptive statistics of Prevalence of Stroke and Related variables by Province in Indonesia, 2018

<table>
<thead>
<tr>
<th>Mode</th>
<th>8.3</th>
<th>18.7a</th>
<th>33.7</th>
<th>10.3a</th>
<th>22.1a</th>
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<tbody>
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<td>Std. Deviation</td>
<td>2.7091</td>
<td>4.2801</td>
<td>5.7920</td>
<td>11.1558</td>
<td>2.6014</td>
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<tr>
<td>Variance</td>
<td>7.339</td>
<td>18.319</td>
<td>33.547</td>
<td>124.452</td>
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<td>Range</td>
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<td>22.6</td>
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<tr>
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<td>10.3</td>
<td>25.2</td>
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<td>18.8</td>
</tr>
<tr>
<td>Maximum</td>
<td>14.7</td>
<td>30.2</td>
<td>47.8</td>
<td>58.4</td>
<td>28.1</td>
</tr>
</tbody>
</table>

Source: The 2018 Indonesia Basic Health Survey

Figure 1 shows a map of the prevalence of stroke by the province in Indonesia. Based on this spatial information, the figure indicates that most stroke tends to be lower in Eastern Indonesia. The result shows Papua, North Maluku, and East Nusa Tenggara have a lower stroke prevalence.

Figure 1. Map of the Prevalence of Stroke by Province in Indonesia, 2018

Source: The 2018 Indonesia Basic Health Survey
Figure 2 is a scatter plot of the prevalence of obesity and the prevalence of stroke by Indonesia’s province. The study result indicates that the relationship between the two variables shows a positive trend. The work means that the higher the prevalence of obesity in a province, the higher the prevalence of stroke.

The analysis results shown in Figure 2 are in line with previous research, which found that 56.5% of respondents who had a stroke had risk factors for obesity. In another meta-analysis study, the result found that being overweight and obese in young adulthood was associated with increased stroke risk. The risk effect will gradually increase as you gain weight. Being overweight and obese is associated with an increased risk of high blood pressure, diabetes, heart disease, and stroke.

Figure 3 shows the Scatter plot of the population's percentage with less physical activity and stroke prevalence by the province in Indonesia. The results of the scatter plot indicate that two variables tend to have a positive relationship. The condition means that the higher the population with less physical activity in a province, the higher the stroke prevalence.

Lack of physical activity causes blood circulation to become less smooth. Blood functions to carry oxygen and nutrients to body cells. Besides, lack of activity can lead to obesity, which is a risk factor for stroke. Exercise and activity can reduce the risk of stroke. Increased activity can reduce 80% of non-communicable diseases such as stroke, which is the largest contributor to death globally.
Figure 3. Scatter Plot of Percentage of Population with Less Physical Activity and Prevalence of Stroke by Province in Indonesia, 2018

Source: The 2018 Indonesia Basic Health Survey

Figure 4. Scatter Plot of Percentage of Population with Fatty/Cholesterol/Fried Food Consumption Habits ≥1 per day and Prevalence of Stroke by Province in Indonesia, 2018

Source: The 2018 Indonesia Basic Health Survey
Moreover, Figure 4 shows the relationship between the percentage of the population with fatty/cholesterol/fried food consumption habits ≥1 per day and the prevalence of stroke by Indonesia’s province. The result indicates that the two variables' relationship shows a positive trend based on the scatter plot. The situation means that the higher the percentage of the population with fatty/cholesterol/fried food consumption habits ≥1 per day in a province, the higher the prevalence of stroke in that province will be.

The research results from Alchuriah, most respondents experienced an increase in cholesterol levels in the high and high category borderline due to a diet and lifestyle that consumed a lot of foods with high cholesterol and saturated fat. If the intake of cholesterol in food that enters the body is too high, the blood amount will increase. Cholesterol build-up in the blood can accumulate to form plaque and cause blood clots to form (atherosclerosis), leading to stroke.

Respondents with high cholesterol levels were 2.4 times more likely to have a stroke than respondents with low cholesterol levels. The situation may be because cholesterol directly impacts obstruction of blood circulation and can lead to stroke. In contrast, in the Japanese Adult Health Study, higher cholesterol intake was associated with a lower risk of ischemic stroke.

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Figure 5. Scatter Plot of Percentage of Population of Daily Smokers and Prevalence of Stroke by Province in Indonesia, 2018

Source: The 2018 Indonesia Basic Health Survey

Meanwhile, Figure 5 is the scatter plot of daily smokers' percentage and stroke prevalence by the province in Indonesia. The figure shows that the two variables' relationship shows a positive trend based on the scatter plot. The result means that the higher the percentage of daily smokers in a province, the higher the prevalence of hypertension.

Nicotine and carbon monoxide in cigarette smoke damage the cardiovascular system. Free radicals produced by smoking can increase the risk
of atherosclerosis. Smoking doubles the risk of stroke associated with a dose-response relationship between pack-years\textsuperscript{11,22}. If the initial age of tobacco before or when the age of 20 years increases the risk of atherosclerotic disease, it will also increase the risk of stroke\textsuperscript{21}. Smoking contributes to 15\% of all stroke deaths per year. A previous study said stroke risk would decrease if it is 2 to 4 years after quitting smoking\textsuperscript{11}.

**Conclusions**

Based on the results, the study concluded that there is a positive relationship between obesity, population with less physical activity, population with fatty/cholesterol/fried food consumption habits ≥1 per day, and daily smoker with the prevalence of stroke in Indonesia.

**Acknowledgments:** The author would like to thank the Ministry of Health of the Republic of Indonesia. The institution was providing the report, which was the source of the data in this study.

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**Ethical Clearance:** The study conducted using secondary data from published reports. Ethical clearance is therefore not required in the conduct of this study.

**Conflicting Interests:** The authors declared no potential conflicts of interest concerning the research, authorship, and publication of this article.

**References**


The Diagnostic Dilemma of a Large Infected Cyst in Anterior Maxilla- A Case Report

Roopika Handa¹, Arpan Manna², Tanha Khan², Monika Singh², Aiman Mahfooz², Dhruv Garg²
¹Professor, ²PG Student, Department of Oral Medicine and Radiology, Teerthanker Mahaveer Dental College and Research Centre, Moradabad

Abstract
Among all the odontogenic origin cyst, radicular cyst is the commonest. It is generally associated with nonvital tooth which have necrotizing pulp as it is an inflammatory cyst. Radicular cyst usually occurs in the periapical region of tooth. As a true cyst it consists of pathologic cavity which is lined by epithelium and commonly filled with fluid. Treatment option for the radicular cyst depends on the size, location and duration of the lesion which includes endodontic treatment, enucleation of the lesion, extraction of the offending tooth and marsupialization. In this case report, we are describing a case of infected radicular cyst in anterior maxillary region.

Keywords: Radicular cyst, Odontogenic Cyst, Cell rest of Malassez, Fine needle aspiration cytology (FNAC), Enucleation, Marsupialization, Occlusal radiograph

Introduction
Radicular cyst is also referred as periapical cyst, dental cyst, apical periodontal cyst or root end cyst. As a sequel of pulpal necrosis or trauma, the epithelial cell rests of malassez in the periodontal ligament proliferates and give rise to the radicular cyst.¹ Most often the radicular cyst involves the apices of the infected tooth but it can also occur along the accessory or lateral root canal of the infected tooth.² It accounts for 52% to 68% of all other cyst found in the jaw.³ There is male predilection in third to fifth decades of life. Maxillary anterior region is the commonest involved site.² Shear et al describe it as usually asymptomatic and there may be no visible extraoral swellings.⁴

Radicular cysts are generally ranging from 0.5 to 1.5 cm in size,² although, larger radicular cyst have been reported in literature.⁵ Treatment of the radicular cyst depends on the size and extension of the lesion. Although, endodontic therapy alone can heal the smaller lesions. For the larger or multiple lesion, enucleation, marsupialization or decompression along with endodontic intervention is the treatment of choice.⁶

Case Report
A 22 years old male patient reported to the department of oral medicine and radiology with the chief complaint of pus discharge from upper front tooth region for 15 days. Patient gave similar history of pus discharge 2 years back and subsequently undergone root canal treatment in upper front tooth region.

There was no related swelling and tenderness on extraoral examination.

On intraoral examination 11 was discoloured with pus discharge from the anterior labial sulcus. The previous dental treatment had resulted in reduced crown length and an edge to edge bite of 11 with 41. The margins of the gingiva were rolled out and inflammed (Fig 1). On palpation, there was no associated tenderness.
and fluctuancy with respect to related palatal and labial vestibular region. Based on the chronicity and recurrence of the lesion provisional diagnosis of radicular cyst in the affected region was given.

Radicular cyst, nasopalatine duct cyst, odontogenic keratocyst, pindborg tumor and central giant cell granuloma were evaluated as differential diagnosis.

The radiographic investigation with anterior cross-sectional occlusal radiograph revealed a mixed radiopaque-radiolucent lesion extending from the apices of 11 and 21 to the palatal aspect of 16 region crossing the midline measuring approximately 3x4 cm in size. The lesion had an ill-defined corticated margin and lateral root displacement of 11, 12, 21, 22 was seen (Fig 2).

Patient was then subjected to fine needle aspiration cytology (FNAC) as a part of chair-side investigation, which did not yield any aspiration. The vitality test of 12, 11, 21, 22 showed no response with respect to 12, 11, 21 and 21.

After correlating all the clinical and radiological findings, it was concluded to be an infected radicular cyst as final diagnosis.

![Fig 1](image1.png)  ![Fig 2](image2.png)

**Fig 1** Discoloured 11 with pus discharge from the anterior labial sulcus.

**Fig 2** Occlusal radiograph revealing a mixed radiopaque-radiolucent lesion extending from the apices of 11 and 21 to the palatal aspect of 16 region crossing the midline measuring approximately 3x4 cm in size.

**Discussion**

The radicular cysts are generally asymptomatic and not commonly noticed until they are secondarily infected and become symptomatic or as an accidental finding on routine radiographic investigation. Sometimes long standing lesions become symptomatic due to acute exacerbation. Cortical expansion is a common clinical feature of radicular cyst in maxilla, as compared to mandible. Consistency of the cystic swelling changes depending on the thinning of the bony covering from hard to springy to fluctuant. Resorption of the affected root, cortical expansion, adjacent teeth displacement are the common radiological features of radicular cyst. In our case no such features were appreciable. According to the available literature, the lesion adjacent to the teeth to the lesion frequently become nonvital as the lesion enlarges, and our present case also gave similar features. The epicentre of the lesion in non-vital tooth, usually located at the root apex where as in case of accessory canals it is usually located on the mesial or distal aspect of
However in our case the epicentre was located on the medial aspect of the offending tooth root and the associated teeth were non-vital. Usually radicular cyst have a well-defined cortical border until and unless it is secondarily infected in which case the cortical border becomes sclerotic. The outline of the cyst is curved or circular unless it is influenced by surrounding structures and sometimes dystrophic calcifications may develop in long standing conditions which appears as radiopacity sparsely distributed in the lesion.

The pathogenesis of radicular cysts includes three phases- i) phase of initiation ii) phase of cyst formation iii) phase of enlargement. Pathogenesis of the radicular cyst was described by Torabinejad (1983) according to the “breakdown/nutritional deficiency theory” and “abscess cavity theory.” The “breakdown” theory suggests that of pulpal necrosis the noxious stimuli continue to provoke the epithelial cells which are derived from epithelial cell rests of Malassez. These epithelial cells continue to proliferate and the lesion become larger and the central cells become deprived of nutrition from the connective tissue and as a result central cells undergo liquefactive necrosis, leading to the development of microscopic cyst. According to the “abscess cavity theory”, the epithelial cell tends to line the exposed connective tissue and these cells proliferate and lined the cavity.

Cystic fluid plays an important role for diagnosis in case of the odontogenic cysts as it can vary from lesion to lesion such as clear fluid, yellow coloured fluid which mainly contains protein. Generally, in radicular cyst, cystic fluid contains 5-11g protein per 100 ml of fluid which is quite higher as compared to other cysts of odontogenic origin such as odontogenic keratocyst, dentigerous cyst. Concentration of globulin (both α1 and β) in radicular cyst is higher than other odontogenic cysts.

In most studies it is seen that the healing process of this lesion is dependent on the lesion size. In most of the studies it is stated that treatment protocol depends on the size and extent of the lesion. If the lesion is small conservative treatment is sufficient and however in larger lesions which include decompression, marsupialization or enucleation of the cysts as the treatment choice.

**Conclusion**

Radicular cyst is one of the most common odontogenic cyst which can be encountered during dental practice though usually it is asymptomatic. Malignant transformation of radicular cyst is very rare. Non-surgical, conservative approach is the treatment of choice for smaller lesions nowadays. However surgical intervention might be necessary sometimes.

**Conflict of Interest:** There is no conflict of interest.

**Donation:** Nil

**Ethical Clearance:** Taken from ethical committee of the college.

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Detection of Enterotoxigenic \textit{Staphylococcus aureus} in Patients with Gastroenteritis in Erbil/Iraq

Rozhalat Khudhur Jarjees
Lecturer, Department of Pharmacy, Erbil Medical Technical Institute, Erbil Polytechnic University, Erbil, Iraq

Abstract

Gastroenteritis is one of the words serious public health problem, and enterotoxigenic \textit{Staphylococcus aureus} is one of the causes of gastroenteritis. The aim of this study was the molecular detection of enterotoxigenic genes in \textit{Staphylococcus aureus}, then detection the association of resistance patterns with the toxigenicity of strains. Microbiological analysis of fecal samples from patients with gastroenteritis was performed for detection of \textit{S. aureus}. The samples were cultured and identified by routine bacteriological methods and VITEK 2 system. Subsequently PCR amplification of enterotoxin genes (\textit{sea} and \textit{seb}) was carried out on all \textit{S. aureus} strains. The results indicated that in total of 417 fecal samples taken from patients with acute gastroenteritis, 30 grew \textit{S. aureus} (7.19%), and of 417 fecal samples 96 yielded bacterial pathogens (23.02%), \textit{S. aureus} was the most common bacterial pathogen found in 30/96 cases (31.25%). In 30 isolated of \textit{S. aureus}, penicillin sensitive \textit{S. aureus} were 2 (6.67%), methicillin sensitive \textit{S. aureus} were 19 (63.33%), multidrug resistant \textit{S. aureus} were 12 (40%), methicillin resistant \textit{S. aureus} were 11 (36.67%), Extensive drug resistance were 3 (10%), and none of them was pan drug resistant, and all isolates were sensitive to vancomycin. The PCR results showed that 7/30 (23.33%) of the \textit{S. aureus} isolates possessed the \textit{sea} gene, and 3/30 (10%) had the \textit{seb} gene. In total of 30 isolates Staphylococcal enterotoxin genes \textit{sea} and \textit{seb} were more prevalent among methicillin resistant \textit{S. aureus} were 6/11 (54.54%), 3/11 (27.27%) respectively, then in multidrug resistant \textit{S. aureus} which were 6/12 (50%), and 3/12 (25%) respectively, and in Extensively drug resistance \textit{S. aureus} \textit{sea} was 1/3 (33.33%) and there is no \textit{seb} genes, while in methicillin sensitive \textit{S. aureus} \textit{sea} was 1/19 (5.26%), and there is no \textit{seb} genes, and there was no Staphylococcal enterotoxin genes \textit{sea} and \textit{seb} in penicillin sensitive \textit{S. aureus}. The result confirmed the involvement of enterotoxigenic \textit{S. aureus} in the occurrence of gastroenteritis, and isolates with enterotoxin genes have a higher drug resistance rate.

\textbf{Keywords:} Gastroenteritis, \textit{Staphylococcus aureus}, \textit{sea} and \textit{seb} Enterotoxin genes.

Introduction

Gastroenteritis is a common clinical syndrome. It poses one of the words major clinical and public health problems. The principal clinical manifestation of gastroenteritis is diarrhea. The Word Health Organization Syndromic definition of gastroenteritis is “three or more abnormally loose or fluid stools over 24 hours”\(^1\). Diarrhea is the second disease that causes death in children in the world. Every year, around there are 1.7 million cases of diarrhea and cause around 525,000 deaths in children under the age of five in the world\(^2,3\). Overall, 1.7 billion global cases of diarrheal disease are reported annually and are associated with an estimated 2.2 million deaths. The burden of diarrheal disease is most critical in developing countries, facilitated by unsafe water supplies, poor sanitation, and nutritional deficiencies. While less common in high-income countries, diarrheal diseases remain a significant health concern. There are an estimated 211 to 375 million episodes of diarrheal illnesses each year in the United States, with 1.8 million

Corresponding Author:
Rozhalat Khudhur Jarjees;
E-mail: rozhhalat.jarjees@epu.edu.iq.
Contact No: 07504685692
hospitalizations and 3,100 deaths\textsuperscript{4,5}.

A wide variety of viruses, bacteria, and protozoa may cause gastroenteritis\textsuperscript{1}. Acute bacterial gastroenteritis (ABG) can cause more severe symptoms than acute viral gastroenteritis in children\textsuperscript{6}, and the list of bacteria that cause gastroenteritis is continuously growing, include \textit{Campylobacter jejuni}, \textit{Staphylococcus aureus}, \textit{Bacillus cereus}, \textit{Escherichia coli}, \textit{Vibrio cholera}, \textit{Shigella dysenteries}, \textit{Salmonella enteriditis}, \textit{Yersinia enterocolitica}, \textit{Clostridium perfringens}, and \textit{Clostridium difficile}. Micro-organisms cause gastroenteritis by a number of mechanisms. They may release performed toxins prior to ingestion, multiply and produce toxins within the gastrointestinal lumen, directly invade the bowel wall, or use a combination of toxins and invasion\textsuperscript{1}.

On 21 September 2006, the Austrian media reported the occurrence of a cluster of gastroenteritis cases in a boarding school in Eisenstadt, capital of the province Burgenland in eastern Austria. \textit{S. aureus} was identified as the causative agent. From 45 stool samples, none of them contained \textit{Salmonella}, \textit{Campylobacter}, enterohaemorrhagic \textit{Escherichia coli} and \textit{Yersinia}. Of those 45 stool samples, 44 were positive for \textit{S. aureus}\textsuperscript{7}. \textit{S. aureus} may cause diarrhea, gastrointestinal carriage of MRSA has been associated with antibiotic associated diarrhea\textsuperscript{8,9}.

The existence of several virulence factors such as staphylococcal exotoxins have an important role in \textit{S. aureus} pathogenicity. One of these factors is enterotoxins which proved to be more tolerance to high temperature\textsuperscript{10}. Staphylococcal enterotoxins are low molecular weight proteins (MW 26.900–29.600D)\textsuperscript{11}. All enterotoxins are superantigens which are encoded by mobile genetic elements including phages, plasmids and pathogenicity islands\textsuperscript{12,13}. Also \textit{S. aureus} is one of the most important causes of food poisoning due to its ability to produce more than 22 different enterotoxins\textsuperscript{14,15}.

The \textit{S. aureus} enterotoxins (SEs) can be separated into two groups; the 5 serological classical types (SEA–SEE)), and the newer (SEG–SE/Y and counting) enterotoxin groups. Many members from both these groups contribute to the pathogenesis of several serious human diseases. Only enterotoxins with demonstrated emetic potential in monkeys were designated “SE,” whereas enterotoxins that failed to do so or have not been evaluated in non-human primate models of emesis are designated enterotoxin like (SEL-) toxins\textsuperscript{14,16}. Among these Staphylococcus enterotoxins, five of them (A, B, C, D and E) are known to be responsible for 95% of Staphylococcal food poisoning cases\textsuperscript{17,15}. \textit{S. aureus} strains can produce one or several of the five major SEs (SEA, SEB, SEC, SED, and SEE)\textsuperscript{18}. Staphylococcal enterotoxin SEA and SEB are two of the most important gastroenteritis causing agents. SEA and SEB are the most food poisoning agents (> 60\%) in USA and England. For the above-mentioned reason, these toxins can cause epidemic gastroenteritis\textsuperscript{19,11}. While the majority of MRSA isolated from food products in Denmark was negative for major virulence factors such as enterotoxins\textsuperscript{20}.

Staphylococcal enterotoxins (SE) A and B are two of the most important gastroenteritis causing agents in USA and England. There are several methods for detection of enterotoxigenic bacteria. The phenotypical methods are not reliable in specificity, because staphylococcal enterotoxins serotypes are antigenically similar. Therefore, molecular techniques such as PCR are recommended for detection of \textit{S. aureus} enterotoxins genes\textsuperscript{13}. Despite the potential role of \textit{S. aureus} enterotoxins in causing diarrhea a limited number of studies have focused on multidrug resistant \textit{S. aureus} colonized diarrhea. Herein we explored the prevalence of \textit{S. aureus} in the fecal of patients with gastroenteritis.

**Materials and Methods**

The study was carried out for six months from June to November 2020. Fecal samples were taken from patients admitted to Erbil Hospitals suffering from acute diarrhea and gastroenteritis infection.

**Bacterial Identification:**

Fecal samples under aseptic conditions were inoculated into a tube containing 10ml Tryptic soy broth.
The broth was incubated at 37°C for 24h then streaked from the enriched broth onto a selective mannitol salt agar medium, and incubated at 37°C for 24h. Suspected *S. aureus* colonies from each sample were evaluated based on morphology. All isolates were further identified for their species assignment by the automated VITEK2 compact system (bioMérieux, France). After the screening, the presumptive *S. aureus* isolates were stored at -20°C in Tryptic soy broth (TSB) plus 20% v/v glycerol.

**Criteria for finding multidrug resistance, methicillin - resistant *S. aureus*, extensively drug resistance, and pan drug resistance in *S. aureus***

Colonies of *S. aureus* were inoculated in 5ml of 0.85% saline, and the turbidity was adjusted to match 0.5McFarland standard. Then sterile cotton swabs were dipped into the inoculums and spread evenly on to Mueller Hinton agar, MHA. The following antimicrobial discs were applied aseptically to the MHA plates and incubated overnight at 37°C: penicillin(P,10U), amoxicillin (AX,25μg), gentamycin (CN, 10μg) ,vancomycin (VA,30μg), erythromycin (E, 15μg), tetracycline (TE,30μg), methicillin (MET,10μg ), cefoxitin (30μg), ciprofloxacin (CIP,5μg), amoxicillin-clavulanic acid (AMC,20μg/10μg), oxacillin (OXA, 1μg), fusidic acid (FA, 10μg), rifampicin (RA, 5μg), clindamycin, (DA, 2μg), and trimethoprim-sulfamethoxazole (SXT,1.25μg/23.75μg).

We categorized a specimen as penicillin-sensitive *S. aureus* (PSSA) if it was susceptible to penicillin and methicillin, methicillin - sensitive *S. aureus* (MSSA) if it was resistant to penicillin and susceptible to methicillin, and methicillin - resistant *S. aureus* (MRSA) if it was resistant to both penicillin and methicillin. Resistance to methicillin was inferred by testing for oxacillin and cefoxitin resistance. Multidrug resistance (MDR) one or more of these have to apply: (i) an MRSA is always considered MDR by virtue of being an MRSA (ii) non susceptible to at least one agent in three or more antimicrobial categories. Extensively drug resistance (XDR): non-susceptible to at least one agent in all but two or fewer antimicrobial categories. Thus, bacteria isolate that is characterized as XDR will also be characterized as MDR. Pan drug resistance (PDR) non-susceptible to all antimicrobial agents in all antimicrobial categories.

**DNA extraction**

Bacterial DNA was extracted from *S. aureus* using a DNA extraction kit (geneaid/Korea), according to the manufacture’s instruction. Nanodrop was used for measuring the concentration and the purity of nucleic acid. The DNA extracted samples were stored at -20°C for further use as template for PCR.

**Detection of Staphylococcal enterotoxin**

Amplification of Staphylococcal enterotoxin genes was done by specific primers and polymerase chain reaction method. Two PCR primers sets were used to detect the staphylococcal enterotoxin *sea* and *seb* encode SEA and SEB respectively, as described in Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Oligonucleotide sequence (5'-3')</th>
<th>Amplicon size</th>
</tr>
</thead>
<tbody>
<tr>
<td>sea</td>
<td>SEA1,</td>
<td>5' TTG CGA AAA AAG TCT GAA TTG C 3'</td>
<td>552 bp</td>
</tr>
<tr>
<td></td>
<td>SEA2</td>
<td>5' ATT AAC CGA AGG TTC TGT AGA AGT A 3'</td>
<td></td>
</tr>
<tr>
<td>seb</td>
<td>SEB1,</td>
<td>5' TCG CAT CAA ACT GAC AAA CG 3'</td>
<td>477 bp</td>
</tr>
<tr>
<td></td>
<td>SEB2</td>
<td>5' AGG TAC TCT ATA AGT GCC TGC CT 3'</td>
<td></td>
</tr>
</tbody>
</table>
The total of 25µl PCR master mix reaction volume was performed containing 1µl of genomic DNA, 12.5µl of 2x Go Tag green master mix (Promega, USA), and 1µl was added from each of the forward and reverse primer and completed with nuclease free water to 25 µl.

The PCR condition were as follows: a total of 32 cycles was performed with the first denaturation at 94°C for 5min, then consisted of 30 cycles, for denaturation at 94°C for one-minute, specific annealing temperature 55°C for one minute, extension at 72°C for one-minute, and the final extension at 72°C for 5min. The PCR products were separated in a 1% agarose gel and stained with Safe dye (GenetBio, Korea). The gel was visualized under UV trans-illuminator (Synegene, UK).

Results

In total of 417 fecal samples taken from patients with acute gastroenteritis, 30 grew *S. aureus* (7.19%), and of 417 fecal samples 96 yielded bacterial pathogens (23.02%), *S. aureus* was the most common bacterial pathogen found in 30/96 (31.25%) cases.

![Figure (1): *Staphylococcus aureus* on selective mannitol salt agar medium](image1)

These isolates showed different susceptibility towards antibiotics. All isolates of *S. aureus* were sensitive to vancomycin. However, the effect of other antibiotics was variable among the isolates, the heigh effective antibiotics were clindamycin, fusidic acid, ciprofloxacin, gentamycin, and trimethoprim-sulfamethoxazole. Adversely, the less effective antibiotics were penicillin, and amoxicillin, as depicted in Figure 2.

![Figure (2): Antibiotic susceptibility test](image2)
Of 30 isolated *S. aureus*, penicillin sensitive *S. aureus* were 2 (6.67%), methicillin sensitive *S. aureus* were 19 (63.33%), multidrug resistant *S. aureus* were 12 (40%), methicillin resistant *S. aureus* were 11 (36.67%), extensive drug resistance (XDR) *S. aureus* were 3 (10%), Bacteria isolates that is characterized as MRSA and XDR will also be characterized as MDR, and none of them was pan drug resistant *S. aureus* because all isolates of *S. aureus* were sensitive to vancomycin, as depicted in Table 2.

**Table 2**: Percentage of penicillin-sensitive *S. aureus* (PSSA), methicillin- sensitive *S. aureus* (MSSA), multidrug-resistance (MDR), methicillin resistance *S. aureus* (MRSA), extensive drug-resistance (XDR), and Pan drug-resistance *S. aureus* (PDR). Bacteria isolates that is characterized as MRSA and XDR will also be characterized as MDR.

<table>
<thead>
<tr>
<th>Type of antimicrobial resistance</th>
<th>S. aureus (30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Penicillin- sensitive S. aureus (PSSA)</td>
<td>2 (6.67%)</td>
</tr>
<tr>
<td>2- Methicillin- sensitive S. aureus (MSSA)</td>
<td>19 (63.33%)</td>
</tr>
<tr>
<td>3- Multidrug-resistance (MDR)</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>4- Methicillin resistance S. aureus (MRSA)</td>
<td>11 (36.67%)</td>
</tr>
<tr>
<td>5- Extensive drug-resistance (XDR)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>6- Pan drug-resistance (PDR)</td>
<td>0%</td>
</tr>
</tbody>
</table>

The PCR results showed that 7/30 (23.33%) of the *S. aureus* isolates possessed the sea gene, and 3/30 (10%) had the seb gene. In total of 30 isolates Staphylococcal enterotoxin genes *sea* and *seb* were more prevalent among methicillin resistant *S. aureus* (MRSA) were 6/11 (54.54%), 3/11 (27.27%) respectively, then in multidrug resistant *S. aureus* (MDR) which were 6/12 (50%), and 3/12 (25%) respectively, and in Extensively drug resistance (XDR) *S. aureus* *sea* was 1/3 (33.33%) and there is no *seb* genes, while in methicillin sensitive *S. aureus* (MSSA) *sea* was 1/19 (5.26%), and there is no *seb* genes, and there was no Staphylococcal enterotoxin genes *sea* and *seb* in penicillin sensitive *S. aureus* (PSSA), as depicted in Table 3, and Figures 3, 4.

**Table 3**: Prevalence of Staphylococcal enterotoxin genes among penicillin- sensitive *S. aureus* (PSSA), methicillin- sensitive *S. aureus* (MSSA), multidrug-resistance (MDR), methicillin resistance *S. aureus* (MRSA), extensive drug-resistance (XDR), and Pan drug-resistance (PDR) *S. aureus*. Bacteria isolates that is characterized as MRSA and XDR will also be characterized as MDR.
<table>
<thead>
<tr>
<th>Type of antimicrobial resistance</th>
<th>No.</th>
<th>Staphylococcal enterotoxin genes sea</th>
<th>Staphylococcal enterotoxin genes seb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Penicillin- sensitive S. aureus (PSSA)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2- Methicillin- sensitive S. aureus (MSSA)</td>
<td>19</td>
<td>1/19 (5.26%)</td>
<td>0/19</td>
</tr>
<tr>
<td>3- Multidrug-resistance (MDR)</td>
<td>12</td>
<td>6/12 (50%)</td>
<td>3/12 (25%)</td>
</tr>
<tr>
<td>4- Methicillin resistance S. aureus (MRSA)</td>
<td>11</td>
<td>6/11 (54.54%)</td>
<td>3/11 (27.27%)</td>
</tr>
<tr>
<td>5- Extensive drug-resistance (XDR)</td>
<td>3</td>
<td>1/3 (33.33%)</td>
<td>0</td>
</tr>
<tr>
<td>6- Pan drug-resistance (PDR)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure (3): Electrophoresis of the PCR products in 1% agarose gel. Lane 1: 100bp DNA ladder, Lane 2: Negative control, Lane 3 *sea* gene (552bp).

Figure (4): Electrophoresis of the PCR products in 1% agarose gel. Lane 1: 100bp DNA ladder, Lane 2: Negative control, Lane 3 *seb* gene (477bp).
Discussion

*S. aureus* is one of the major pathogens able to infect every organ, damaging tissues and causing severe, chronic, and/or recurrent infections, which require prolonged antimicrobial treatment, with a dramatic impact on the patient’s quality of life\(^{30,31}\). The result revealed that in total of 417 fecal samples, 30 grew *S. aureus* (7.19%), and of 417 fecal samples 96 yielded bacterial pathogens (23.02%), *S. aureus* was the most common bacterial pathogen found in 30/96 cases (31.25%).

The PCR results showed that 7/30 (23.33%) of the *S. aureus* isolates possessed the sea gene, and 3/30 (10 %) had the seb gene. In total of 30 isolates Staphylococcal enterotoxin genes sea and seb were more prevalent among methicillin resistant *S. aureus* (MRSA) were 6/11 (54.54%), 3/11 (27.27%) respectively, then in multidrug resistant *S. aureus* (MDR) which were 6/12 (50%), and 3/12 (25%) respectively, and in Extensively drug resistance (XDR) *S. aureus* sea was1/3 (33.33%) and there is no seb genes, while in methicillin sensitive *S. aureus* (MSSA) sea was 1/19 (5.26%), and there is no seb genes, and there was no Staphylococcal enterotoxin genes sea and seb in penicillin sensitive *S. aureus* (PSSA). *S. aureus* resistance against a wide range of antimicrobials is another important factor in spread of infections by this bacterium that is increasing worldwide.

Different results have been reported in other countries for the prevalence of sea and seb genes in *S. aureus*. isolated from fecal samples.

Our results consistent with the findings of other studies, in Seoul, Korea; Kim et al., 2017, they reported that out of 664 stool samples taken from children with acute gastroenteritis from age (1-18), 183 (27.6%) yielded bacterial pathogens, and *S. aureus* was the most common bacterial pathogen, found in 72 cases (39.3%)\(^6\). In a similar study in China hospitals the overall colonization prevalence of *S. aureus* and MRSA were 20.0% and 4.5% respectively, in fecal samples from patients with age 1day to 17 years old\(^{22}\).

Other studies reported different prevalence of *S. aureus*, in 2007, Flemming and Ackermann, reported that in German out of 2,727 stools investigated, 198 grew *S. aureus* (7.265%). A total of 114 *S. aureus* strains (57.58%), produced the following enterotoxins in vitro: A, 36 (31.58%); B, 20 (17.54%). Twenty-nine (14.6%) *S. aureus* strains were identified as methicillin-resistant\(^{32}\).

In 2014, Kadhim in Iraq detected *S. aureus* in (6.7 %) diarrhea samples, and (66.7%) were found to be positive for production of enterotoxin B\(^{21}\). In United States of America out of 625 human stool samples, 58 were positive for *S. aureus* (9.3%). A high number of isolates were resistant to multiple antibiotics including oxacillin (43.1%), and erythromycin (51.7%), all isolates were susceptible to vancomycin\(^{33}\). In developed countries, Salmonella was the most frequently detected bacteria found in German patients with acute gastroenteritis AGE, followed by enterotoxin gene-carrying *S. aureus* and *Campylobacter spp.* Enterotoxin-encoding *S. aureus* was 1.7% \(^{34}\).

Infection with *S. aureus* was detected in 64/345 fecal samples (18.6%), taken from Iranian patients with gastroenteritis. In total, 50% of the strains (32/64) carried the enterotoxin genes; the most common was sea (56.1%). These genes were more prevalent among MDR-MRSA (58.8%) compared with methicillin-sensitive *S. aureus* (15.4%) strains. *Staphylococcus aureus* should be considered an etiological agent of gastroenteritis. Infection with MRSA resistant to multiple antimicrobial classes (MDR-MRSA) is common among gastroenteritis patients\(^{35}\). In Iranian outpatients with underlying inflammatory bowel disease *S. aureus* were detected in 15.8%. In *S. aureus* isolates, only positivity for the presence of sea enterotoxin was detected\(^{36}\).

The prevalence of *S. aureus* and genes encoding enterotoxins in diarrhea samples in other studies were higher or lower than our results, these variation between our results and other studies, can be attributed to several factors, including the size of the samples tested, the season in which the sampling was done, and geographical
difference. Our result revealed that vancomycin is still the main antibiotic of choice for treatment of infection caused by methicillin resistant S. aureus (MRSA) and extensively drug resistance (XDR) S. aureus. Also, some strains of S. aureus remain susceptible to penicillin. Thus, this antimicrobial agent remains the treatment of choice for patients infected with penicillin sensitive S. aureus (PSSA).

In China, Liang and his colleague did research in 2018, in their result the highest rate of resistance was detected for penicillin (PEN) (93.9%), followed by erythromycin (ERY) (58.8%), clindamycin (CLI) (55.7%), tetracycline (TCY) (31.3%), sulfamethoxazole-trimethoprim (SXT) (7.6%), ciprofloxacin (2.3%), rifampicin (1.5%), nitrofurantoin (2.3%), and gentamicin (0.8%). All of the isolates were susceptible to vancomycin. In Italy, Rifampicin is one of the major drugs used on its own and also in combination to treat numerous infections sustained by methicillin-resistant Staphylococcus aureus (MRSA), but rifampicin resistance (RIF-R) is increasing in multidrug-resistant-MRSA isolates (16.4%), with respect to Europe (5.7%). Some S. aureus strains remains susceptible to penicillin. Thus, this antimicrobial agent remains the treatment of choice for patients infected with penicillin susceptible isolated. In Guangzhou, China, Ai and his colleague did research in 2020, they found that all MRSA strains were resistant to cefoxitin screening, also all MRSA strains were resistant to penicillin. The S. aureus strains exhibited highest rate of resistance to penicillin (PEN, 84.2%), followed by erythromycin (ERY, 38.8%), the resistance rates of antibiotics were lower for gentamicin (GEN, 2.7%), ciprofloxacin (CIP, 1.9%), and rifampicin (RIF, 0.7%); however, all isolates were susceptible to vancomycin.

Patients with MRSA colonized diarrheal stool impact significantly on environmental contamination. The epidemiology of prevalent MRSA is changing dynamically and geographically. Furthermore, having a gastrointestinal condition would increase the risk of intestinal S. aureus carriage.

Conclusions

These results confirmed the involvement of S. aureus in the occurrence of gastroenteritis, and the enterotoxigenic genes sea and seb were more prevalent among methicillin resistant S. aureus (MRSA), then multidrug resistant S. aureus (MDR), and extensively drug resistance (XDR) S. aureus. Vancomycin is still the main antibiotic of choice for treatment of infection caused by methicillin resistant S. aureus (MRSA) and extensively drug resistance (XDR) S. aureus. Our findings suggest that PCR is a rapid, specific, and inexpensive method for detecting SE.

Conflict of Interests: None.

Source of Funding: Self.

Ethical Clearance: The Research Ethics Committee of Erbil Polytechnic University approved the study proposal and approved also by Erbil Teaching Hospital.

References

6- Kim SY, Kim H, Shi EH, Eun BW, Ahn YM,


Lipoteichoic Acid as Antibiofilm against *Staphylococcus aureus*

Ruaa SH¹, Suhad M²

¹Doctor of Immunology, ²Doctor of medical microbiology. Kufa University, Collage of Science, Laboratory of Investigation, Kufa City, Iraq

**Abstract**

**Background:** It is a polysaccharide substance produced by a group of microorganisms that helps it adhere to living and non-living surfaces and it is responsible for many diseases, as it has a role in staphylococcal diseases such as pneumonia, urinary tract infection and endocarditis. Although lipoteichoic acid is a component of biofilm. However, it has a role in preventing biofilm formation

**Materials and Methods:** The methods of this study included isolated and identification of bacteria from UTI samples and identified by morphology characters and biochemical test to identify *S.aureus*. Then discover their ability to formbiofilm and Use lipoteichoic acid in different concentration as antibiofilm

**Results:** The results of identification of bacterial isolatesshowed the colonies grown on blood agar causing the typical B hemolytic state, these samples not grown on MacConkey agar and under the microscope showed gram positive cocci, a cluster like grape, these samples were identified as *Staphylococcus* when grown on mannitol medium converted it to yellowish color and appeared round, smooth, raised, mucous and gelatinous. The *staphylococcus aureus* formed biofilms with different concentrations, where the fourth isolate was the lowest percentage (0.065), while the second and fifth isolates were given (0.120 and 0.153) and the first isolation was given (0.220),however the highest percentage was given by the third isolation (0.357). So this study showed that the concentrations of LTA (200) µg/mlare MIC. The results of this work showed that after 24 hours of treatment with LTA it impeded the formation of biofilms of *Staphylococcus aureus* and the result after treatment was the concentration 50 and 100µg/ml gave less percentage of inhibition, while 500µg/ml appeared 100% of inhibition, so (200,300 and 400 gave 61%, 80% and 90% inhibition to biofilm respectively.

**Keyword:** Biofilm, MIC, gram positive and UTI

**Introduction**

*Staphylococcus aureus* is a gram-positive bacterium that has the ability to adapt to living in various non-living environments. This bacterium is characterized by being an important factor in causing a variedcollection of clinical infections bacteremia and infectious endocarditis, osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections¹. These bacteria are distinguished by their ability to attach to non-living materials by producing a polysaccharide matrix, which is a virulence factor that gives bacteria the ability to resist antibiotics and the immune systemin hospital; this leads to the emergence of antibiotic resistance². This leads to chronic and destructive infections, as these membranes allow them to adhere to tissues such as heart valves and bones, and cause infective endocarditis and osteomyelitis, or on implanted medical devices such as catheters, artificial joints, artificial heart valves, and bone implants in hospitalized patients in the healthcare environment³. The first step is to create a biofilm at the place where the cells adhere to surfaces and reinforce
several mechanisms of irreversible adhesion\textsuperscript{4}.  

Lipoteichoic acids (LTA), are connected with the membrane of the cell through a glycolipid anchor in a Gram-positive bacterium such as \textit{S. aureus}, \textit{enterococci}, \textit{Listeriamonocytogenes}, \textit{Streptococcus pneumoniae}, and \textit{Bacillus subtilis}. LTA is an anionic 1,3-glycerolphosphate holding polymer attached to the cell wall\textsuperscript{5}.  

It has an important role in bacterial growth, cell wall working, membrane stability, and virulence\textsuperscript{6}. Anti-biofilm causes were developed as replacements to antibiotics to protect against infection associated with biofilms to prevent these membranes from developing without bacterial resistance. Bacteria-derived amphiphilic have been largely studied for their anti-biofilm properties\textsuperscript{7}.  

\textbf{Materials and Methods}  
\textbf{Collecting and diagnosing specimens}  

50 specimens of bacteria were collected from women suffering from urinary tract infection and who did not take any antibiotics, in Al-Sadr Hospital in AL-Najaf Governorate. Samples were cultured in pre-prepared media including (MacConkey agar, blood agar, and mannitol salt agar) then incubated at 37 c° for 24 h. the bacterium was diagnosed based on the phenotypic properties using biochemical tests, where was staphylococcus aureus diagnosed by gram staining, catalase after growing on mannitol salt agar medium, coagulase test\textsuperscript{8}, in order to check the diagnosis.  

\textbf{Preparation of lipoteichoic acid}  

It was the ready prepared solution (according to Sigma Aldrich, Germany) and supplied in a glass vial (5 mg/ml) provided with water (1ml) as a stock solution. The stock solution was diluted by adding distilled water in proportion to 100 µl of lipoteichoic acid: 900 µl D.W.  

\textbf{Minimum inhibitory concentration (MIC) of lipoteichoic acid}  

The minimum inhibitory concentration (MIC) of lipoteichoic acid was defined as the microdilution broth method. Briefly, serial concentrations of lipoteichoic acid (50, 100, 200, 300, 400, and 500 µg/ml) were set with tryptic soy broth. The lowest concentration that inhibits the growth of bacteria is considered as the MIC\textsuperscript{9}.  

\textbf{Biofilm formation}  

The biofilm assay defined by\textsuperscript{10}, with some alterations: 10 ml of trypticase soy broth (TSB) with 1% glucose was inoculated with a loopful of test bacterium from an overnight culture on nutrient agar. The flat bottom tissue culture plates (96 wells) were filled with 200µl of diluted cultures separately. uninoculated sterile broth assisted as blank. The control bacteria were also diluted and incubated. The culture plates were incubated at 37˚C for 24 hours. After incubation, kind tapping of the plates was finished. The bores were washed with 200 µl of Normal saline four times to eliminate free-floating bacteria. Biofilms that remained adherent to the walls and the bottoms of the wells stained with 0.1% crystal violet for 10 min. another stain was washed with Normal saline and plates were dried properly then adding 200 µl of the destaining solution (95% ethanol) for 10 min. Lastly, 200µl from each well was moved to a new microtiter plate and measured at 570 nm by a microplate reader. The biofilm degree was calculated as follows: Biofilm degree=Mean OD570 of tested bacteria- Mean OD570 of control.  

\textbf{Effects of lipoteichoic acid on biofilm formation}  

The method described by\textsuperscript{11} was accepted to investigate the effect of LPT on biofilm formation. An overnight bacterial culture (in trypton soya broth) was attuned with McFarland standard No. 0.5. Tryptone soya broth containing MIC of LPT was inoculated with a before prepared bacterial suspension and incubated for 24 hours at 37°C. An amount of 200 µl of the culture was transferred in triplicate into the vertical rows of a polystyrene microtiter plate well for each isolate and served as control. A volume of 200 µl of culture containing the MIC concentration of the LPT was transferred into another three wells. All plates were incubated at a temperature of 37°C for 24 hours. Subsequently, the
biofilm formation protocol was followed as mentioned earlier. Percentage of biofilm inhibition was calculated following the equation: 
\[ \text{percentage of inhibition of biofilm formation} = 1 - \left( \frac{\text{O.D of treatment}}{\text{O.D of control}} \right) \times 100 \]

**Results and Discussion**

**Collecting and diagnosing specimens**

50 samples were collected from women suffering from urinary tract infection and the results of the bacterial culture showed 5 samples grown on blood agar causing the typical B hemolytic state, these samples not grown on MacConkey agar and under the microscope showed gram positive cocci, a cluster like grape, these samples were identified as Staphylococcus when grown on mannitol medium converted it to yellowish color and appeared round, smooth, raised, mucous and gelatinous. The results of the current study showed that all mannitol fermenters 5(10%) were coagulant and catalase test positive, and therefore they were considered *S. aureus*.

**Biofilm formation**

In this study, the result was that the five isolates of *Staphylococcus aureus* formed biofilms with different concentrations, where the fourth isolate was the lowest percentage (0.065), while the second and fifth isolates were given (0.120 and 0.153) and the first isolation was given (0.220), however, the highest percentage was given by the third isolation (0.357) which was used in the subsequent experiments (table -1).

**Table-1)** show the percentage of the biofilm formed by *S. aureus*

<table>
<thead>
<tr>
<th>No of isolate</th>
<th>Biofilm formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.220</td>
</tr>
<tr>
<td>2</td>
<td>0.153</td>
</tr>
<tr>
<td>3</td>
<td>0.357</td>
</tr>
<tr>
<td>4</td>
<td>0.065</td>
</tr>
<tr>
<td>5</td>
<td>0.120</td>
</tr>
</tbody>
</table>

**3.3 minimum inhibitory concentration (mic) of lipoteichoic acid**

The result of this study showed that the concentrations (500) µg/ml were lethal to *Staph aureus*, while (200) µg/ml were inhibitor and the concentrations (50,100 µg/ml) gave were not effective, (table-2).

**(Table-2)** minimum inhibitory concentration (mic) of lipoteichoic acid

<table>
<thead>
<tr>
<th>Con of lipoteichoic acid</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0%</td>
</tr>
<tr>
<td>100</td>
<td>0%</td>
</tr>
<tr>
<td>200</td>
<td>20%</td>
</tr>
<tr>
<td>300</td>
<td>40%</td>
</tr>
<tr>
<td>400</td>
<td>70%</td>
</tr>
<tr>
<td>500</td>
<td>100%</td>
</tr>
</tbody>
</table>

**3.4. Effects of lipoteichoic acid on biofilm formation**

The results of this work showed that after 24 hours of treatment with LTA it impeded the formation of biofilms of *Staphylococcus aureus* and the result after treatment was the concentration 50 and 100µg/ml gave less percentage of inhibition, while 500µg/ml appeared 100% of inhibition, so (200,300 and 400 gave 61%, 80% and90% inhibition to biofilm respectively, (table-3)

**(Table-3)** Explain the influence of LTA on biofilm formation

<table>
<thead>
<tr>
<th>Lipoteichoic acid concentration</th>
<th>Biofilm inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>4%</td>
</tr>
<tr>
<td>100</td>
<td>7%</td>
</tr>
<tr>
<td>200</td>
<td>61%</td>
</tr>
<tr>
<td>300</td>
<td>80%</td>
</tr>
<tr>
<td>400</td>
<td>90%</td>
</tr>
<tr>
<td>500</td>
<td>100%</td>
</tr>
</tbody>
</table>
found that the total 355 urine samples of UTI suspected patients were screened 119 (33.52%) sample showed grow out of which *Staphylococcus aureus*, while 13 found the *Staph aureus*(1.9%) from UTI. found the effect of *Lactobacillus plantarum* lipoteichoic acid on developed biofilm, *E. faecalis* was grownup on glass-bottom plate’s treatment with Lp.LTA for 24 h. The 3-week-old *E. faecalis* biofilm was condensed by Lp.LTA in a dose-dependent manner. In addition, appeared that *Lactobacillus plantarum* LTA could inhibit *S. aureus* biofilm development.

*S. aureus* is a bacterium that causes many inflammatory infections and biofilm development is closely related to antibiotic resistance and adhesion to surfaces. The inhibitory influence on the development of *Staph aureus* biofilm is a communal characteristic of LTA of gram-positive bacteria including *S. aureus*, *S. pneumoniae*, *S. gordonii*, *E. faecalis*, and *B. subtilis*. D-Alanine moieties in the LTA play an important role in inhibiting biofilm development, and many explanations have been found for this inhibition. First, the presence of the positive charge resulting from the presence of de-alanine has an inhibitory role as the positive charge prevents biofilms from forming. For example, an increase in the degree of N-deacetylation causing an increased positive charge. Second, D-amino acids interfere with the formation of biofilms as they break down biofilms. For example, *S. aureus* biofilm development was inhibited by D-phenylalanine, D-proline, and D-tyrosine. The inhibitory influence of LTA on *S. aureus* biofilm development is not only due to competition with bacterial cell membrane-anchored LTA, but is also due to bacterial sensing and signaling in response to LTA because LTA could finish the pre-formed biofilm.

**Conclusion**

The aim of this study was to investigate antibacterial activities and disruption of biofilm structure by Lipoteichoic acid. *Staph aureus* was chosen because its ability to form biofilm on surfaces makes the cells impervious to therapeutic concentrations.

**Conflict of Interest:** Nill

**Source of Funding:** Self

**Ethical Clearance:** Taken from Hospital Ethical Committee at Medical, Collage, Kufa

**References**


Survey of Puree Users without Smoking History of Households in Kosebo Village, Angata District, Konawe Selatan District

Ruslan Majid1, Elma Prasetyaningsih1, Fikki Prasetya1, Jumakil1, Fifi Nirmala1
1Faculty of Public Health, Halu Oleo University, Kendari Southeast Sulawesi, Indonesia

Abstract

“Puree” in the local language is a process of concocting or mixing tobacco and whiting, finally forming a black paste and then rubbing it on the lips, the user. “Me puree” in local language terms is using “Puree”. The purpose of this study was to determine the survey of Puree Users with no smoking history among housewives in Kosebo Village, Angata District, South Konawe Regency in 2019. This research method used a descriptive method, carried out in Kasebo Village, Angata District, South Konawe Regency. The population of all housewives was 203. The sampling technique used was proportional random sampling, and obtained a number of 54 respondents. The results of the research after analyzing the frequency distribution showed that the respondents’ knowledge was lacking, the history of using “puree” was sufficient, the tradition of “puree” users was good, the income of “Puree” users was less and the previous disease history of “puree” users was less. It is recommended that a primitive program to provide public health education by conducting outreach to identify risk factors that can increase the incidence of both smokeless and smoked tobacco use.

Keywords: Puree, Knowledge, User History, Tradition, Income

Introduction

“Puree” The term local language is a process of concocting or mixing tobacco and whiting, finally forming a kind of black paste which is smeared on the user’s lips. “Me puree” means using “Puree”. Tobacco is a type of plant that is well known among Indonesian people. In addition, people use tobacco as chewing, especially among mothers in rural areas. The dangers of tobacco use include diseases related to the heart and lungs such as heart attack, stroke, chronic obstructive pulmonary disease, emphysema and cancer. Reproductive disorders and pregnancy can also result from tobacco use.

Dependence on tobacco has become a global epidemic that can cause disability, disease, decreased productivity and death. This is because in the tobacco leaves there are several kinds of alkaloids that can give pleasure to the user, namely nicotine, nicotine and myosin. So that this alkaloid gives an addictive effect for those who use tobacco. Some of the negative effects caused by the habit of consuming chewing tobacco are cataracts, pneumonia, leukemia, stomach cancer, pancreatic cancer, cervical cancer, kidney and other diseases. These diseases add to a long list of diseases caused by consuming tobacco, such as cancer of the lungs, esophagus, larynx, mouth and throat. Lung disease, emphysema and bronchitis, stroke, heart attack and other cardiovascular diseases. Nearly 90% of lung cancers are caused by tobacco consumption. Tobacco can also damage the reproductive system, contributing to miscarriage, premature birth, low birth weight, and diseases in children such as hyperactivity.

Smokeless Tobacco or smokeless tobacco is the use of tobacco without having to be burned like cigarettes. Smokeless tobacco contains several carcinogenic compounds that have been linked to various diseases.
such as oral cancer, hypertension, heart disease and other diseases. Smokeless tobacco is also used in several countries and various uses for each country such as dipping tobacco. Smokeless tobacco products such as: snus, tobacco cream or toothpaste, chewing tobacco, dissolved tobacco, topical tobacco paste, water tobacco, smokeless tobacco herbs, and others.

The use of smokeless tobacco is more common in Asian, African and Middle Eastern countries than in Europe and America. The prevalence of smokeless tobacco uses in the United States according to the Surveillance System Study of use ranges from 1-9% in different countries and more in young men with low educational status, and in countries with a high smoking prevalence. The United States Population Survey reports a significant reduction in smokeless tobacco use from 1992 to 20023.

The US National Health Interview Survey (1991-2003) also reported a decrease in smokeless tobacco use. The prevalence of smokeless tobacco use has also been reported from European countries. This is almost similar to the United States with rates ranging from 1-9% in 3 different countries.

“Puree” is one of the products used by the residents of Angata for their smoking cessation efforts which contain tobacco or in the palace it is called Smokeless Tobacco. “Puree” as a substitute for cigarettes during smoking cessation. The fact shows the result that Puree’s herb is effective as a therapy to quit smoking. Puree is seen as local wisdom in Angata District for smokers who are motivated to quit. The residents of Angata support the use of Puree as a smoking replacement therapy for adult smokers, they engage with this product on a smoking cessation pattern without knowing the carcinogenic effects of Puree. The puree is made from tobacco water and whiting then becomes a kind of paste and rubbed on the user’s lips. The health hazards posed by Puree come from tobacco components that contain harmful substances to health such as nicotine1.

Puree is a smokeless tobacco product, apart from having a positive impact, puree also has a negative impact on health so that puree as one of the smokeless tobacco products certainly has a negative impact that needs to be researched. By knowing the survey of puree users without a smoking history among housewives, they can take the right steps in an effort to improve public health by changing wrong behavior habits.

Method

This research uses descriptive method so that the main objective is to make an objective analysis of the situation. Determination of respondents used Probability sampling technique to take the subject from each stratum or each area that is determined balanced with the number of subjects in each stratum or region. By using the proportional random sampling technique, the total sample size is 54 respondents.

Results

1. Knowledge

Based on respondents’ knowledge of using Pure without a history of smoking, the results show that from a total of 54 respondents, the majority of respondents said Yes as much as 58.95% and respondents who answered No were 41.05%.

2. Pure Usage History

Based on the history of the use of “Pure” without cigarette production, the respondents who answered “Pure” without the product of cigarettes, as for the results of a total of 54 respondents, the majority said Yes as much as 57.93% and respondents who answered No were 42.07%.

3. Tradition

In accordance with the traditional results of using “Pure” without a history of smoking, from a total of 54 respondents, the majority answered Yes as much as 58.95% and respondents who answered No were 41.05%

4. Income

Respondents’ income of “Pure” users without cigarette production, can be seen in the following table:
Based on the table, it shows that of the total 54 respondents, most of them had less income, namely 38 (70.4%) and the number of respondents who had sufficient income was 16 (29.6%).

5. Previous Disease History

The distribution of respondents based on previous history of using “Pure” without history. The history of the respondent’s illness in the last 6 months can be seen in table 2 as follows:

Table 2. Distribution of Respondents Based on Previous Disease History Using “Pure” Without Cigarettes.

<table>
<thead>
<tr>
<th>Number</th>
<th>Previous Disease</th>
<th>Total (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diarrhea</td>
<td>9</td>
<td>40.91</td>
</tr>
<tr>
<td>2</td>
<td>Stomach</td>
<td>13</td>
<td>59.09</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>22</td>
<td>100</td>
</tr>
</tbody>
</table>

Based on the table, it shows that of a total of 22 respondents, most of them suffered from gastric disease as much as 13 (59.09%) and as many as 9 (40.91%) had diarrhea.

Discussion

1. Knowledge

Knowledge or cognitive is a very important domain in shaping one’s actions (overt behavior). From experience and research, it is proven that behavior which is conscious of knowledge will be more lasting than behavior which is not based on knowledge. In the person, a sequence of processes occurs, namely: Awareness, Interest, Evaluation, Trial, Adoption. Knowledge, namely 34 (63.0%). This is because respondents do not know the impact of pure. And not always someone’s knowledge can allow someone not to use puree.

As many as 20 respondents (37.0%) had less knowledge. This is because some respondents do not know about pure both in terms of benefits and in terms of its impact. This is in accordance with the research conducted by Rahayu regarding “The Relationship Between Knowledge of the Dangers of Smoking and Smoking Behavior in Students at the Muhammadiyah University of Surakarta” states that there is a significant
relationship between the level of knowledge and smoking behavior. This shows that there is a tendency that low knowledge will shape smoking behavior, whereas high knowledge is more likely to have non-smoking behavior. The behavior of students who often smoke in the Pakuan University campus environment is influenced by a lack of knowledge about the dangers of smoking.

2. Usage History pure

Tobacco is a plant that can cause addiction because it contains nicotine and also carcinogens and other toxic substances. After being processed into a product, whether it is cigarettes or other products, the added chemicals have the potential to cause tissue damage and cancer. The main toxins in tobacco are tar, nicotine and CO₂.

Pure is made from tobacco water and whiting then becomes a kind of paste and rubbed on the user’s lips. The health hazards posed by Pure come from tobacco components that contain harmful substances for health such as nicotine.

Based on the results of research on the history of the use of pure respondents, it is known that all respondents have a real history of using pure, namely as many as 54 respondents (100%), with an average weight of puree that they consume per day is 157.97 g / day. Researchers assume that respondents use puree because puree does not contain the ingredients used in the cigarette which they know are very harmful to the body. This is what becomes a benchmark for people in China that it is better to use puree than cigarettes besides that puree is easier to obtain and cheaper than cigarettes.

Smokeless tobacco is much lower in continuous risk than burnt products, but the risk varies within product classes for example: Snus, Swedish type nitrosamines are low compared to other smokeless tobacco with high nitrosamine levels. It is thought that the safety risks of smokeless tobacco are similar to those of e-cigarettes. There is no safe level of using smokeless tobacco. This disease is associated with a number of adverse effects such as dental disease, oral cancer, esophageal cancer and pancreatic cancer, as well as adverse reproductive effects including stillbirth, premature birth and low birth weight. Smokeless tobacco contains cancer-causing chemicals. About 28 chemical elements present in smokeless tobacco are carcinogenic, of which nitrosamines are the most prominent. The results of research conducted by Fikki P. in 2018 show that pure ingredients are effective as a therapy to quit smoking. It is something very different, but has a big impact, on the success of quitting smoking. Pure is seen as local wisdom in Angata for smokers who are motivated to quit. Angata residents support the use of pure as a smoking therapy for adult smokers. They are involved with this product in a smoking cessation pattern, without knowing the carcinogenic effects of the pesticide.

3. Tradition

Tradition has become part of the life of a group. The most important thing in a tradition is the existence of information from generation to generation, both oral and written. Community behavior is often influenced by the culture or customs that prevail in the community itself. One of the cultural factors found in society is the habit of chewing. Betel nut is the process of concocting a mixture of several ingredients such as betel, areca nut, lime and Gambier which are then chewed together. Betel behavior has generally been practiced since a long time ago in the South East Asia and Asia Pacific regions. The origin of this culture of betel nut is not known exactly when it started, but it is thought to have existed since approximately 2000 years ago.

Based on the results of research on the tradition of respondents, it is known that most of them have good traditions, namely as many as 51 respondents (94.4%) and the number who have less traditions is as many as 3 respondents (5.6%). This is because respondents still consider beetching or using puree to be good for their health because many of their parents who use puree are more likely to live a long life than parents who do not use puree.

The most commonly used ingredients of betel are betel, areca nut, lime and Gambier. Some regions
also add tobacco to the betel mixture. The betel nut seeds used for betel contain phenolic compounds. This phenolic content is relatively high. When the process of chewing betel nut seeds in the mouth, reactive oxygen or what is commonly known as free radicals will form phenolic compounds. The mixture of betel nut and whiting will produce alkaline pH conditions. This will more quickly stimulate the formation of recommended oxygen. This oxygen causes DNA or genetic damage to epithelial cells in the oral cavity.

Several countries in the world chewing by concocting a mixture of betel ingredients together with tobacco. In Indonesia, betel behavior follows local culture. Indonesian society has long recognized the behavior of beetling. They believe that chewing can strengthen teeth, heal small sores in the mouth, get rid of bad breath, stop bleeding gums, and as a mouthwash. Betel leaf is also used as an antimicrobial against Streptococcus mutants which is the bacteria that most often causes tooth decay.

This is in line with the research conducted by Kamisorei, entitled “An Overview of Beliefs About the Efficacy of Chewing in Papuan Communities in Adipura 1 Jaya Pure Selatan Village.” feels fresh, eliminates sleepiness and betel as a form of friendship.

4. Income

Income can be defined as revenue generated for a business or activity. According to Iskandar Putong, income is all types of income, including income obtained without providing any activity that is received by the population of a country.

Cigarette consumption in Indonesia continues to increase, increasing cigarette consumption worsens the economy of a family, especially for poor families. The income they earn is mostly used for consuming cigarettes and will reduce the consumption of other foods that will support health. The higher the consumption of cigarettes it will endanger health When sick, spending will increase and will worsen the economy and will result in a cycle of poverty.

Tax is used as an alternative to reduce cigarette consumption, the higher the tax, the higher the sales price, with the aim that the community can reduce cigarette consumption. In addition, on the one hand, cigarette tax revenues can be used as income to finance health losses caused by smoking or can be used as funds for the development of facilities and infrastructure such as the development of smoke-free areas.

In the poorest households in some low-income countries, more than 10% of total household expenditure is on tobacco (de Beyer, et al., 2001; Efroymson, et al., 2001; Mary Assunta, 2010; WHO, 2004b, 2004d). This means that families have less money to spend on basic items such as food, education and health care. It is even worse in Bangladesh, that the poorest households in Bangladesh spend nearly 10 times as much on tobacco than on education.

5. Previous Disease History

Smokeless Tobacco or smokeless tobacco is a tobacco product that is used for other than smoking. Uses include chewing, sniffing or placing this product between the gum and the cheek or lips. Smokeless tobacco products are produced in various forms such as: Chewing tobacco, Snus tobacco and soluble tobacco products. Smokeless tobacco products usually contain more than 3000 constituents. All smokeless tobacco products contain nicotine and are therefore very addictive. Quitting smokeless tobacco is just as difficult as quitting smoking.

Smokeless tobacco is much lower on the risk continuum than burnt products, but the risk varies within product classes for example: Snus, Swedish type nitrosamines are lower than other smokeless tobacco with high nitrosamines. It is thought that the safety risks of smokeless tobacco are similar to those of e-cigarettes. There is no safe level of using smokeless tobacco. This disease is associated with a number of adverse effects such as dental disease, oral cancer, esophageal cancer and pancreatic cancer, as well as adverse reproductive effects including stillbirth, premature birth and low birth weight. Smokeless tobacco contains cancer-
causing chemicals. About 28 chemical elements present in smokeless tobacco are carcinogenic, of which nitrosamines are the most prominent.

The negative impact caused by the habit of consuming smokeless tobacco is the emergence of gastric cancer, lung cancer, kidney cancer, stroke and heart attacks. Nearly 90% of lung cancers are caused by tobacco consumption.

Based on the results of research on the respondent’s previous medical history, it is known that most of the respondents did not have a previous history of disease, namely 46 respondents (85.2%) and the number of respondents who had previous disease histories was 8 respondents (14.8%). Researchers assume that pear user/pure user does not have the disease before using puree, which can make the disease worse if the respondent uses puree.

People are not fully aware of the risk of disease and premature death due to their decision to buy tobacco products, because several factors cause it, among others, it takes 20-25 years since people start smoking and symptoms of the disease appear. Most of the smokers or people who use puree are those who already know the health effects of tobacco and they are aware of the powerful addictive effects of nicotine which increases and makes it difficult for people to stop consuming it.

The study by Gupta, entitled “Smokeless tobacco and cardiovascular disease in low and middle income countries”. Smoking is an important cardiovascular risk factor, but smokeless tobacco use is also important but not well researched. smokeless tobacco use is high in South and Southeast Asian countries, Africa and Northern Europe. The use of smokeless tobacco accelerates Atheros thrombosis much like smoking cigarettes. The importance of this study for improving public health and clinical interventions to reduce smokeless tobacco smokeless tobacco addiction.

**Conclusion**

Based on the results of research conducted on “PURE” users without a history of smoking. So it can be concluded several things, namely

1. Knowledge “Pure” users without smoking history, have less knowledge
2. User History “Pure” No Smoking History, having a user history of “pure” is sufficient.
3. Tradition of “Pure” User with No Smoking History has a good tradition.
4. Income “Pure” User without Smoking History has less income.
5. Previous Disease History “Pure” user without smoking history, has no previous medical history.

**Suggestion**

Based on the results of research conducted on “PURE” users without a history of smoking. Then the following suggestions can be made: It is necessary to increase public awareness that using tobacco even though in the term pure packaging will not be free from disease, it can even get worse and it is recommended to relevant agencies to always provide education/counseling regarding the dangers of cursing pure to the community.

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**Conflict of Interest:** Author declares that there is no any conflict of interest within this research.

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Evaluation of Incisor Index as a Forensic Tool in Gendural Dimorphism – A Study in South Indian Population

S.Prasanna¹, L.S.Makesh Raj², P.Sai Krishna³, V.Jai Santhosh Manikandan⁴, Srikant.N⁵
¹Senior Lecturer, ²Associate Professor, ³Professor & Head, ⁴Senior Lecturer, Department of Oral and Maxillofacial Pathology, Tagore Dental College & Hospital, Chennai, Tamil Nadu, India, ⁵Professor & Head, Dept. of Oral Pathology and Microbiology, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India

Abstract

Introduction: Forensic odontology plays a vital role in investigation and presenting the dental evidences to the court of law. The challenges that are encountered by the forensic odontologist are identifying the individual which includes certain characteristic features to define an individual. Gendural dimorphism means systemic difference in form (shape or size) between individual of different genders in the same species. Teeth being considered as one of the stable part which can withstand any environmental changes even after death of an individual in situations where there are minimal supporting evidences. Odontometrics is the field involving measurements of a tooth.

Aim and Objective: This study aims in determining the sex of an individual using incisor index of maxillary and mandibular arches as a tool in forensic investigation with brief review on literature.

Material and Method: A total of 1200 permanent maxillary and mandibular teeth from 150 study models with an age range of 18 – 25 yrs were measured using manual divider and scale. The Incisor index (Ii) was analysed by using the formula as given by Aitchison (1964). The values were noted separately for maxillary and mandibular dental arches with regards to central and lateral incisor and these data were subjected for statistical analysis.

Result: Maximum mesio-distal dimension of the all the incisors (maxillary and mandibular) were higher in males than females. Incisor index of maxilla was higher in female when compared to male which was not statistically significant. The Incisor index of mandible was higher in male when compared to females which was also statistically non-significant.

Key words: Incisor index, forensic odontology, Gendural dimorphism, odontometrics

Introduction

Forensic odontology is that branch of dentistry which deals with application of dental findings in legal situations. Dental evidence can be preserved for indefinite period and can be presented before the justice. Tooth can be analysed and recorded during ante-mortem and post-mortem. Major problem in acquiring and interpreting the ante-mortem records are due to poor record maintenance and variation with time duration.

Dental remains will be the sole identification when there is no information about the deceased and remains one of the important and crucial evidence in the analysis. Teeth are the important component of the masticatory apparatus of skull which are considered as good source of aid for civil and medico legal identification. As they are resistant to bacterial decomposition, fire, to some
extent in acid attacks they serve a valuable source of information in forensic investigation.\textsuperscript{4}

Gender identification is one of the most crucial steps in the forensic investigation. Sex determination is the first and foremost challenge for a forensic expert in identifying the mutilated bodies beyond recognition in mass disasters, chemical and nuclear bomb explosions.\textsuperscript{2} The systemic difference which are seen with regards to shape or size between individuals of different genders within the same species are known as Genderual dimorphism. This dimorphism is greatly appreciated with regards to tooth.\textsuperscript{5}

Numerous articles were published in the literature regarding dimorphism with respect to tooth size. Among these, very few publications were related to incisors. Present study aimed in determining the gender of an individual employing incisal index as the forensic tool in a South Indian population and to determine the percentage of sexual dimorphism.

**Materials and Methods**

The study was carried out in Department of Oral and Maxillofacial Pathology, Tagore Dental College and Hospital, Vandalur, Chennai during the period between 2019 – 2020. A total of approximately 1200 permanent maxillary and mandibular teeth from 150 study models with an age range of 18 – 25 yrs were measured. Patient who have teeth that were periodontally weak, affected with dental caries, fracture, restored with crown, spacing, diastema and previous orthodontic treatment were excluded from the study. Maximum Mesio-distal diameter of the lateral incisor (MDI\(_2\)) and central incisor (MDI\(_1\)) was measured using divider and scale. The Incisor index (Ii) was analysed by using the formula as given by Aitchison (1964):\textit{Ii} = \left\{\frac{MDI_2}{MDI_1}\right\} \times 100

The values were noted separately for maxillary and mandibular dental arches with regards to central and lateral incisor and these data were subjected for statistical analysis.

**Results**

Maximum mesio-distal dimension of the all the incisors (maxillary and mandibular) were higher in males than females. Mesio-distal dimension of maxillary central incisor (male = 8.63±0.859, female =8.33±0.763) and mandibular lateral incisor incisor (male = 5.84±0.624, female =5.54±0.609) showed a statistically significant difference between males and females with a p value of 0.029 and 0.008 respectively. (Table 1)

Incisor index of maxilla was higher in female compared to male with a t value of 1.537 and is statistically non-significant. While Incisor index of mandible is higher in male when compared to females with a t value of -1.879 which is also statistically non-significant. (table 2)

**Table 1: Independent T test for comparison of the gender for maxillary and mandibular incisors**

<table>
<thead>
<tr>
<th></th>
<th>GENDER</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>t</th>
<th>DF</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAXILLA MDI1</td>
<td>FEMALE</td>
<td>101</td>
<td>8.33</td>
<td>0.763</td>
<td>-2.21</td>
<td>148</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>MALE</td>
<td>49</td>
<td>8.63</td>
<td>0.859</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAXILLA MDI2</td>
<td>FEMALE</td>
<td>101</td>
<td>6.47</td>
<td>0.715</td>
<td>-0.332</td>
<td>148</td>
<td>0.741</td>
</tr>
<tr>
<td></td>
<td>MALE</td>
<td>49</td>
<td>6.51</td>
<td>0.893</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MANDIBLE MDI1</td>
<td>FEMALE</td>
<td>101</td>
<td>5.16</td>
<td>0.596</td>
<td>-0.653</td>
<td>148</td>
<td>0.515</td>
</tr>
<tr>
<td></td>
<td>MALE</td>
<td>49</td>
<td>5.22</td>
<td>0.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MANDIBLE MDI2</td>
<td>FEMALE</td>
<td>101</td>
<td>5.54</td>
<td>0.609</td>
<td>-2.711</td>
<td>93.02</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>MALE</td>
<td>49</td>
<td>5.84</td>
<td>0.624</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Independent T test for comparison of the gender for Incisor index in maxilla and mandible

<table>
<thead>
<tr>
<th>GENDER</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>t</th>
<th>Df</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>INCISOR INDEX MAXILLA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEMALE</td>
<td>101</td>
<td>77.94555</td>
<td>8.68208</td>
<td>1.537</td>
<td>148</td>
<td>0.126</td>
</tr>
<tr>
<td>MALE</td>
<td>49</td>
<td>75.57755</td>
<td>9.189334</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INCISOR INDEX MANDIBLE</td>
<td></td>
<td></td>
<td></td>
<td>-1.879</td>
<td>127.18</td>
<td>0.063</td>
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<tr>
<td>FEMALE</td>
<td>101</td>
<td>108.419</td>
<td>13.7836</td>
<td></td>
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</tr>
<tr>
<td>MALE</td>
<td>49</td>
<td>112.114</td>
<td>9.868</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Graph 1: Independent T test for comparison of the gender for maxillary and mandibular incisors)

(Graph 2: Independent T test for comparison of the gender for Incisor index in maxilla and mandible)
Discussion

Tooth shows coronal completion at the early stages of tooth development and the dimensions are unaltered during further growth and development, except in situations like disorders in functionality, pathology and nutrition which can alter the dimensions of the crown. Hence odontometrics can be used in situations where osseous features are not yet defined.6

Sexual dimorphism in humans are a result of survival strategy which is a balancing action between high degree of biological variation within species and narrow range of variation in females whose physical structure focuses on supporting an infant prenatally and postnatally.7

So these variations are mirroring the on-going events happening in the evolution process and the genetic background has been explained which is put forth by the polygenic model of inheritance and there by this forms the reason behind the sexual dimorphism in the morphological and metric attributes of males and females.4

The results of our study regarding mesio-distal dimension of the maxillary central incisors higher in males than females which is statistically significant with p value 0.029. Our results were inaccordance with those of Mohammed Nahidh (2014)4, Khangura RK et al (2011)8, Bakkannavar SM et al (2012)9, Kalia S (2006)10, Al-RifaiyMQ(1997)11 in contrary SandipamuThabitha Rani (2017)12 showed a similar mean value as that of our study but with a statistically non-significant p-value (0.098).

The mesio –distal dimensions of the mandibular lateral incisors were higher in males than females which was statistically significant with a p value of 0.008. Our results were in accordance with that of Islam et al(2012)13 but SandipamuThabitha Rani (2017)12 showed a similar mean value with a statistically non-significant p-value (0.098).

Tooth proportions are considered for gender assessment, so Aitchison (1964)14 have given the Incisor index (Ii) which tends to be higher in males than females. With regards to our study, the incisor index in maxilla, females have a higher index value than males which was not in agreement with Aitchison (1964)14 and no supporting article was found on through literature search. The present study results can be because of lesser mesiodistal width of lateral incisors in males which is not inaccordance with SchrantzDT(1963)15 who reported that females have smaller lateral incisor than central incisor.

Our study results regarding incisor index in mandible, males had a higher incisor index value than females. Our study results were in accordance with ST Rani et al (2017)12, Cristiana Pereira (2010)16, Aitchison (1964)14.

Conclusion

Forensic odontology is important branch which will help in victim identification in a situation where it is difficult to identify. Out of various modalities in sex determination, incisor index is considered as a valuable tool. The present study shows that incisor index is high in females with regard to maxilla and higher in males with regard to mandible.

To further validate the accuracy of this forensic tool, future studies can be carried out with more study subjects, different geographic locations, population and other parameters for consideration of study samples.
References

Emotional Intelligence Training Program and its Effect on Nursing Students Problem Solving Skills

Sabah Mohamed Ragab1, Mona Mostafa Shazly2, Hemat Abdel-Azeem Mostafa3

1Master degree of Nursing Administration, 2Professor and head of Nursing Administration, 3Assistant Professor of Nursing Administration, Nursing Administration Department Faculty of Nursing Ain Shams University, Egypt.

Abstract

**Background:** Emotional intelligence is comprised of individual, emotional, and social abilities. It includes the competency of an individual to manage their relationships with others, and regulate emotions and efficiently solved their problem. **Aim:** this study aims to assess the effect of emotional intelligence training program on nursing students problem solving skills. **Design:** A pretest- posttest one group quasi-experimental study design was used. **Setting:** The study was conducted at the Technical Nursing Institute at El-Fayoum University. **Subjects:** included all the available nursing students at second year was 200 nursing students. **Tools of data collection:** Data were collected by using two tools emotional intelligence questionnaire and problem solving skills questionnaire. **Results:** 48.5% of the nursing students had high emotional intelligence before the intervention. This increased to 86.5% at the post intervention phase and the improvement continued through the follow up phases reaching to 89.5%. Half of nursing students in the study sample were high problem solving skills before the intervention. This rose to 87.5% at the post intervention phase and reached 90.5% at the follow-up phase. **Conclusion:** There were highly statistically significant correlations between emotional intelligence and problem solving skills. **Recommendations:** The Technical Institute of Nursing should adopt the emotional intelligence, educational approach in all its different grades and Enhance nursing problem solving skills through providing them with supportive and positive training environment.

**Key words:** Emotional intelligence - Nursing students- Problem solving skills

Introduction

Emotional intelligence is defined as a set of emotional and social competencies and knowledge regarding other mental skills. This concept was introduced by John Mayer, emphasizing on the ability of individuals to control their feelings and those of the others, accept the views of others, and control social relations and feedback. Emotional intelligence is the capability of individuals to explain and interpret their emotional status. In fact, emotional intelligence is a form of competence, which determines the processes through which skills could be utilized optimally13.

Goleman states that EI consists of four fundamental capabilities: “self-awareness, self-management, social awareness and social skills”. Self-awareness is understanding one’s own personal preferences, strengths and limitations. Self-management is the ability of one person to manage personal internal states and intuitions to assist in pursuing goals. On the other hand, social awareness is the ability of a person to sense and understand what other people are going through including their feelings and concerns. Social skill is the ability to encourage appropriate responses in other people15.

Emotional intelligence can assist student nurses in managing their own and their patients’ emotions,
showing genuine emotional responses, being empathetic and communicate emotions without introducing conflict. It also helps in dealing with instinctive feelings, such as anger and dissatisfaction, a nurse–patient relationship. By attempting to view the circumstance patients’ points of view and empathizing with their feelings, nursing students can manage many clinical situations. When patients are cared for by a nurse who demonstrates emotional intelligence skills, they feel that the nurse is actually concerned about their welfare and health, which is the essence of nursing and caring17.

Emotional intelligence is the ability to understand feelings and emotions, put them in a ways that make emotion and intelligent mature and healthy. Emotional intelligence is comprised of individual, emotional, and social abilities. It includes the competency of an individual to manage their relationships with others, and regulate emotions and efficiently solved their problem (Saleh, 2020).

Nursing students face unique problems which are specific to the clinical and therapeutic environment, causing a lot of stress during clinical education. This stress can affect their problem-solving skills. They need to promote their problem-solving to meet the complex needs of current health care settings and should be able to respond to changing circumstances and apply knowledge and skills in different clinical situations. Institutions should provide this important opportunity for them 3.

A problem is defined as the difference between the actual situations and desired situation. In other words, a problem is the gap between where one is and where one wants to be. A problem occurs when there is deviation from standards or routines which cause conflict and becomes a barrier to the achievement of organizational goals 20.

Problem-solving is defined as a cognitive and behavioral process that involves creating effective options, selecting and applying the most appropriate one to cope with a particular situation. Problem-solving skill means acquiring knowledge to reach a solution, and a person’s ability to use this knowledge to find a solution requires critical thinking. The promotion of these skills is considered a necessary condition for nursing students’ performance in the nursing profession3.

Problem-solving skills are essential in nursing education at undergraduate and graduate nursing schools are faced with the critical challenge of preparing students to safely and effectively perform nursing tasks in a complex, ever-changing healthcare environment. However, since these skills have not been systematically integrated in the nursing curriculum, nurses who are expected to offer the best and fastest solutions in response to patient needs and problems have been reported to lack sufficient problem-solving skills22.

To solve the problem at hand, nursing students will take steps related to the problem-solving process. The problem-solving process is explained as a complex process that requires a lot of skills in applying it. Steps that must be passed by nursing students in solving problems there are six steps are namely: 1) determines the existence of the problem, 2) identify and define the problem, 3) find solutions (generate alternative), 4) evaluate the solutions (prioritization), 5) implement the chosen solution and 6) evaluate the outcome5.

Emotional intelligence in the person health students is proven to have a big share in the ability solve the problem (Problem Solving Skill). This is because health students while studying and become a health worker later will be faced with a variety kinds of human emotions so health students must have abilities that are smart in terms of emotionality and fast and firm in solving problems. Ability in solving problems cannot be measured by only looking at cognitive abilities someone, but must be to see emotional intelligence abilities when something happens crisis or problem14.

**Significance of the Study:**

Nursing profession can be regarded as a difficult profession because of the fact that it requires making vital decisions and due to the negative feelings during this process. Nursing students need to have advanced
problem solving skills so as to be able to cope with these negative emotions. Improved decision making and problem solving performance of nursing students vary depending on their emotional intelligence. In addition to the development of nursing students professional knowledge and skills, it is suggested that attempts to increase and improve their emotional intelligence should be made.

The Aim of the Study

This study aims to assess the effect of emotional intelligence training program on nursing students problem solving skills.

Hypothesis:

Emotional intelligence training program will improve nursing students problem solving skills.

1) Research design

A pretest- posttest one group quasi-experimental study design was carried out in the study.

Subjects and Methods

Setting:

The study was conducted at Technical Nursing Institute at El- Fayoum University, where nursing students were having their training. Technical Nursing Institute at El- Fayoum University consists of two classrooms, Medical Surgical lab, Obstetric and Pediatric lab., Computer lab, and library in addition to two administration offices. The Period of study at the Technical Institute of Nursing is two years and six months internship.

Subjects of the study:

Study subjects included all 2nd year nursing students who were enrolled in the Technical Nursing Institute at El-Fayoum University at the time of study during the data collection period (academic year 2019-2020). The study sample was 200 nursing students

Tools for data collection

Data for this study was collected by using two tools, namely emotional intelligence questionnaire and problem solving skills.

Emotional intelligence Questionnaire (Appendix I): This tool consisted of three parts:

Part I: It was included data related to personal characteristics of nursing students such as; age, gender, marital status, residence, attended training about emotional intelligence and problem solving.

Part II: This part was developed by the researcher based on a literature review Motamedi & El Ghoul. It aimed to assess nursing student knowledge regarding emotional intelligence and problem solving skills. It included (2) basic dimensions contained 35 questions (15) multiple choice question, (10)Matching and (10) True and False questions covering different aspects of emotional intelligence and problem solving skills.

Part III: This tool was developed by Abo Hashem, 2008 based on Mayer &Salovey. It aimed to assess the emotional intelligence among nursing students. It was included (5) basic dimensions contained (61) items

2-Problem solving skills questionnaire

It was developed by Abuzaitoun & Banat. It aimed to assess perceived problem-solving skills among nursing students. It consists of 40 items grouped under five dimensions.

2) Operational Design:

The current study was carried out on three phases: preparation, pilot study and field work.

A- Preparatory phase

This stage lasted from May to the end of August. It extend for four months, before constructing the program. The researcher reviewed of related literatures and theoretical knowledge of various aspects of the study using books, articles, internet, periodicals and magazines on the review, the researcher prepared the
tools for data collection.

**B- Pilot Study**

A Pilot study was done on “20” nursing students selected randomly which represents approximately 10% of the main study subjects at the mid September. A pilot study was done for testing the clarity and applicability of tools and their relevance to study. It also helped to estimate the time needed to complete the data collection forms that approximately ranged from 25 – 30 minutes. Since there wasn’t any change done in the tools, the pilot study subjects were included in the main study sample.

**C- Field Work**

The fieldwork of the study was performed in the period from the beginning of October 2019, and was completed by the March 2020. The study involved four phases (assessment, planning, implementation and evaluation phases).

§ **assessment phase.**

Permission to conduct the study was obtained from the director of technical nursing institute El-Fayoum University. This questionnaire was distributed three times throughout the study pre, post program and after three months of the program implementation. In the beginning, the researcher met the nursing students to explain the purpose and the benefits of the study. This phase was conducted at the beginning of October 2019. The researcher explained to the participants the questionnaire sheets (Knowledge assessment, Emotional intelligence questionnaire and problem solving skills questionnaire). The researcher, distributed data collection tools to the respondents individually in the class, each respondent had adequate time to complete the questionnaire sheets. The time needed to complete the data collection forms that approximately ranged from 25 – 30 minutes. The researcher was present during this time to clarify any inquiries. Each participant filled the tools and back it to the researcher to check for completeness. These constituted The baseline pre-test data of the study intervention. After completing of the data collection during the Assessment pre test phase, analysis was done in order to identify all the nursing students needs

§ **Planning Phase.**

During this phase, the researcher developed the content of the training program. This was based on the pertinent literature from text books, articles, magazines, internet search and guided by the results of the assessment pre test phase. The suitable place and time for conducting the sessions determined after took the approval with the director and study subjects agreement, as well as the program schedule were prepared accordingly. This phase was conducted at the end of October 2019. The program consisted of two main parts. The first theoretical part (7) sessions covered knowledge regarding objectives and content of the program, as concept of emotional intelligence, importance of emotional intelligence, types of intelligence, characteristics of emotional intelligent personnel, components of emotional intelligence and problem solving skills knowledge as concept of problem solving, importance of problem solving skills, types of problem and characteristics of problem, process of problem solving. The second part was (8) practical sessions included small group activities to apply rules of emotional intelligence, role play to apply components of emotional intelligence and brainstorming for applying problem solving techniques.

§ **implementation phase.**

The program was implemented at Technical Nursing Institute at El- Fayoum University where nursing students were having their study.

The program was implemented throughout two months (November and December 2019). The time allowed for achieving the program was “30 hours for two groups”: (21 theoretical and 9 practical). Two days weekly, one session was offered daily, two hours for each session.

At the beginning of each session, the objective of the session was explained. Daily feedback was done about the previous session and at the end of each session. In
the first session the researcher explained the aim of the study, program objectives, plan and content.

The researcher used various teaching methods to attract students attention and motivate them to participate. The teaching methods used during the implementation of the program were lecture, brainstorming, discussion, role play, group activities, while the teaching media used were data show, white board and marker, video, poster and flip chart. The researcher distributed a handout about content of the program to all nursing students. At the end of last session; a post test was done to assess the effect of training program.

§ Evaluation phase

A post test was done at the end of the program implementation at the last week of december using the same tools used as in the pre-program assessment to evaluate the effect of emotional intelligence training program on nursing students problem solving skills.

§ Follow up phase

Was done after Three months after implementing of the program at the march 2020 using the same evaluation tools.

Ethical Considerations: Prior study conduction, an approval was obtained from the scientific research and “ethical committee of the faculty of nursing, Ain Shams University” The researcher clarifies the aim of the study to all nursing students who agree to participate in the study prior to data collection. Respondents were assured that anonymity and confidentiality would be guaranteed, and were informed about their right to refuse or withdraw from the study at any time. The study procedures do not entail any harmful effects on participants.

3) Statistical design

Data entry and statistical analysis were done by using (SPSS) Version 20 statistical software package. Data were presented using descriptive statistics in the form of frequencies and percentages for qualitative variables, and Means and standard deviation and range of quantitative variables. Student t-test (t) was used for comparisons between two-independent quantitative variables. Cronbach alpha coefficient was calculated to assess the reliability of the developed tool through its internal consistency. Spearman rank correlation was used for assessment of the interrelationships among quantitative variables and ranked once in order to identify the independent predictors of the scores of knowledge and multiple linear regression analysis was used and analysis of variance for the full regression models was done. Statistical significance was considered at p-value <0.05 and highly significant at p-value <0.001. Correlation Coefficient (r) test was used to test the closeness of association between two variables.

Results

Table (1): Socio-demographic characteristics of nursing students in the study. (n=200)

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>168</td>
<td>84</td>
</tr>
<tr>
<td>21</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td>Mean±SD</td>
<td></td>
<td>20.16±0.37</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>82</td>
<td>41</td>
</tr>
<tr>
<td>Female</td>
<td>118</td>
<td>59</td>
</tr>
</tbody>
</table>
The study sample consisted of 200 nursing students whose age ranged between 20 and 21 years as shown in Table 1. 59% of them were female. The great majority was single (89.5%). 61% live in Rural area. Majority of the participants haven’t previous training on emotional intelligence and problem solving skills (92% & 85.5%) respectively.

**Table (1): Socio-demographic characteristics of nursing students in the study. (n=200)**

<table>
<thead>
<tr>
<th>Marital status</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>179</td>
<td>89.5</td>
</tr>
<tr>
<td>Married</td>
<td>21</td>
<td>10.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Residence</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural</td>
<td>122</td>
<td>61</td>
</tr>
<tr>
<td>Urban</td>
<td>78</td>
<td>39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Attendance of training about emotional intelligence</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>No</td>
<td>184</td>
<td>92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Attendance of training about problem solving skills</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>29</td>
<td>14.5</td>
</tr>
<tr>
<td>No</td>
<td>171</td>
<td>85.5</td>
</tr>
</tbody>
</table>

Figure (1): Total knowledge of emotional intelligence among nursing students throughout the intervention phases.
Figure 1 demonstrates that only 28.5% of the nursing students had satisfactory knowledge of emotional intelligence before the intervention. This increased to 89.5% at the post intervention phase and slightly declined to 81.5% at the follow-up phase.

Figure (2): Total knowledge of problem solving skills among nursing students throughout the intervention phases.

Figure 2 demonstrates that only 30.5% of the nursing students had satisfactory knowledge of problem solving skills before the intervention. This increased to 88.5% at the post intervention phase and slightly declined to 81.5% at the follow-up phase.

Table 2: Nursing students Emotional Intelligence throughout intervention phases (n=200)

<table>
<thead>
<tr>
<th>Emotional Intelligence Dimensions</th>
<th>Time</th>
<th>X2 (P-value) Pre &amp; Post</th>
<th>X2 (P-value) Pre &amp; Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Follow Up</td>
</tr>
<tr>
<td>N  %</td>
<td>N  %</td>
<td>N  %</td>
<td>N  %</td>
</tr>
<tr>
<td>104 52</td>
<td>178 89</td>
<td>181 90.5</td>
<td>65.825 (&lt;0.001**)</td>
</tr>
<tr>
<td>95 47.5</td>
<td>167 83.5</td>
<td>174 87</td>
<td>57.351 (&lt;0.001**)</td>
</tr>
<tr>
<td>87 43.5</td>
<td>173 86.5</td>
<td>178 89</td>
<td>81.275 (&lt;0.001**)</td>
</tr>
<tr>
<td>108 54</td>
<td>175 87.5</td>
<td>182 91</td>
<td>54.230 (&lt;0.001**)</td>
</tr>
<tr>
<td>92 46</td>
<td>174 87</td>
<td>180 90</td>
<td>75.457 (&lt;0.001**)</td>
</tr>
</tbody>
</table>

Highly statistically significant p<0.001**
As regards emotional intelligence Table (4) shows that there was highly statistically significant improvement in the post and follow up phases p<0.001** as compared to the pre intervention phase. The improvement continued through the follow up phases reaching to 89.5% as a total.

Table 3: Nursing students problem solving skills throughout intervention phases (n=200)

<table>
<thead>
<tr>
<th>Problem Solving Skills dimensions</th>
<th>Time</th>
<th>X2 (P-value) Pre &amp; Post</th>
<th>X2 (P-value) Pre &amp; Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Follow Up</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>General orientation</td>
<td>97</td>
<td>48.5</td>
<td>174</td>
</tr>
<tr>
<td>Definition of Problem</td>
<td>112</td>
<td>56</td>
<td>170</td>
</tr>
<tr>
<td>Generating alternatives</td>
<td>94</td>
<td>47</td>
<td>183</td>
</tr>
<tr>
<td>Make decision</td>
<td>89</td>
<td>44.5</td>
<td>176</td>
</tr>
<tr>
<td>Evaluation</td>
<td>110</td>
<td>55</td>
<td>173</td>
</tr>
</tbody>
</table>

Highly statistically significant p<0.001**

Concerning the problem solving skills among nursing students in the study sample, Table 5 show that half of them has high problem solving skills at the pre-intervention phase. Highly statistically significant improvement at the post and follow-up phases of the intervention(p<0.001**) in all areas of skills.

Table 4: Correlation matrix between knowledge, emotional intelligence, and problem solving skills dimensions scores (n=200)

<table>
<thead>
<tr>
<th>Total knowledge</th>
<th>Total Emotional Intelligence</th>
<th>Total Problem Solving Skills</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>P-value</td>
<td>R</td>
</tr>
<tr>
<td>Total knowledge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Emotional Intelligence Questionnaire</td>
<td>0.482</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Total Problem Solving Skills</td>
<td>0.169</td>
<td>0.035*</td>
</tr>
</tbody>
</table>
Table 4 presents an correlation matrix between knowledge, emotional intelligence, and problem solving skills dimensions scores. It shows highly statistically significant correlation between Total knowledge and total emotional intelligence questionnaire\(r=0.482\). Meanwhile statistically significant strong positive correlation between total knowledge and total problem solving skills and between total emotional intelligence questionnaire and total problem solving skills.

Discussion

Emotional intelligence is a concept that fascinates academic scholars and health care professionals. Emotions affect thinking and are essential for people to make the right decisions, to best solve problems, to cope with change, and to succeed. Emotional intelligence is actually a mixture of abilities to identify emotions, integrate emotional information into problem solving processes, perceive and cope with the complexity of emotions and the regulation of emotions in oneself and one’s environment. Nursing is a stressful occupation. Therefore, nursing students need to develop the ability to control emotions and channel their moods in a beneficial way Ayala & Keren\(^7\).

According to the present study results, nursing students knowledge of emotional intelligence was deficient at the pre intervention phase. However, at post and follow-up program phase majority of nursing students had adequate knowledge. From the researcher point of view, this result may be due to most of nursing students were the first time they knowing and hearing about the emotional intelligence.

In this respect, Mousa \(^19\) in a study on Ain Shams University in Egypt who studied “The Effect of Emotional Intelligence training Program on stress among nursing students” and reported that less than one fifth of nursing students had adequate knowledge regarding emotional intelligence at preprogram. However, at post and follow-up program phase majority of nursing students had adequate knowledge.

In further confirmation of the positive effect of the current study intervention on nursing students total knowledge, the multivariate analysis identified it as the main significant positive predictor of knowledge score. This shows the independent influence of the intervention on the nursing students knowledge scores regardless other confounding factors. The positive effect of the intervention might explained by its focus on problem solving skills so that the attendance realize the importance of applied content to close the gap between theory and practice.

This study result is consistent with Ancel, et al.\(^4\) in a study in Turkey reported that most of the students had unsatisfactory knowledge level pre training program about concepts related problem solving because they had not received PS education before the training program.

The implementation of the present study intervention led to significant improvement in nursing students emotional intelligence. Such improvement could be attributed to the effect of the intervention as evidenced by multivariate analysis, which identified it as the main positive predictor of the study score. This success of the intervention is undoubtedly due to its content which was tailored to fit students’ needs and the process of intervention which entailed true hands-on training the emotional intelligence.

In agreement with the foregoing current study findings, Bikmoradi et al.\(^8\) in a study in Iran conducted a study to identify the correlation between emotional intelligence and leadership style of nursing managers. These study findings revealed that, nursing managers had high level of emotional intelligence. It also revealed that emotional intelligence of nursing managers was at good ability level.

The current study intervention has also led to a significant improvement on nursing students problem solving skills. This continued to increase reaching ninety and half percent at the follow-up phase. Such improvement
is attributed to the effect of intervention. problem solving skills help nursing students to be innovative, responsible, flexible, courageous, adventurous, different thinker, self-confident, logical, objective, comfortable, emotional, energetic, effective, creative and producer has a structure. These characteristics are important for the success of one’s life.

The finding of this study supported by Ebrahem who said that the studied participants who trained on problem-solving skills were able to mention the cause of the problem, define problem, discuss alternative solutions and make decision toward solution and evaluate the solution. This may be due to the efficacy of the program for improving the students’ ability to solve the problems and the effective role of the researchers in using real life situations and helped students express reactions to problems and develop alternative and more adaptive behaviors by using role-playing, video modeling, skill practice, and reinforcing desired behaviors.

as an evidence the present study demonstrated significant positive correlations between emotional intelligence dimensions and problem solving skills dimensions. This result may be due to the implementation of the intervention helped students to recognize own emotions and effects easily, always take responsibly of personal performance and aware of own strengths and limits, they learn how to judge self-worth and capabilities, exercise effective tactics for urging and work with others toward mutual goals and easily solve problems.

On the same line the study conducted by Arefnasab in a study in Iran found that People with high emotional intelligence significantly solve problems better than people with low emotional intelligence.

On the same line the study conducted Shahbazi who reported that training emotional intelligence and problem-solving skills can be successful in controlling the emotional reactions of individuals and can reduce adverse reactions to problem and reported that teaching emotional intelligence and problem-solving skills and can both play a significant role in reducing students’ aggression. Conversely, Ergin in a study in Turkey reported that there is a negative relationship between emotional intelligence and problem solving.

**Conclusion**

In the light of the study findings, it is concluded that, the majority of nursing students in the study setting lack knowledge of emotional intelligence and problem solving skills. Implementing emotional intelligence training program for these students is effective in improving their emotional intelligence as well as problem solving skills. A number of student characteristics as age, gender, marital status do influence their emotional intelligence and problem solving skills and the extent of their improvement after the intervention. There is statistically significant relation between emotional intelligence and age and gender of nursing students. There were highly statistically significant correlations between emotional intelligence and problem solving skills.

**Recommendations:**

- The Technical Institute of Nursing should adopt the emotional intelligence educational approach in all its different grades.
- Emotional intelligence should be applied in all nursing curricula to enhance life-long self-directed learning and improve professional performance of future nurses.
- Giving students the opportunity to express their feelings and discuss situations that may cause problems and effective communication between students and teachers.
- Enhance nursing problem solving skills through providing them with supportive and positive training environment.
- Continuing assessment of the application of the problem solving skills should be done regularly through soliciting the feedback from students.

**Source of Funding:** Self funding
Conflict of Interest: Not present any conflict.

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Noise Sensitivity (Hyperacusis) Due to Covid-19: The First Report of a New Corona Symptom

Safoura Khodaei¹, Arash Momeni Safarabadi¹, Fatemeh Mehrabi Rad¹, Pegah Shakib², Maede Nilechi³

¹Masters Student, Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran, ²Assistant Professor, Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran, ³Researcher (Ph. D), Department of Biology, School of Basic Science, Research Branch, Islamic Azad University Tehran, Iran

Abstract

Background: Coronaviruses are a large family of viruses and a subset of Coronaviridae. The present study is the first report of voice sensitivity in patients with Covid-19.

Case Report: In this case report, the patient was a 35-year-old man who referred to a private clinic specializing in infectious and febrile diseases on October 26, 2020. According to the clinical symptoms and laboratory test, the coronary test of the patient SARS-COV-2 (RdRp gene) and (E gene) SARS-COV-2 was positive. The patient suffers from severe noise sensitivity from the first day of infection until 10 days after treatment.

Conclusions: The patient was so sensitive to sound that different sounds had painful and excruciating effects on the patient’s mind and psyche. Therefore, sensitivity to sound can be one of the symptoms of Covid-19.

Keywords: COVID-19, Noise Sensitivity, Case Reports, Clinical Symptoms

Introduction

Coronaviruses are a large family of viruses and a subset of Coronaviridae that range from the common cold virus to the cause of more severe illnesses such as SARS and Mercovid-19 [1]. In December 2019, in the Chinese city of Wuhan, people contracted pneumonia for no apparent reason, a new type of coronavirus was identified that could spread to humans. SARS-Cov-2 (Covid-19) is a new virus in the coronavirus category. The virus is an emerging natural and biological hazard that originated in Wuhan, China, and now pervades the entire planet [2]. Covid-19 disease is a highly contagious infectious disease caused by the new coronavirus and can be transmitted from person to person through close contact [3]. There is currently no drug or vaccine for this disease [4]. The most important clinical symptoms at the onset of patients with Covid-19 include fever, cough, sputum, headache, vomiting, diarrhea, fatigue, rhinorrhea and chest pain, the most important of which are fever and cough [2-4].

Case Report

The patient is a 35-year-old man who referred to a private clinic specializing in infectious and febrile diseases on September 26, 2020. The patient’s problem is with symptoms of fever, severe headache, cough, voice change, body aches, nausea, lethargy, sweating, difficulty breathing and sensitivity to sound. The patient’s body pain was so severe that he had severe insomnia for 10 days and nights, so much so that he slept...
for a maximum of about 1 hour a day. The patient has an underlying allergy (respiratory) disease and cough with a history of 16 years of allergies. Clinically, the patient had fever, cough, lethargy and difficulty breathing, and the patient’s blood oxygen was 93. Finally, according to the clinical symptoms and laboratory test, the patient’s coronary test SARS-COV-2 (RdRp gene) and SARS-COV-2(E gene) were positive. The patient suffers from severe noise sensitivity from the first day of infection until 10 days after treatment.

The patient was severely sensitive to sound. The sound of dripping water, the sound of television, etc. had affected the patient’s nervous system and had painful and excruciating effects on the patient’s mind and psyche.

**Discussion**

Although the virus is known as acute respiratory syndrome, it has recently been confirmed that in addition to systemic respiratory symptoms, it also causes symptoms in other parts of the body. For example, the results of a study showed that 36.4% of patients with Covid-19 show neurological symptoms such as headache, paresthesia and confusion in consciousness [5]. The results of autopsy studies in patients with coronavirus-induced death (Covid-19) indicate inflammation of brain tissue and partial nerve degeneration [6]. There has also been a case report of viral encephalitis due to Covid-19 [7]. In Covid-19 disease, fear of illness and fear of death, along with disruption of daily activities, cause anxiety [8]. Other symptoms that may occur due to nervous system involvement due to Covid-19 include a lack of sense of smell and taste [9]. It can probably be said that this virus, like pathogenic bacteria, destroys the blood-brain barrier and causes a secondary infection inside the skull, which are the symptoms of these injuries [10]. Finally, it can be concluded that sensitivity to sound is due to nervous system involvement as well as insomnia caused by Covid-19 pain. There have been many reports of epidemics and symptoms of Covid-19 disease. This speech was written in November 2016 and about 9 months after the onset of Covid-19 disease, the above report (severe sensitivity to sound) is reported for the first time.

With the high rate at which this disease is occurring and new symptoms are being reported every day, there is no doubt that our scientific knowledge contributes very little to our ignorance or ill-knowledge about this disease. However, there is no doubt that for the decisive fight against the epidemic of the present century (Covid-19), the publication and dissemination of all scientific knowledge is crucial.

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**Acknowledgments:** We kindly acknowledge from deputy for research and technology of Lorestan University of Medical Sciences, Khorramabad, Iran for technical support.

**Informed Consent:** Written informed consent was obtained from patient who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – SKH; Design – MN; Supervision – AM, FM; Data Collection and/or Processing – MN, PSH; Analysis and/or Interpretation – All authors; Literature Search – MN, PSH; Writing – All authors; Critical Reviews – MN.

**Conflict of Interest:** The authors have no conflict of interest to declare.

**Financial Disclosure:** The authors declared that this study has received no financial support.

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Presentation and Sources of Pediatric Odontogenic Infection

Sahar Abdulkader Ismaeel¹, Saif Saadedeen Abdulrazaq², Reem Fadhil Abbas³

¹Lecturer, ²Assistant Professor, Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Baghdad, Iraq, ³Specialist, Department of Periodontics, Al-Shaikh Omar Specialist Dental Center, Baghdad, Iraq

Abstract

Background and Objectives: The causes, clinical presentation and management of the odontogenic infection in children during the mixed and premixed dentition period should be thoroughly studied as it differs from that of adults. The study aimed to identify the common sources and presentation of dental infection in those children.

Methods: The selected cases were 122 (54 females and 68 males). All cases presented with odontogenic infection, examined meticulously to diagnose the source of infection.

Results: The mean age was 6.5 years old (The age range was 3-10 years). The most common source of infection in primary teeth was the second primary molars (38 cases; 31.1%). The most common source of infection in permanent teeth was the first permanent lower molars (30 cases; 24.6%). The most common presentation was vestibular swelling (56 cases; 45.9%).

Conclusion: Dental care should be provided early especially to the permanent first molars in children to avoid losing them. Teamwork between the oral surgeon and the general pediatrician is necessary for better management.

Keywords: Odontogenic infection; dental abscess; surgical drainage; fascial spaces.

Introduction

Dental caries is a chronic disease affecting especially children, with more aggressive behavior than in adults, causing tooth decay. The modern lifestyle fill of high-sugar content sweeties with the unwillingness of the children to brush their teeth makes tooth decay more destructive. Lack of parents monitoring of the dental health of their children makes the tooth decay having a poor prognosis.¹, ²

Untreated dental caries may end with an odontogenic infection presented clinically as an oral and/or facial swelling along with the tenderness of the accused tooth on eating, pus discharge from oral sinus or orocutaneous fistula. An oral and maxillofacial surgeon must manage the infection by providing antibiotic coverage either orally or intravenously in the hospital if the acute status of infection present, the surgical part include pus drainage by incision, tooth extraction or through the root canal. Despite appropriate antibiotic coverage, severe odontogenic infection or its complications such as Ludwig angina may occur.³, ⁴

The most common causative source of odontogenic infection is dental caries; other causes include periodontal abscess, pericoronitis, unsuccessful endodontic treatment or deep-seated tooth fillings. All these sources end with bacterial invasion of the root canal to form thereafter a periapical lesion that spread to the adjacent fascial spaces.⁵, ⁶
The aim of the study was to identify the sources and the clinical presentation of odontogenic infection in children during the age of 3-10 years old.

**Materials and Methods**

The study involved 122 patients received at the department of oral and maxillofacial surgery with the initial presentation of intra- or extra-oral swelling or draining sinuses; the diagnosis of all cases was an odontogenic infection. There were 54 females (44.3%) and 68 males (55.7%).

All the cases pass through the routine examination protocol; history, clinical and radiological examinations to locate the exact causative tooth and to define the appropriate treatment plan.

**Results**

The age range of the cases was (3-10 years); the mean age was 6.5 years old. The majority of cases (117 cases; 95.9%) were treated as outpatient, while five cases (4.1%) needed admission to the hospital for better management. The primary teeth were the source of the infection in 74 cases (60.67%), while the permanent teeth were the source of infection in 48 cases (39.3%).

The majority of cases (92 cases; 75.4%) were managed by tooth extraction, while the other cases (30 cases; 24.6%) were managed by root canal therapy.

The total number of the primary teeth involved was 74 (60.7%), while the permanent teeth were 48 (39.3%). The most common cause of infection was caries (109 cases; 89.3%) followed by traumatic pulp necrosis to upper incisors (13 cases; 10.7%).

As shown in table 1, the most common source of infection in primary teeth was the second primary molars (38 cases; 31.1%). The most common source of infection in permanent teeth during mixed dentition was the first permanent lower molars (30 cases; 24.6%).

The most common chief complaint was swelling (110 cases; 90.2%) (Table 2). During clinical examination, the most common presentation was vestibular swelling (56 cases; 45.9%) (Table 3) (Fig. 1).

![Figure 1: Vestibular swelling associated with the primary lower second molar](image-url)
**Figure 2: Orocutaneous fistula associated with the primary upper second molar**

**Table 1: The sources of infection.**

<table>
<thead>
<tr>
<th>Involved tooth</th>
<th>No.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st primary molar</td>
<td>20</td>
<td>16.4%</td>
</tr>
<tr>
<td>2nd primary molar</td>
<td>38</td>
<td>31.1%</td>
</tr>
<tr>
<td>Upper primary incisors</td>
<td>15</td>
<td>12.3%</td>
</tr>
<tr>
<td>Lower primary canine</td>
<td>1</td>
<td>0.82%</td>
</tr>
<tr>
<td>Upper permanent central incisors</td>
<td>13</td>
<td>10.7%</td>
</tr>
<tr>
<td>1st permanent upper molar</td>
<td>5</td>
<td>4.1%</td>
</tr>
<tr>
<td>1st permanent lower molar</td>
<td>30</td>
<td>24.6%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>122</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: The most common complaint of the patients**

<table>
<thead>
<tr>
<th>Chief Complain</th>
<th>No.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>10</td>
<td>8.2%</td>
</tr>
<tr>
<td>Swelling</td>
<td>110</td>
<td>90.2%</td>
</tr>
<tr>
<td>Limited Mouth Opening</td>
<td>1</td>
<td>0.8%</td>
</tr>
<tr>
<td>Extra-oral Fistula</td>
<td>1</td>
<td>0.8%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>122</strong></td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Types of lesions at presentation

<table>
<thead>
<tr>
<th>Type of Lesion</th>
<th>No.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra Oral Sinus</td>
<td>27</td>
<td>22.1%</td>
</tr>
<tr>
<td>Vestibular Swelling</td>
<td>56</td>
<td>45.9%</td>
</tr>
<tr>
<td>Facial Swelling</td>
<td>38</td>
<td>31.1%</td>
</tr>
<tr>
<td>Extra-oral Fistula</td>
<td>1</td>
<td>0.82%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>122</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

The first line of antibiotics for odontogenic infection is amoxicillin or clindamycin, erythromycin is less effective especially with severe odontogenic infection\(^7\). Good choice of antibiotics hasten the healing, shorten the hospital stay and delay dental or surgical procedures\(^5\). The study protocol was to use amoxicillin/clavulanic acid, if the child sensitive to penicillin, clindamycin is the drug of choice as a substitute. Antibiotic coverage is not enough if surgery does not treat the accused tooth or drain the pus.\(^8\)

If the treatment plan involves tooth extraction, the accused tooth (the source of infection) extracted as soon as possible after the acute phase of infection disappeared for better resolution of the infection and to decrease the hospital stay\(^5\). Management of the dental abscess or odontogenic infection is similar in both primary as well as permanent teeth except in the way of treatment of the accused tooth; we try to save the permanent tooth by root canal therapy rather than a tooth extraction.

Diagnosis of the mild to moderate orofacial infection is straightforward by history, clinical as well as radiological examinations (the study used orthopantomograph screening imaging technique), whereas in severe type of infection computed tomography with contrast is the ideal technique to focus on the most dependent area of the pus for easy surgical access.\(^9\)

Whether to treat the patient as an outpatient or plan for admission to the hospital, authors put criteria to select admission, include severe cellulitis, shortness of breath, difficulty in swallowing, involvement of deep spaces, high fever, severe limitation of mouth opening, uncooperative patients and immunodeficiency\(^{10, 11}\). We admitted five cases to the hospital, as they were uncooperative and with limited mouth opening, one case with multiple space involvement. Infection with multiple space involvement increase the severity of the infection and in most cases warrant the admission to the hospital\(^{12}\). Only one case (0.8%) of multiple space infection in our study was observed.

The family motivation to seek dental care was the appearance of swelling intra- or extraoral which indicates delay in providing proper early dental care. Differential diagnosis of orocutaneous fistula (Fig. 2) include many skin lesions, this is one of the main causes of treatment delay as the parents and the general pediatricians deal with it as a skin lesion\(^{13}\). To avoid misdiagnosis and mismanagement, teamwork between parents, general pediatricians and oral surgeons should be made whenever extraoral facial draining fistula present.

**Conclusion**

The study concluded the following: dental care should be provided to the primary and permanent carious lesion in children as early as possible to prevent
further spread of infection especially to the permanent first molars. Teamwork between the oral surgeon, the parents and the general pediatrician is necessary for better management of the facial draining fistula. It is better to do tooth extraction for the badly carious primary tooth rather than lengthy conservative treatment if the tooth is associated with a dental abscess, while the opposite is correct for the permanent teeth; it is better to do conservative treatment rather than extraction.

**Declarations**

Conflict of Interest the authors declare that there are no potential conflicts of interest related to the study.

**Source of Funding:** Nil

**Ethical Clearance:** This research has exemption as it a routine treatment (no new materials were used).

**References**


Improvement of Self-Efficacy of Mothers Inpostpartum Period Through Home Visit by Health Workers in Aceh

Said Usman¹, DewiMarianthi², Irwan Saputra¹, T.M. Rafsanjani³
¹Assistant Professor, Universityof Syiah Kuala, Magister Program of Public Health, Medical Faculty, Banda Aceh, Aceh, Indonesia,²Associate Professor, Department of Nursing, Health Polytechnic Faculty of Health Minister, Aceh Besar, Aceh, Indonesia, ³Instructor, University of SerambiMekkah, Public Health Faculty, Banda Aceh, Aceh, Indonesia

Abstract

World Health Organization recorded that about 44% of infant deaths in 2012 happened in the first 28 days after birth, and 78.5% of deaths happen in the first week after births. It was estimated that 80% of infant deaths happen at home (with little or no contact with health workers). Good quality care service for infants especially newborns at home is a serious challenge, thus an effort that could be performed is to optimize the role of health workers in the community by doing home visit. The goal of this research is to identify the self-efficacy of postpartum mothers before home visit and after home visit, to compare the self-efficacy of intervention group and control group on newborn care by means of health workers role during home visit. This research use quantitative research with quasi-experimental non-equivalent control group design method. The sample of the research was health workers; each with responsibility of one fostered family of a mother in postpartum period. The research site for the intervention group was the working area of UleeKareng Health Center, and for the control group was the working area of Baiturrahman Health Center. The result of the research revealed that there was a change in self-efficacy of postpartum mothers on newborn care after three home visits by the health workers. The difference of the average efficacy score was 12.5, with p value of 0.0001. When the efficacy of postpartum mothers from the intervention group (who received home visits) was compared to that of mothers from the control group, there was a significant difference found, with p value of 0.003. Home visit executed by the health workers can improve self-efficacy of the postpartum mothers on newborn care. This can be seen from the difference between self-efficacy of the postpartum mothers before and after home visit by the health workers.

Keywords: Health workers, home visit, newborn care, self-efficacy.

Introduction

World Health Organization (¹) recorded that about 44% of infant deaths in 2012 happened in the first 28 days after birth. The deaths of newborns, especially of age one day to two months, are relatively higher than older infants, both with or without complication, and 78.5% of deaths happen in the first week afterbirths (²).

Health profile of Aceh in year 2015 (³) documented that the NMR (Neonatal Mortality Rate) was 11 per 1.000 live births, IMR (Infant Mortality Rate) was 15 per 1.000 live births, and UFMR (Under-Five Mortality Rate) was 16 per 1.000 live births.

In National average, data obtained from Indonesia ministry of health 2017 revealed that IMR (Infant Mortality Rate) have started to decline from 2015 to the first semester of 2017. IMR decreased from 33.278 cases in 2015 to 32.005 in 2016. Meanwhile, in first semester of 2017 there were 10.295 cases of infant mortality. According to WHO, in year 2019, Indonesia recorded in the top 10 countries with the highest cases of neonatal mortality (NMR) with 60.000 cases.

Moreover, UFMR was recorded decrease in 2017 with 32 cases out of 1000 lives compared to UFMR in 2012 in which 36 cases out of 1.000. Meanwhile, the
MMR (Maternal Mortality Ratio) was 149 per 100,000 live births. While the coverage of neonatal visit (NV) in Aceh in 2015 the NV-1 obtained was 83.67%, with the complete NV coverage of 80.53%. This data shows a number slightly below the Indonesian standard, which is 83.74%. However, the coverage of neonatal complications treatment in Aceh is still very low, at 50.51%.

The profile of Banda Aceh City Health Office (2016)(4) stated that the cause of infant deaths in the city of Banda Aceh was asphyxia and low birthweight. There are also other causes that are not easily identifiable. Various factors allegedly contributed to this include the lack of skilled health workers and lack of appropriate equipment in the health facilities, the lack accessibility and the willingness of the community to change their traditional unhealthy life patterns to healthier lifestyles.

According to WHO and UNICEF (2), 80% of infant deaths happen at home (with little or no contact with health workers). Good quality care service for infants especially newborns at home is a serious challenge. An effort that can be made to optimize the role of health workers in a community is by visiting houses (homevisit) to give education, provide counseling and perform early detection of newborns at risk (5).

Home visit executed by the health workers is expected to be a solution to the problems stated above. In Aceh, there is a culture for the mothers named ‘madeung’, in which the mothers are prohibited to step out of home 44 days after the birth delivery. In mean time, health workers are expected to visit mothers at homes as a way to improve the healthcare access for the mothers and their babies. The purpose of home visit is to maintain the health status of and prevent disease among the infants through growth monitoring, breastfeeding monitoring and immunization. One of the indicators of a healthy infant is that the growth is in synchronization with the age. Additionally, home visit by the health workers also can improve mothers’ knowledge of postpartum, these visits are also to ensure that the postpartum mothers are capable of taking care of themselves and their babies (6).

The capability of postpartum mothers in taking care of themselves and their babies cannot be separated from the efficacy of the mothers themselves. Self-efficacy is the postpartum mothers’ belief of their own capability to perform the tasks or actions needed to achieve certain outcomes. Bandura (7) explained that self-efficacy is the result of cognitive process in the form of decisions, beliefs, or rewards on how far an individual estimate his or her own ability in performing a certain required task or action to achieve the desirable outcome.

People with high self-efficacy believe that they are able to do something in order to change their situations, while people with low self-efficacy regard themselves as incapable of doing anything (8). This is proven in a study by Rahayuningsih (9) on the relation between preparation training for postpartum period with self-efficacy of the postpartum mothers in the Regency of Sragen, Central Java. The result of the study revealed that the preparation training for postpartum period effectively improved the self-efficacy of postpartum mothers.

As stated above, self-efficacy of postpartum mothers linked with mothers’ abilities in taking care of their babies and themselves. One of the factors that can increase the postpartum mothers’ self-efficacy is by home visiting. However, the quantity of health workers is limited to cover all of the home visiting, hence, social workers are prepared to cover this shortage. This research was obtained to identify self-efficacy of postpartum mothers can be increased by social workers home visit.

**Method**

This is a quantitative research with quasi-experimental non-equivalent control group design (10) method. The sample of the research was 48 health workers in the working area of Ulee Kareng Health Centre, Banda Aceh. A self-administered questionnaire was used in this study to measure postpartum mothers’ Self-efficacy level. The measurement was consist of 22 items, adapted from Bandura’s General Self-Efficacy Scale and has been tested for reliability and validity. Further, the design of the research can be depicted as follows:
Pretest Home-visit posttest

Intervention Group $O_1 \longrightarrow X_{1,2,3} \longrightarrow O_2$ Control Group $O_3 \longrightarrow O_4$

from the scheme above, self-efficacy questionnaire was given two times (in the beginning and ending of the experiment) to the both intervention and control group of postpartum mothers. However, in the intervention group, three times of home visit was done by social workers before the second test of self-efficacy was administered.

This research assessed any change in efficacy of the postpartum mothers on newborn care before and after three home visits by the health workers. The health workers, accompanied by local midwives, visited the homes of the postpartum mothers in their own villages. The schedule of the visits was adjusted to the schedule of neonatal visits, where NV-1 is the first visit executed on the 3rd to the 7th day after childbirth. NV-2 is the second visit on the 8th to the 28th day. NV-3 is the last neonatal executed on the 29th day to the 44th day after childbirth (11).

On the home visits, the health workers accompanied by the midwives held a discussion on condition during postpartum period by using the mother and baby’s health book (pink book) (12). Meanwhile, for the control group, the postpartum mothers studied the materials in the mother and baby’s health book given by the health centre during their pregnancy on their own.

The research for the inter vention group was conducted for post partum mothers in the area of Ulee Kareng Health Centre, while for the control group was in the area of Baiturrahman Health Centre. The sampling was taken using samplesize meanin dependent (13) technique. 44 samples were required from the calculation. To anticipate drop out, 10% was added which resulted in 48 samples.

Results

In this research, there was a change in the number of the research subjects, due to dropouts in every stage of the execution. On the first visit, according to the sample calculation, the supposed number of the research subjects was 48 respondents, but only 45 respondents acquired fostered family. Meanwhile, out of 48 respondents for the control group, only 46 respondents agreed to join their research.

Furthermore, to learn the characteristics of the research subjects, the result of the demographic data of the research subjects is displayed in the table below.

Table 1: Characteristics of the Research Samples, Health Workers of Intervention group and Control group in Ulee Kareng and Baiturrahman Health Center

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sub Variable</th>
<th>N</th>
<th>%</th>
<th>Mean + SD Min-Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health workers Group</td>
<td>Intervention</td>
<td>45</td>
<td>49,5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>46</td>
<td>50,5</td>
<td></td>
</tr>
<tr>
<td>Health workers education</td>
<td>Low</td>
<td>11</td>
<td>12,1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>65</td>
<td>71,4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>15</td>
<td>16,5</td>
<td></td>
</tr>
<tr>
<td>Health workers Employment</td>
<td>Not working</td>
<td>79</td>
<td>86,8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Working</td>
<td>12</td>
<td>13,2</td>
<td></td>
</tr>
<tr>
<td>Health workers age</td>
<td></td>
<td></td>
<td></td>
<td>40,1 ± 8,5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23-63</td>
</tr>
<tr>
<td>Length of time being health workers</td>
<td></td>
<td></td>
<td></td>
<td>7,7 ± 8,2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-40</td>
</tr>
</tbody>
</table>
In the table 1 above, the number of health workers for the intervention group was 45 respondents and 46 respondents for the control group. The education level of most of the respondents was middle school, which was up to 71.4%. As for the employment status, most of the respondents were not working, as 86.8% of them were housewives. The average age of the respondents was 40.1 years, with the youngest being 23 years old, and the oldest being 63 years old. The average length of time of being health workers were 7.7 years, with the shortest time of 1 year and the longest time of 40 years.

Next, the characteristics of the postpartum mothers as the research subjects in intervention group and control group during home visits by the health workers is shown in the table 2 below.

Table 2: Characteristics of the Research Samples, Postpartum Mothers of Intervention Group and Control Group in Ulee Kareng Health Center and Baiturrahman Health Center Banda Aceh

<table>
<thead>
<tr>
<th>Variable</th>
<th>NV-1 Intervention</th>
<th>NV-1 Control</th>
<th>NV-3 Intervention</th>
<th>NV-3 Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>7</td>
<td>58.3%</td>
<td>5</td>
<td>41.7%</td>
</tr>
<tr>
<td>Middle</td>
<td>18</td>
<td>54.5%</td>
<td>15</td>
<td>45.4%</td>
</tr>
<tr>
<td>High</td>
<td>17</td>
<td>53.1%</td>
<td>15</td>
<td>46.9%</td>
</tr>
<tr>
<td>Employment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not working</td>
<td>34</td>
<td>51.5%</td>
<td>32</td>
<td>48.5%</td>
</tr>
<tr>
<td>Working</td>
<td>8</td>
<td>72.7%</td>
<td>3</td>
<td>27.3%</td>
</tr>
</tbody>
</table>

From the characteristics description it was revealed that before the home visit, the number of the postpartum mothers who were present on NV-1 from the intervention group were 42, while from the control group were 35 mothers. Most of their education level was middle to high level; with most of them not working, only staying at home as housewives. After home visit, the number of the postpartum mothers who were present on NV-3 from the intervention group were still 42 mothers, while from control group were 44 postpartum mothers. Their education level was middle to high level; with most of them not working.

To discover the change in self-efficacy of the mothers on newborn care during postpartum period, before and after three home visits by the health workers (NV-1, NV-2, and NV-3), paired t test (14) was conducted. Paired t test is a paired test on the same subject using the same measuring instruments.

In this research, the researcher only conducted test on the intervention group because no home visit was made by the health workers for the control group. The postpartum mothers from the control group were given the mother and baby’s health book (pink color) by health workers after their baby delivery. The researcher divided the control group following the same characteristics of
the intervention group, where the pretest group was postpartum mothers of day 3 to day 7 after childbirth (NV-1) and posttest group was postpartum mothers of day 29 to day 44 after childbirth (NV-)

The result of the home visits executed by the health workers was self-efficacy of the postpartum mothers on newborn care. Pretest measurement was conducted before NV-1, and posttest was conducted after NV-3. The test result of the influence of the home visit for the postpartum mothers is displayed in table 3 below:

![Table 3: The Score of Self-Efficacy of the Postpartum Mothers of Intervention Group Before and After Home Visit in UleeKareng Health Center Banda Aceh (n = 42)](image)

Based on the table 3 above, it was revealed that the self-efficacy of mothers on newborn care during postpartum period before and after the home visit has the p value of 0.000. Therefore, it can be concluded that there was a change in the mothers’ self-efficacy on newborn care after three home visits by the health workers.

Then, to find out the difference between the intervention group and the control group, independent t test was conducted. The data of self-efficacy of the mothers on newborn care for the independent t test from the intervention group was only taken after home visit. This is because the data of the mothers in the control group was only taken once, with the division of categories of postpartum mothers who were in NV-1 and NV-3.

The result of the independent t test of self-efficacy of the postpartum mothers on NV-1 and NV-3 is displayed in the table below:

![Table 4: The Score of Self-Efficacy of the Postpartum Mothers from Intervention Group and Control Group](image)
Analysis of the independent t test of self-efficacy of postpartum mothers from the intervention and the control group revealed that there was a significant change with the p value of 0.000 in NV-1 and 0.003 in NV-3. Hence, it can be concluded that there was score difference in self-efficacy of postpartum mothers from the intervention group and the control group on newborn care.

Discussion

From the test result, it was discovered that there was a change in attitude of the postpartum mothers on newborn care after three home visits executed by the health workers. The difference of the average score of the mothers’ self-efficacy was 12.5 with p value of 0.0001. Based on this result, it can be concluded that there was a change in self-efficacy of the postpartum mothers before and after home visit by the health workers.

When the scores of self-efficacies of the mothers from both groups were compared, a significant difference was found between mothers from the intervention group who received home visit and mothers from the control group, with p value of 0.003.

Self-efficacy is one’s self perception of whether or not he can function well in certain situation. Efficacy is also correlated with the self-belief that one has the ability to perform the expected actions (15). Parental efficacy is the belief of a mother of being capable of doing something according to her ability in certain conditions, as well as the ability of parents to plan and carry out specific actions that can produce results (16).

Self-efficacy of postpartum mothers on newborn care is a crucial aspect in facilitating a mother’s adaptation and experience. One of a mother’s characteristics that influences self-efficacy is maternal confidence (17). Self-efficacy is also obtained from self-confidence by influence of other people. Health workers as a part of the community and have experiences on childbirth and infant care are considered to have experienced similar condition that the postpartum mothers are experiencing. Thus, this condition can improve someone’s self-efficacy (18).

Home visit executed by health workers can improve self-efficacy of the postpartum mothers. Moreover, in correspondence with previous study by Salonen (19), it was discovered that there was a significant improvement of parenting self-efficacy of postpartum mothers who joined parenting program compared to mothers who did self- treatment at home. A visit by people who have capacity in health subject and to be given direct lesson on health care is one way to improve self-efficacy and strengthen the belief of the community that they have capacity to succeed Systematic literature conducted by Warren et al (20) on self-efficacy of mothers who have just given birth during postpartum period. The result of the study was that self-efficacy of a mother who has just given birth is the mother’s satisfaction in taking care of the baby. A good self-efficacy of a postpartum mother can also be associated as a mean to minimize depression of a postpartum mother.

The result of this research can be implied in Puskesmas Aceh/Health care system by regulating a new policy towards home visit to the postpartum mothers in each area covered by each Puskesmas, in which social workers are prepared as the home visitors for helping, monitoring, and educating postpartum mothers in the health care area.

Conclusion

Generally, it can be concluded that there is a correlation between home-visit executed by health workers with self-efficacy of postpartum mothers on newborn care. Specifically, the result of the research showed improvement of self-efficacy of the postpartum mothers after home visits by the health workers in the area of UleeKareng Health Center, Banda Aceh. There was a difference between self-efficacy improvement of mothers in the intervention group and of ones in the control group.

Suggestion

The health workers in the UleeKareng Health Centre Banda Aceh are expected to conduct home visit
as a way to improve self-efficacy of postpartum mothers in the community. The health workers could also build partnership with local health workers through referral program during home visit in the community.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Ethical Approval**

Ethical clearance for publishing the results of this study has been given by the Department of Ethic, Medical Faculty at Syiah Kuala University where the writer-researchers work.

**Conflicts of Interest:** The authors have all declared that no conflicts of interest exist.

**Acknowledgement:** The Head of Ulee Kareng Health Center and the Head of Baiturrahman Health Center who had permitted the researcher to utilize the working area of the health centers as the research sites. Also, to all the health workers and the postpartum mothers who agreed to participate in this research.

The authors thank the Aceh Health Polytechnic for funds provided to do this research amounting to Rp.30,900,000{about US$ 2,060}.

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Prosthetic Status and Needs among Head and Neck Cancer Irradiated Patients Suffering from Xerostomia in Delhi, India

Sakshi Gupta¹, Pankaj Dhawan², Piyush Tandan³, Meena Jain⁴

¹Post graduate Student, ²Professor & Head, ³Professor, ⁴Associate Professor & Head, Department of Public Health Dentistry & Research & Innovation Catalyst, Manav Rachna Dental College, FDS, MRIIRS, Faridabad, Haryana, India

Abstract

Aim: To assess the prosthetic status and prosthetic needs of Head and Neck Cancer Irradiated Patients suffering from Xerostomia in Delhi India.

Materials and Method: A multi-centric, cross-sectional observational study was conducted among 120 head and neck cancer irradiated patients suffering from xerostomia in Delhi. The information related to socio-demographic data, the prosthetic status, and prosthetic need was obtained using a proforma based on WHO oral health assessment form 1997.

Results: Out of 120 participants, 103 were males and 17 were females. The mean age of the study participants was 47.68 ± 10.26 years. Stage 1 carcinoma was diagnosed in 69 (57.5%) of the participants while stage 2 carcinoma was diagnosed in 51 (42.5%). Out of 120 individuals, 7 (5.8%) needed single unit prosthesis, 33 (27.5%) needed multi-unit prosthesis, 48 (40%) needed a combination of a single and multi-unit prosthesis, and 18 (15%) needed a full prosthesis.

Conclusion: The need for prosthesis was high among Head and Neck Cancer Irradiated Patients. There is a need to emphasize dental service utilization among this group of people.

Keywords: Head and neck cancer, prosthetic status and needs, radiotherapy, xerostomia

Introduction

Each year, approximately 500,000 new cases of head and neck cancer (HNC) are diagnosed worldwide.¹ Oral cancers are the most common cancers among head and neck cancers that determine and modify the prosthetic needs of the patient.² Head and neck cancers are the eighth most prevalent form of cancer worldwide, usually destructive, and cause extensive tissue damage related to both hard and soft tissues.² Treatment modalities include surgery, radiation, and combined surgery and radiation.³,⁴

RT plays a pivotal role in the management of patients with HNC, but it is also associated with several side effects. Commonly, the salivary glands, oral mucosa, and jaws will inevitably be included in the RT field. Changes induced by exposure to radiation occur during and after completion of therapy, which include xerostomia, mucositis, candidiasis, osteoradionecrosis, and radiation caries.⁵,⁶

Xerostomia or dry mouth is a major side effect of radiotherapy. It is also the most common and permanent...
side effect. Radiation-Induced Xerostomia often occurs as a late effect, however, acute xerostomia after radiotherapy is also known.7,8 Xerostomia in head and neck cancer patients is associated with a reduced quality of life.9 This may be due to various complications which include difficulty in swallowing, eating and problems in speaking as well as tooth decay. These patients invariably need dental prosthesis for restoring the lost form and function and thereby enhance their life quality. Further, xerostomia also modifies the ease and compliance for wearing denture prosthesis. However, there is limited data available in the scientific literature on the prosthetic status and needs concerning to cancers of head and neck irradiated patients in India. Hence, this research was done to assess prosthetic status and needs in Head and neck irradiated patients suffering from xerostomia in Delhi.

Materials and Method

This cross-sectional observational study was carried out in the head and neck cancer irradiated patients suffering from xerostomia to understand the prosthetic status and prosthetic needs of such patients. This multi-centric study was conducted among three tertiary care cancer hospitals in Delhi, India. Ethical committee approval and protocol approval was obtained from the Institutional Ethics Committee (IEC) of Manav Rachna Dental College, Faridabad, vide letter number MRDC/IEC/2018/11.

Patients with the following characteristics were included in the study:

1) Head and neck cancer irradiated patients after 3 months of completion of radiotherapy.

2) Patients who provide informed consent for participation in the study.

3) Patient whose normal whole salivary flow rate < 0.7 ml/min.

Patients who had either learning, audio-visual, psychiatric/ intellectual disability or disorder were excluded from the study. A list of cancer hospitals in Delhi was made to choose the hospitals for study. Out of a total of 10 hospitals, 3 hospitals were chosen using the lottery method. After hospital selection, the permission for conducting the study was taken from these hospital authorities. The research was conducted for 12 months from April 2019 to February 2020 at (Rajiv Gandhi Cancer Hospital), (Balaji Action Cancer Institute) and (Max Hospital, Shalimar Bagh) in Delhi, India.

Sample size:

Sample size calculation was done using a formula for cross-sectional epidemiological studies.

\[ N = \frac{4 \times P \times (1-P)}{d^2} \]

\( N = \) Sample size

\( P = \) Prevalence of Prosthetic need among the head and neck cancer patients.

\( d= 10 \) (Relative Precision of 20%)

Level of Significance- 5%

An earlier study by Jham et al (2008), on the oral health status of 207 head and neck cancer patients before, during, and after radiotherapy found the prevalence of prosthetic needs among these patients as 50.2%.10 This prevalence rate was used to calculate the sample size for the present study. A non-response rate of 20% was considered and was added to the minimum sample size. A sample size of 120 patients was established for the present study.

Participants who were to be included in the research were briefed about the research by the researcher (SG) and provided with a patient information sheet containing the details of the study, confidentiality, benefits, and risks. The patients were encouraged to clarify any doubts about the research. Those who decided to participate in the study were provided with an informed consent document and asked to sign it in front of an independent witness to ensure that the informed consent process was free and non-coercive. The informed consent document and patient information sheet were made available both in Hindi as well as in English. For the patients who could
not read and write, a person who was independent of the study was made available to read out the patient information sheet and informed consent document. For such patients, a thumbprint was used instead of a signature.

Based on convenience non-probability sampling, all the eligible patients present on the day of the study and gave consent were included. A full medical history of the patient was taken at the outset. The details about the oral cancer staging and grading as well as the diagnosis were obtained from the patient records. The treatment provided including chemotherapy and radiotherapy was noted. The number of cycles of radiotherapy undergone till the date of examination was also noted from the patient records.

For assessment of xerostomia, the patients were instructed to brush their teeth using a soft toothbrush and toothpaste one hour before saliva collection. They were asked to refrain from eating after brushing their teeth. The collection was done in the morning time usually a few hours after breakfast time. Not more than 10-12 patients were examined in a day to avoid examiner fatigue.

The Swab method was used to measure xerostomia. Three pre-weighed cotton swabs were placed into the mouth of the patient. The swabs were placed at three locations on the floor of the mouth to facilitate maximum possible saliva absorption. The patient was asked not to swallow saliva for 5 minutes. The cotton swab was then retrieved and collected in a corked test tube and weighed. A digital scale (Kerro P5B,BL 3000) was used to weigh the cotton swab before and after the procedure. The difference of weight between dry and saliva-soaked pellet was recorded. The rate of salivary flow was expressed in the units of ml per minute. A weight of one gram was considered to be equal to one ml.

The enrolled patients were then interviewed to obtain data on socio-demographic variables ie education, occupation, annual income. Socio-economic status was calculated using the Kuppuswamy scale updated for 2019. This was followed by oral examination, which was performed by a single calibrated examiner who assessed the dental prosthetic status and treatment needs according to the criteria described in the WHO Oral health assessment form 1997.11

Intraoral examination of the patient was done in the type II setting. All the data were tabulated in Microsoft Excel. Data were analyzed through IBM SPSS™ Statistics for Windows, version 20.0 (IBM Inc., Armonk, NY, USA). Descriptive statistics and differences among prosthetic status and prosthetic needs, according to gender, age, grade, stage of cancer, and socio-economic status were calculated using Chi-square test. All the statistics were considered significant at a P-value of equal to or less than 0.05.

Results

The total number of participants included in this study was 120. The mean age of the study sample was 47.68 + 10.26 years. The largest number of individuals belonged to the age group of 51 to 60 years (n=43, 35.8%). The number of participants in the 18-30 years age group was 7 (5.8%), 31 to 40 years age group was 25 (20.8%), 41 to 50 year age group was 37 (30.8%) and 61 to 70 years was 8 (6.7%). The sample was predominantly men as 103 (85.8%) of the study participants were men. On the other hand, 17 participants (13.3%) were women. Most of the participants belonged to upper-middle and upper-lower socio-economic strata. The study sample comprised of 8 (6.7%) participants from lower SES, 23 (19.2%) from lower-middle SES, 42 (35.0%) from upper-lower, 45 (37.5%) from upper-middle, and 2 (1.7%) from upper SES.

Stage 1 carcinoma was diagnosed in 70 (57.5%) of the participants while stage 2 carcinoma was diagnosed in 50 (42.5%) population. While 44 (36.7%) participants received concomitant chemotherapy, 76 (63.3%) participants did not receive chemotherapy. The mean number of radiotherapy cycles obtained by the respondents was 18.4 (SD = 8.881) with a range from minimum of 2 cycles to a maximum of 35 cycles.
The majority of the people in the study sample brush once a day (n = 116, 96.7%) while 2 people each do not brush their teeth or brush twice a day. About 81% (n=126) patients had not visited the dentist in past 12 months while 18.2% (n = 28) participants had visited dentist in past 12 months.

According to table 1, the proportional difference in prosthetic status among genders was not found to be significant in the present study sample ($\chi^2 = 3.440$, p=0.969). The proportional difference in prosthetic status among different socioeconomic strata was not found to be significant in the present study sample ($\chi^2 = 21.768$, p=0.353). The proportional difference in prosthetic status among patients with stage 1 and stage 2 cancer was not found to be significant in the present study sample ($\chi^2 = 1.228$, p=0.746). The proportional difference in prosthetic status among patients who received and did not receive chemotherapy was found to be statistically significant in the present study sample ($\chi^2 = 13.568$, p=0.019). (Table 2)

The highest percentage of individuals (n=38, 31.7%) had 14 teeth in their upper dentition, while only 1 person (0.8%) had 4 teeth. Fourteen teeth were present in 36.7% of the population (n=44) which was the highest number, while 8 teeth were present only in 1 person (0.8%). The minimum number of teeth present was 5.

Table 3 shows the distribution of population according to the number of teeth requiring replacement. A total of 46 participants did not need any replacement of teeth. Of the other participants who needed replacement, the highest number (n=16),13.3% needed replacement of 4 teeth, followed by 14 participants needing replacement of 7 teeth and 12(10%) each needing replacement of 6 and 8 teeth. ( Table 3)

Of a total of 120, 75% (n=90) did not have any prosthesis, 10.8% (n=13) had one bridge while 3.3% (n=4) had more than one crown and bridge prosthesis in their mouth. Partial dentures were seen in n=10 (8.3%) participants, (n=2) (1.7%) had bridge and partial denture both while (0.8%) n=1, person had a complete denture prosthesis.( Table 4)

Only (11.7%) n=14of the total study sample had no prosthetic needs. Of the total of 120 individuals, n=7 (5.8%) needed a single unit prosthesis, n=33 (27.5%) needed multi-unit prosthesis, n=48 (40%) needed a combination of a single and multi-unit prosthesis, and n=18 (15%) needed a full prosthesis. (Table 5)
Table 1. Prosthetic status according to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Prosthetic Status</th>
<th>Tests &amp; P value</th>
<th>( \chi^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Prosthesis</td>
<td>Bridge &gt;1 bridge Partial Denture Both Bridge and Partial Denture Complete Denture</td>
<td>( \chi^2 = 3.440, p = 0.969 )</td>
<td>( \chi^2 = 13.568, p = 0.019 )</td>
</tr>
<tr>
<td>Male</td>
<td>75</td>
<td>11</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>13</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Socioeconomic Status</th>
<th>Prosthetic Status</th>
<th>Tests &amp; P value</th>
<th>( \chi^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lower Middle</td>
<td>17</td>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Upper</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Upper Lower</td>
<td>29</td>
<td>4</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Upper Middle</td>
<td>35</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>13</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage of cancer</th>
<th>Prosthetic Status</th>
<th>Tests &amp; P value</th>
<th>( \chi^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>61</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Stage II</td>
<td>29</td>
<td>9</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>13</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>Prosthetic Status</th>
<th>Tests &amp; P value</th>
<th>( \chi^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>62</td>
<td>8</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Yes</td>
<td>28</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>13</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 2. Prosthetic needs according to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>No Prosthesis Needed</th>
<th>Need For One Unit Prosthesis</th>
<th>Need For Multi Unit Prosthesis</th>
<th>Need For Combination Prosthesis</th>
<th>Need For Full Prosthesis</th>
<th>Tests &amp; P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>10</td>
<td>7</td>
<td>29</td>
<td>41</td>
<td>16</td>
<td>( \chi^2 = 7.115, p=0.524 )</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>7</td>
<td>33</td>
<td>48</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>( \chi^2 = 21.096, p=0.175 )</td>
</tr>
<tr>
<td>Lower Middle</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>4</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Upper Lower</td>
<td>5</td>
<td>1</td>
<td>9</td>
<td>20</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Upper Middle</td>
<td>5</td>
<td>4</td>
<td>15</td>
<td>18</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>7</td>
<td>33</td>
<td>48</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Stage of Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>( \chi^2 = 1.228, p=0.746 )</td>
</tr>
<tr>
<td>Stage 1</td>
<td>13</td>
<td>2</td>
<td>15</td>
<td>32</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>1</td>
<td>5</td>
<td>18</td>
<td>16</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>7</td>
<td>33</td>
<td>48</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>( \chi^2 = 11.446, p=0.022 )</td>
</tr>
<tr>
<td>No</td>
<td>9</td>
<td>7</td>
<td>26</td>
<td>25</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>0</td>
<td>7</td>
<td>23</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>7</td>
<td>33</td>
<td>48</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>
### Table 3 Number of teeth needing replacement

<table>
<thead>
<tr>
<th>Number of teeth to be replaced</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>46</td>
<td>38.4%</td>
</tr>
<tr>
<td>1.0</td>
<td>1</td>
<td>0.8%</td>
</tr>
<tr>
<td>2.0</td>
<td>2</td>
<td>1.7%</td>
</tr>
<tr>
<td>4.0</td>
<td>16</td>
<td>13.3%</td>
</tr>
<tr>
<td>5.0</td>
<td>2</td>
<td>1.7%</td>
</tr>
<tr>
<td>6.0</td>
<td>12</td>
<td>10%</td>
</tr>
<tr>
<td>7.0</td>
<td>14</td>
<td>11.7%</td>
</tr>
<tr>
<td>8.0</td>
<td>12</td>
<td>10%</td>
</tr>
<tr>
<td>9.0</td>
<td>1</td>
<td>0.8%</td>
</tr>
<tr>
<td>12.0</td>
<td>5</td>
<td>4.2%</td>
</tr>
<tr>
<td>14.0</td>
<td>1</td>
<td>0.8%</td>
</tr>
<tr>
<td>15.0</td>
<td>2</td>
<td>1.7%</td>
</tr>
</tbody>
</table>

### Table 4 Prosthetic Status of the Population

<table>
<thead>
<tr>
<th>Prosthetic Status</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Prosthesis</td>
<td>90</td>
<td>75.0</td>
</tr>
<tr>
<td>Bridge</td>
<td>13</td>
<td>10.8</td>
</tr>
<tr>
<td>More than one bridge</td>
<td>4</td>
<td>3.3</td>
</tr>
<tr>
<td>Partial Denture</td>
<td>10</td>
<td>8.3</td>
</tr>
<tr>
<td>Bridge and Partial Denture</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>Full Removable Denture</td>
<td>1</td>
<td>.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>120</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
Table 5. Prosthetic needs of study sample

<table>
<thead>
<tr>
<th>Prosthetic Needs</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Prosthesis Needed</td>
<td>14</td>
<td>11.7</td>
</tr>
<tr>
<td>Single Unit Prosthesis</td>
<td>7</td>
<td>5.8</td>
</tr>
<tr>
<td>Multi Unit Prosthesis</td>
<td>33</td>
<td>27.5</td>
</tr>
<tr>
<td>Combination of One And / Or Multi Unit Prosthesis</td>
<td>48</td>
<td>40.0</td>
</tr>
<tr>
<td>Full Prosthesis</td>
<td>18</td>
<td>15.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>120</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

**Discussion**

Prosthetic needs increase with age and the presence of health-related conditions. Head and neck cancers may be directly responsible for tooth loss due to the infiltration of cancer cells into the surrounding dental tissues including the support structures of teeth. Additionally, radiotherapy in head and neck cancers may be one of the major reasons for xerostomia or dry mouth and subsequently for dental caries leading to loss of a tooth. Thus, it is premised that prosthetic status and needs of head and neck cancer irradiated subjects may differ from those of individuals not under radiotherapy as well as healthy individuals.12–14

The present study showed that there was a statistically significant difference in the proportion of patients using prosthesis among those who had undergone radiotherapy and those who were not given radiotherapy. The proportion of people using prosthesis was lower in the irradiated patients as compared to radiated ones. On the other hand, the prosthetic needs of irradiated patients were higher than of the non-irradiated patients. Many of these patients needed multi-unit or a combination of a single and multi-unit prosthesis. It may be explained that since there are higher needs and lower use of prosthesis in irradiated patients, much of the need that has arisen might be a recent one. This may be due to the impact of radiotherapy on the health of oral tissues. The causes of this difference among irradiated and non-irradiated patients need to be affirmed through the establishment of sound evidence indicating the cause.

A significantly higher percentage of subjects in the upper SES categories had some kind of prosthesis as compared to subjects in the lower SES category. The social pressure of maintaining the esthetics and function could be the driving force that influences the subjects in the upper class to get their missing teeth replaced.15 In addition to this, the attitude and awareness toward dental care, and the cost of dental treatment might also be the significant factor that determines the prosthetic status of a person.

According to database data available online, the present study is the first one to assess the prosthetic status among head and neck cancer patients who underwent treatment during the time of the study. The study showed that about 25% of the sample used one or the other kind of prosthesis. Of those who needed prosthesis, about 67% of the individuals required a multi-unit or a combination prosthesis. Hence there was an extensive tooth loss in the population with a large number of those who needed prosthesis requiring
long span and complex prosthesis. This indicates the complex process of tooth loss among head and neck cancer patients. Many causative elements may work in tooth loss including the effect of pathology as well as the treatment including radiotherapy and chemotherapy on the teeth and their supporting structures. All these factors should be considered as a part of the multifaceted etiology leading to tooth loss in such patients.

Geographical as well as demographic features of the population under study have an important influence on the results. The present study was conducted in the capital of India. It is predominantly urban area with high availability of healthcare facilities and higher health literacy among the residents. However large variations are seen among the health parameters within this geographical region. These are mainly related to socio-economic status. These factors may result in different health-related status among different population groups. For a health-related condition such as head and neck cancer, these variations may be the primary reasons for the difference in dentition status among various groups. Since, upper-middle and upper-lower socio-economic strata formed the majority of the study sample, this may affect the overall prosthetic status of patients in the present study.

The results of this study show that almost 64% of individuals had at least one missing tooth except for third molars in the lower arch, while this percentage was close to 70% of individuals having at least one missing tooth in the upper arch. The percentage of patients with some kind of prosthesis was 25%. Prosthetic status was better than those of healthy elderly in Kerala as seen in a study conducted by Joseph et al (2016) where 18.2% had dentures. However, the prosthetic need in the present study was also higher at almost 88% of patients requiring prosthesis as compared to about 63% in Joseph et al study. According to the another study conducted by Yadav et al (2016) among institutionalized elderly in Delhi in which the prosthetic status and needs were determined, about 25% of participants required prosthesis as compared to 88% in the present study. This reflects the difference between prosthetic needs of healthy and head and neck cancer irradiated individuals. The higher prosthetic needs in cancer irradiated patients may be attributed to the pathologic process of cancer as well as conditions such as xerostomia that lead to enhanced tooth loss after cancer radiotherapy.

The present study could not compare the differences among males and females because only 17 (15%) of the study sample were females. However, various studies assessing prosthetic needs in India showed that females have higher prosthetic needs when compared to their male counterparts. There were also studies showing results in contrast.

A weakness of the study was that patients at various doses of radiation therapy were included in the study. There may be differences in the dentition status between various doses of radiotherapy concerning their oral health and oral healthcare needs.

**Conclusion**

The present study established a high oral health risk as well as a high oral disease burden in the head and neck irradiated population of Delhi. It also indicates towards the adoption of a robust framework integrated with a treatment plan for this population. A collaborative teamwork of oncologist and prosthodontist on a cohesive treatment plan for head and neck cancer will not only help in reducing the oral disease burden in this population but also help in improving oral health-related as well as general health-related quality of life among these patients.

**Acknowledgement** - The authors would like to acknowledge the support and permission given by the managements of the Rajiv Gandhi Cancer Hospital, New Delhi; Balaji Action Cancer Hospital, New Delhi and Max Hospital, Shalimar Bagh, Delhi.

**Conflict of Interest** – Nil

**Ethical Clearance**- Taken from the Institutional ethics committee of Manav Rachna Dental College

**Source of Funding**- Self
References


In Vitro Antifungal Activity of of Pleurotuseryngii against Trichophytonrubrum

Salah M. Dawood1, Ahmed K. Abdulrazzaq2, Kareem T. Shnawa3, Mohammed J. Hanawi4

1MSc Student, Dep. of Microbiology, College of Science, University of Wasit, Iraq, 2Scientific Researcher, Plant Protection Directorate, Ministry of Agriculture, Iraq, 3Dermatologist, Alzahraa Teaching Hospital, Ministry of Health, Wasit Province, Iraq, 4Prof. Dep. of Microbiology, College of Science, University of Wasit, Iraq

Abstract

Biological control represents an important approach for controlling many dermatophyte fungi. Pleurotuseryngiiisa promising and effective bioagent against many pathogenic fungi. In this paper Pleurotuseryngiiwere screened for their efficacy against Trichophytonrubrum. The results had been revealed that the fruiting bodies extract of the bioagentPleurotuseryngiiaffected the radial growth of the dermatophyte fungus Trichophytonrubrum. The extract of test fungus at all test concentrations had inhibitory effect on the radial growth of Trichophytonrubrum. The results had been revealed also that ethanolic extract was more effective than the aqueous extract of Pleurotuseryngii but lower than the affectivity of the antifungal drug clotrimazole.

Keywords: BIOcontrol, Trichophytonrubrum, Pleurotuseryngii, antifungal activity.

Introduction

Dermatophytosis is caused by pathogenic, keratin-digesting fungi in the genera Microsporum, Trichophytonand Epidermophyton. The genus Trichophyton especially T. rubrum was the most frequently agent of dermatophytosis worldwide1.

About 76% of the dermatophyte species isolated from humans are Trichophytonrubrum, 27% are T. mentagrophytes, 7% are T. tonsurans, 3% are T.verrucosum and another dermatophyte fungi (less than 1%)2.

A great attention was made to use microorganism or their metabolities in treatment of various infection and diseases. Mushrooms contain some potential antibacterial and antifungal compounds such as peptide eryngin and polypeptide alveolarin originated from Pleurotuseryngiiand Polyporusalveolaris, respectively which have highly antifungal potential3.

Extract of Pleurotuseryngiǐhas great antifungal activity against fungi Candida albicans, Candidaglabrataand Trichophyton spp4.

The bioactive compounds of mushrooms are responsible for antiviral and antibacterial properties. Examples of such bioactive compounds are polysaccharides, dietary fiber, antioxidants (vitamins C, B12 and D; folate; tocoherols, ergothioneine, carotenoids and polyphenols), phenolics and polyphenolics5,6.

Eun-Ji, et al.7 found that the ethanol extract of Pleurotuseryngiihave antimicrobial activity and contain higher levels of polyphenols and flavonoid compounds than other solvents.

Ligninolytic enzymes that produced by Pleurotuseryngii such as laccase (Lac) and Mnperoxidase (MnP), are able to degrade aflatoxin B1 (AFB1), the most harmful among the known mycotoxins8.

Not much literature is available with regard to their antifungal activities of Pleurotuseryngii against dermatophyte fungi, so this study was
performed to determine the antifungal activity of *Pleurotus eryngii* against *Trichophyton rubrum* in laboratory.

**Materials and Methods**

**Source of *Trichophyton rubrum* isolate**

Specimens were collected from patients in hospital and medical clinic. Specimens then cultured on SDA medium and identified morphologically.

**Source of *Pleurotus eryngii* isolates**

*Pleurotus eryngii* isolates were obtained from Plant Protection Directorate, Ministry of Agriculture, Iraq.

**Source of antifungal drug**

The antifungal drug (Clotrimazole) obtain as a standard solution from nation pharmacy.

**Pleurotus extract preparation**

**Aqueous extract of *Pleurotus eryngii***

To obtain water extract of *Pleurotus eryngii*, 10 g of dry powdered of fruiting bodies was boiled with 100 mL distilled water for 30 min. and then cooled. The cooled solution was filtered through Whatman filter paper No. 1. To obtain dried extract, filtered solution evaporated in the oven (55 °C). The dried extracts kept at 4°C for further studies. To prepare different concentrations, extract was dissolved by distilled water, sterilized by filtration (using Millipore 0.45 filter paper), and the requisite dilutions were prepared.

**Ethanolic extract of pleurotus**

To obtain ethanolic extract of *Pleurotus eryngii*, 10 g of dry powdered of fruiting bodies were mixed with 100 ml of ethanol 70% , the container was shook 2 or 3 times per day and returned to warm dark place. The liquid was filtered through Whatman No. 1 filter paper and leave it to evaporate to obtain dry extract. To prepare different concentrations, dry extract was dissolved by dimethyl sulfoxide(DMSO), sterilized by filtration (using millipore0.45 filter paper), and the requisite dilutions were prepared.

**Effect of *Pleurotus eryngii* on *Trichophyton rubrum* in dual culture technique**

To evaluate the effect of *Pleurotus eryngii* on *T. rubrum* in dual culture technique, both tested fungi were grown on SDA for a week at 30 ± 2°C. 5mm disc of the target fungus cut from the periphery of culture and transferred to the Petri dish contain SDA. 5mm disc of *Pleurotus eryngii* was transferred in the same plate of opposite end of the plate at equal distance and was incubated at 30 ± 2°C for 7 days. In control plates, a sterile agar disc(without *Pleurotus eryngii*) was placed at opposite side of the *T. rubrum* agar disc. The experimental design used was a completely randomized design (CRD) with three replicates for each treatment. Radial colony growth of *T. rubrum* was measured after three and seven days. The percentage of inhibition of the mycelial growth of the test fungus was calculated using the formula of Philippe et al.,

\[
\text{Inhibition of mycelial growth (\%) = \left(\frac{dc - dt}{dc}\right) \times 100}
\]

where \( dc \) is mean diameter of colony in the control sample and \( dt \) is mean diameter of colony in the treated sample.

**Effect of *Pleurotus extract* on growth of *T. rubrum* by disc diffusion method**

To evaluate the effect of *Pleurotus* extract (aqueous and ethanolic) on growth of *T. rubrum* by disc diffusion method, different amount of dry extract of fruiting bodies mixed with distilled water to obtain the concentrations 25 (2gm/80ml), 12.5, and 6.25mg/ml in the case of aqueous extract and with Dimethyl Sulfoxide (DMSO) in the case of ethanolic extract and sterilized by filtration (using Millipore 0.45 filter paper). Paper discs (5 mm) were sterilized by autoclave and soaked in a pleurotus extracts (ethanolic and aquatic extract) solution and putted in a petri plate contain SDA previously inoculated with targeted fungus. The agar plates maintained at room temperature for 2 h allowing for diffusion of the solution. All plates were then incubated at 30 ± 2°C for the specified period of time. The zones of inhibition were subsequently measured in Millimeters.
Effect of clotrimazole on growth of \emph{T. rubrum}

To evaluate the effect of clotrimazole on growth of \emph{T. rubrum} by disc diffusion method, different concentrations of clotrimazole 10, 5 and 2.5 μg/ml were used. Paper discs (5 mm) were sterilized by autoclave and soaked in each concentration and putted in a petri plate contain SDA previously inoculated with targeted fungus. The agar plates maintained at room temperature for 2 h allowing for diffusion of the solution. All plates were then incubated at 30 ± 2°C for the specified period of time. The zones of inhibition were subsequently measured in Millimeters\textsuperscript{12}.

Results

Effect of \emph{Pleurotus} \emph{enyrgii} growth of \emph{T. rubrum}

The results of this study that presented in Table (1) and figure (1) showed that the \emph{Pleurotus} \emph{enyrgii} inhibited the radial mycelial growth of \emph{Trichophyton rubrum}. The Mean of radial growth of \emph{Trichophyton rubrum} after three days and seven days of treatment was 2.5 cm, 2.7 cm respectively as compared with control which were 3.1 cm and 7.8 cm respectively. The percentages of inhibition of radial growth (PIRG) values after three days and seven days were 19.35 % and 65.38 % respectively.

The fungus recorded high antagonistic activity and completely overgrew the test pathogen \emph{Trichophyton rubrum} and grows on the entire surface of petri-plate after 14 days of treatment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Radial growth (cm) of \emph{T. rubrum} after 3 days</th>
<th>Inhibition (%)</th>
<th>Radial growth (cm) of \emph{T. rubrum} after 7 days</th>
<th>Inhibition (%)</th>
<th>Over Growth after 8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleurotussp</td>
<td>2.5 a</td>
<td>19.35</td>
<td>2.7 a</td>
<td>65.38%</td>
<td>+++</td>
</tr>
<tr>
<td>Control</td>
<td>3.1 a</td>
<td></td>
<td>7.8 b</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>NS</td>
<td></td>
<td>0.406</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Each value is a mean of 3 replicates. +++ = High antagonistic activity (61 – 75 PIRG),

PIRG: Percent of Inhibition in Radial Growth (Soytong\textsuperscript{13}).

*Similar letter means no significant difference

Figure (1): A: Radial growth of \emph{T. rubrum} in dual culture
a- control (*T. rubrum*), b- overgrowth of *Plurotussp*(7days), c-overgrowth(14days)

**Effect of Pleurotus extract on *T. rubrum* by Disc diffusion method**

The results of the antifungal activity of culture filtrate of *Pleurotus eryngii* against *T. rubrum* by disc diffusion method are summarized in the figure (2) and figure (3).

All tested concentrations of ethanolic extract affected the growth of *T. rubrum* significantly as compared with the control, and the effect was increased with the increasing of concentration.

The highest zone of inhibition was recorded at the case of ethanolic extract at the concentration (25mg/ml) which was 18.66 mm followed by the concentrations 12.5 and 6.25mg/ml which were 11.83 mm and 7.16 mm respectively.

The result also showed that aqueous extract recorded no inhibition zone at the concentration 6.25mg/ml but recorded 9.33mm and 4.66mm at the concentration 25mg/ml and 12.5mg/ml respectively.

The statistical analysis refers to the significant differences between all treatments as compared with the control except aqueous extract at the concentration 6.25mg/ml.
Figure (2): Effect of *Pleurotus* extract on growth of *T. rubrum* by disc diffusion method

*Different letter means significant difference

Figure (3): Inhibition zones of *T. rubrum* by *Pleurotus* ethanolic extract

Left at concentration 12.5mg/ml, right at concentration 25mg/ml
Effect of clotrimazole drug on growth of *T. rubrum* by disc diffusion method

The results of this study that presented in table (4) and figure (5) had been revealed that the antifungal clotrimazole affected the growth of *T. rubrum* in all tested concentrations and the effect was increased with the increasing of concentration. Highest inhibition zone was recorded in the case of the concentration 10 mg/ml which was 29.33mm and the lowest was 12.33mm at the concentration 2.5 mg/ml.

![Figure (4): Effect of clotrimazole on growth of *T. rubrum* by disc diffusion method](image1)

*Different letter means significant difference*

![Figure (5): Effect of clotrimazole on growth of *T. rubrum*](image2)

2.5 mg/ml, b- 5 mg/ml, c- 10 mg/ml
Discussion

Oyster mushroom produce secondary metabolites which was important against pathogenic fungi. These metabolites include triterpenoids, polysaccharides, proteins and enzymes (Patel, et al. 2012). Also, the ability of oyster mushroom to produce metabolic materials such as enzymes give it inhibition force toward decay cellular walls of pathogenic fungi.

Mycelium of Pleurotus tuber-regium was able to completely overgrow the keratophilic fungus Chrysosporium keratinophilum.

Both fruiting body and mycelium of mushrooms contain compounds with wide ranging antimicrobial activity. They are rich sources of natural antibiotics, where the cell wall glucans are well known for their immunomodulatory properties, and many of the externalized secondary metabolites combat bacteria, fungi, and viruses.

Results of study revealed that the ethanolic extract was more effective than aqueous extract and this results in agreement with the results of Eun-Ji, et al., who found that the ethanolic extract of Pleurotus eryngii were the most effective against tested microbes and contain higher levels of polyphenols and flavonoid compounds. Thillaimaharani, et al., who investigate the antifungal activity of four different solvents extracts of Pleurotus florida against three dermatophyte fungi such as Trichophyton rubrum, Epidermopontflocossumand Microsporum gypseum and they observed that the ethanolic extract of P. florida exhibited highest activity and produced minimum inhibitory concentration.

Kalu and Kenneth, reported the presence of bioactive compounds, saponin, carbohydrates, tannins, flavonoids and proteins in both ethanolic and aqueous extract while glycoside and alkaloids, were found only in ethanolic extract.

The results also agree with Egra, et al., who found the antifungal activity of oyster mushroom against Candida albicans and found that ethanolic extract affect the growth of the fungus and the effect increased with the increasing of concentration and the inhibition zones ranged between 9.3 to 10.8mm.

Clotrimazole also is the most potent agent and oldest antifungal drugs. This antifungal agent showed excellent in-vitro potency against most dermatophyte fungi. Khalaf et al., investigated the antifungal activity of Nystatin, Fluconazole, Griesofulvin, Clotrimazole and Fluconysin against Trichophyton mentagrophytes and found that Clotrimazole and Fluconysin were more effective than the other.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Obtained from Institutional ethical committee

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Genotyping of Histological Cutaneous Leishmaniasis Isolated by Dental Broach Smear in Iraqi Patients

Salah Mahdi Hassan¹, Bushra Qasim Dhumad²

¹Assistance Professor, ²Assistant Lecturer, Department of Medical Laboratory Technology, College of Health and Medical Technology, Baghdad, Iraq

Abstract

Cutaneous leishmaniasis is a major public health problem as a disease endemic in Iraqi population. Many diagnostic tools are used to establish the diagnosis like smear, histopathology and culture. To find more rapid, sensitive and specific diagnostic method for the diagnosis of cutaneous leishmaniasis, and to detect the genotypes of *Leishmania tropica* and *Leishmania major* strains in Iraq. Sixty six patients (43 males and 23 females) with clinical diagnosis of cutaneous leishmaniasis were included in present work during the period between December / 2019 to May / 2020 in Al-Yarmook Teaching Hospital and Al-Karama Teaching Hospital. The age of patients ranged from 6 months to 55 years, with median age 24 years. The following diagnostic techniques were carried out for diagnosis of cutaneous leishmaniasis including dental broach smear, histopathology examination and culture on Roswell park medium institute (RPM I 1640) and on NNN media. In addition to polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), genotype techniques were performed for all patients. Sixty patients with other skin lesions were processed for PCR as a control group.

Key words: Cutaneous leishmaniasis, genotypes, Al-Karama Hospital, PCR, histopathology.

Introduction

Cutaneous leishmaniasis is a clinical manifestation in which the parasite causes one or more slow-healing ulcer on the skin. The diagnostic methods available at present are mostly based on clinical and epidemiological evidence and parasite detection (¹). So far, no single laboratory method has been accepted as a gold standard for diagnosis CL. Parasitological tests of a skin biopsy specimen are not always conclusive in patients with a clinical diagnosis of CL (²). The different species of Leishmania present the same morphology when classical diagnostic methods, such as microscopic examination of Giemsa-stained smears or parasite culture, are used (³). This led to the development of molecular approaches combining high sensitivity for direct detection and identification in clinical specimen with species specificity by amplifying either species-specific DNA sequences or genus-specific sequences that allow for subsequent differentiation of Leishmania species (⁴). Polymerase chain reaction (PCR) is a technique that permits the exponential amplification of a known DNA sequence from minimal sample quantities, increasing the sensitivity of detection (⁵). Due to its high sensitivity and specificity afforded, the PCR has been widely used in the diagnosis and epidemiological studies (⁶). There are in numerous DNA sequences used as targets for the diagnosis and detection of Leishmania in PCR protocols. The principal sequences used are kinetoplast DNA (kDNA), mini-exon, ribosomal DNA, and glucose-6-phosphate dehydrogenase, among others (⁷). Only a few of the assays described in the literature permit identification of the etiological agent of American Cutaneous Leishmaniasis (ACL) at species level, the majority identifies genus and subgenus (⁸). The mini-exon gene of kinetoplastid protozoa, which is involved in the transsplicing process of nuclear mRNA, is present as 100 to 200 tandemly repeated copies per nuclear genome. Each repeat consists of three major parts: a transcribed
region comprising a highly conserved 39-nucleotide exon; a moderately conserved intron, approximately 55 to 101 bp; and a nontranscribed, intergenic spacer of variable length (51 to 1,350 bp) depending on the genus and species (9).

**Material and Methods**

Cutaneous leishmaniasis is a major public health problem as a disease endemic in Iraqi population. Many diagnostic tools are used to establish the diagnosis like smear, histopathology and culture. To find more rapid, sensitive and specific diagnostic method for the diagnosis of cutaneous leishmaniasis, and to detect the genotypes of *leishmania tropica* and *leishmania major* strains in Iraq. Sixty six patients (43 males and 23 females) with clinical diagnosis of cutaneous leishmaniasis were included in present work during the period between December / 2019 to May / 2020 in Al-Yarmook Teaching Hospital and Al-Karama Teaching Hospital. The age of patients ranged from 6 months to 55 years, with median age 24 years. The following diagnostic techniques were carried out for diagnosis of cutaneous leishmaniasis including dental broach smear, histopathology examination and culture on Roswell park medium institute (RPM I 1640) and on NNN media. In addition to polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), genotype techniques were performed for all patients. Sixty six patients with other skin lesions were processed for PCR as a control group. Gene sequences the results were compared with basic local alignment reference sequences.

**Statistical Analysis**

The data were analyzed by using Microsoft Excel 2007 and SPSS version 18. The Chi-square test was used to compare of differences between study groups.

**Results**

In all age groups, males 43(65%) were more frequently infected with CL than females 21 (35%) as shown in table (1). The differences between genders were statistically highly significant (p. value < 0.05). The highest age group infected with CL was (30-44) years. The differences between age groups were statistically significant (p. value < 0.05).

**Table (1): The frequency of cutaneous leishmaniasis according to age and gender in a patients group**

<table>
<thead>
<tr>
<th>Gender Age(year)</th>
<th>male</th>
<th>female</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-14</td>
<td>11</td>
<td>7</td>
<td>18</td>
<td>27.3</td>
</tr>
<tr>
<td>15-29</td>
<td>12</td>
<td>7</td>
<td>19</td>
<td>28.7</td>
</tr>
<tr>
<td>30-44</td>
<td>13</td>
<td>7</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>45-59</td>
<td>7</td>
<td>2</td>
<td>9</td>
<td>13.6</td>
</tr>
<tr>
<td>Total</td>
<td>43 (65%)</td>
<td>23 (35%)</td>
<td>66</td>
<td>100</td>
</tr>
</tbody>
</table>

Chi-square 10.8, P. value = 0.001 for sex. For age Chi-square 8, P. value= 0.0460
Twenty two (33.33%) patients had a single lesion, while forty four (66.66%) patients had multiple lesions. Table (2) showed that the main part of the body infected with CL was at the upper limbs and hands (38.5%), followed by the face (25%), then the lower limbs and feet (21.5%), while the lowest frequency of infection (15%) was in other parts of the body. The statistical difference between the locations of skin lesion was not significant (P. > 0.05).

Table (2): The location of skin lesion in different parts of the body in cutaneous leishmaniasis patients

<table>
<thead>
<tr>
<th>Location of skin lesions</th>
<th>Upper Limbs &amp; hands</th>
<th>Lower Limbs &amp; feet</th>
<th>face</th>
<th>other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>22</td>
<td>33.33%</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Multiple</td>
<td>44</td>
<td>66.67%</td>
<td>65</td>
<td>39</td>
</tr>
<tr>
<td>∑</td>
<td>66</td>
<td>100%</td>
<td>77</td>
<td>43</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>38.5%</td>
<td>21.5%</td>
<td>25%</td>
</tr>
</tbody>
</table>

*Chi-square 2.066, P.value < 0.9561.

When the number of skin lesions was from 1 to 9, it has been found that males were more affected than females, while when the number of lesions was 10 or more females were much more affected as shown in table (3). The statistical difference between numbers of lesions according to gender was highly significant (P. < 0.05)

Table (3) Number of lesions according to gender in patients group

<table>
<thead>
<tr>
<th>No. of lesion</th>
<th>male</th>
<th>female</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>26</td>
<td>7</td>
<td>33</td>
<td>50</td>
</tr>
<tr>
<td>4-6</td>
<td>8</td>
<td>4</td>
<td>12</td>
<td>24.2</td>
</tr>
<tr>
<td>7-9</td>
<td>7</td>
<td>1</td>
<td>8</td>
<td>12.1</td>
</tr>
<tr>
<td>10 or more</td>
<td>2</td>
<td>11</td>
<td>13</td>
<td>19.7</td>
</tr>
</tbody>
</table>

*single skin lesion in 20 cases

*multiple skin lesions in 40 case. *Chi-square 21.324, P. value < 0.0033.

All the positive cases in culture methods was considered as golden standard and compared with other methods. It had been found that PCR was more specific than other methods. All the positive cases in culture method gave positive results in PCR, so the specificity of PCR was 100%, while the specificity of dental broach smear was 75.55% and histopathology was 50%.
Figure (1) PCR products of mini-exon gene of *Leishmania* species from patients group, on 1.5% agarose gel, M1 & M2 molecular marker 1Kbp (1000-25 bp), Lane 1 positive control; Lane 2: negative control (distil water instead of DNA template); Lanes (3-14): samples, with voltage 100 for 30 min. Lanes (3,4,6,7,8,9,11,12,14): positive samples *L. major* at M.W. 430 bp; Lane 13: positive sample *L. tropica* at M.W. 400bp. The mini-exon PCR assay of 60 samples by using specific primers for specific *Leishmania* gene (mini-exon gene) amplification products varied in size which was indicative of different *Leishmania* spp., from 55 positive samples, 33 samples were identified as *L. major* (60%) with amplicon molecular Wight 430bp, and in 22 samples were identified as *L. tropica* with amplicon molecular Wight 400bp. (Fig 2).

Figure (2): Genotyping of *Leishmania* spp. by Restriction Fragment Length Polymorphism (RFLP) analysis after digestion with (*Eae I* and *Hae III*) on 2.5% agarose gel. M1 & M2: 1Kbp (1000-25 bp) molecular marker; Lanes 1, 2, 3, 4, 5 *L. major*; Lanes 8, 9, 10, 11, 12, 13, 14 *L. tropica*. With voltage 100 for 30 min.
Twelve samples were analyzed to make gene sequences. The results were compared with basic local alignment reference sequences.

*Leishmania* tropica isolate 31 isolated internal transcribed spacer 1 and 5.8s ribosomal RNA gene partial sequence.

Sequence ID: KP773410.1Length: 309 number of Matches: 1

Alignment statistic for match # 1

Table (4) showed the mutation that occurred in the numbers of positions in substantiation nucleotides in comparison of reference isolates KY612611.1.

<table>
<thead>
<tr>
<th>No. of sample</th>
<th>Positions</th>
<th>Mutation</th>
<th>parasite</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>218</td>
<td>G&gt;A</td>
<td>Leishmania Major</td>
</tr>
<tr>
<td></td>
<td>224</td>
<td>A&gt;G</td>
<td></td>
</tr>
<tr>
<td></td>
<td>213</td>
<td>C&gt;A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>261</td>
<td>T&gt;C</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>112</td>
<td>T&gt;C</td>
<td>Leishmania Major</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>G&gt;C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>218</td>
<td>G&gt;A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>224</td>
<td>A&gt;G</td>
<td></td>
</tr>
<tr>
<td></td>
<td>233</td>
<td>C&gt;A</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>178</td>
<td>T&gt;C</td>
<td>Leishmania Tropica</td>
</tr>
<tr>
<td></td>
<td>179</td>
<td>G&gt;A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>185</td>
<td>A&gt;G</td>
<td></td>
</tr>
<tr>
<td></td>
<td>194</td>
<td>C&gt;A</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Cutaneous leishmaniasis, also known in Iraq as aghdad boil, is the most common form of leishmaniasis. It is a skin infection caused by a unicellular parasite that is transmitted by sand fly bites. There are about 20 species of Leishmania that may cause cutaneous leishmaniasis. The total incidence of cutaneous leishmaniasis in Iraq varies from 2.3 to 45.5 / 100000 (10). According to age and gender in patients group, the present study showed significant differences between males and females infected with CL in all age groups. In the present study, males were the predominant (65%) than females (35%) were in agreement with previous Iraqi study (11) where males were (57%) and females were (31.7%). But our results were disagree with the other study done by (Saki et al. 2010) in Iran which has found that females were more predominant (54.68%) than males (45.31%). These results are somewhat difficult to explain and may be due to the fact that males are working out-door and also due to men are less covering than women and expose to the insect biting more than females. The more frequency of CL in this study was in age group 0-14 years showing agreement with the other studies done in Iraq (11). This difference in age group playing outdoors for long time and more exposure to the infected sand flies. The most of Cutaneous leishmaniasis lesions were present on the exposed parts of the body; the highest frequency was (38.5%) at upper limbs and hands followed at face (25%) and then lower limbs and feet (21.5%) while the lowest frequency was in other parts of the body (15%). These results were in agreement (12), in which was (57%) in upper limbs followed by face (25%) then lower limbs (15%) and also agreement with other studies done in Sri Lanka (13), which had found that CL lesions occurred mainly on the upper limbs and the lower limbs, and less frequently on the face. When the number of lesions from 1-9, males were suffering more than females, but when the number of lesions 10 or more, females were much more suffering than males and showed the significant difference between male and female with number of lesions.

The present study showed two type of Leishmania Spp. In Iraq, by used mini-exon PCR assay, showed L. major (60%) while L. tropica (40%) and this agreement with other Iraqi studies (14), and other study in nearby countries such as Iran (15) hence the high frequency of L. major may be due to the presence of reservoir animals in large numbers, especially rodents and dogs (reservoir of L. major). Obviously, dense populations of natural hosts of L. major, together with abundant vector sand flies, are the key elements responsible for the high rate of human infection. (16). This study used a new genotype assay for molecular diagnosis of the different Leishmania species and strains in Iraq, it is based on amplification of the mini-exon gene and combined it with restriction digests of PCR product (RFLP technique), a method which is routinely used for genotyping tasks (17). The resulting patterns of restriction fragments were characteristic for each species and achieved a high resolution and high discrimination power. The exon is highly conserved, whereas the intron and non-transcribed spacer region vary in size and sequence among different species. Species identification was performed by digesting mini-exon PCR products with one or two different restriction enzymes (15) Restriction fragment length polymorphism (RFLP) generated species-specific patterns of bands visualized in agarose gels, which allowed differentiating each species and strain unequivocally. This study was in agreement with Saki et al 2010 these all Leishmania species of subgenus Leishmania can be distinguished by mini-exon PCR-RFLP, with Eae I being the most informative restriction enzyme. However, in two L. tropica samples used in our study, Eae I failed to cut the mini-exon PCR products of these samples and we used another restriction enzyme Hae III to digest the products of PCR. The mutation that occurrence in the numbers of positions in substantiation nucleotides in comparison of reference isolates KY612611.1. Leishmania Major: G＞A, A＞G, C＞A, T＞C, T＞G, G＞C, G＞A, A＞G, and C＞A, while Leishmania Tropica: T＞C, G＞A, A＞G and C＞A.

Conclusions

Sampling using dental appeared to be more active
than other sampling techniques used in this study. Histopathological sections have identified the focal presence of the parasites. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) was more specific than other methods.

**Acknowledgment:** The authors would like to acknowledge the staff of the hospital who helped, National center laboratory in AL-Kute hospital. Their support to conduct this research project.

**Ethical Clearance:** No need

**Source of Funding:** Self

**Conflict of Interest:** The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**References**


(15) Faraj Ch., Adlaoui El. B. Ouahabi, S. *et al*,

Correlation of High sensitivity C-reactive Protein Levels with Body Mass Index in a Group of Adults in Erbil City

Salam Naser Zangana¹, Namir G Al-Tawil², Suhad Ali Khazaal³

Abstract

Background and Objectives: The prevalence of overweight and obesity is increasing worldwide. Obesity is related to morbidity and mortality. High sensitivity C-reactive protein (hs-CRP) is an important inflammatory marker to predict cardiovascular diseases. This study aims to correlate between hs-CRP levels and BMI among healthy adults.

Materials and Methods: A cross-sectional study was conducted on 200 healthy adults from May 2019 to October 2019. The study population was divided into four groups based on BMI; normal, overweight, obese I, and obese II. Hs-CRP level was measured in all groups. Multiple linear regression analysis was done to find out the independent factors affecting hs-CRP.

Results: Out of 200 participants, 56 (28%) had normal BMI, 43(21.5%) were overweight, 57(28.5%) were obese group I and 44 (22%) were obese group II. The mean hs-CRP of the normal weight and overweight participants was 1.03 mg/dl and 1.37 mg/dl respectively (p= 0.725), then there was a marked increase to 3.75 mg/dl and 7.69 mg/dl among the obese I and obese II participants respectively (p < 0.001). A positive significant correlation was found between the hs-CRP levels and the BMI (r = 0.436, p < 0.001). The only factor that was independently associated with the high hs-CRP was the BMI (B = 0.357, p < 0.001).

Conclusion: Rising BMI was significantly correlated with hs-CRP. The higher the BMI, the higher will be plasma hs-CRP levels, suggesting the presence of chronic systemic inflammation which might lead to cardiovascular events in the future.

Keywords: High sensitivity C-reactive protein, Body mass index, Obesity.

Introduction

Being overweight or obese means that you have more body fat accumulated to an extent to cause negative health effects.¹ Fat accumulation is multifactorial. It may be due to more calorie intake, lack of exercise and daily activities, hormonal imbalance, genetic factors, or the combination of all.²⁻³ BMI is an accurate measure of body fat content than simply measuring a person’s weight. The BMI is defined as the body mass (weight in kilograms) divided by the square of the body height (in meters) and is expressed in units of kg/m². Overweight and obesity is a presentation of rising BMI.⁴ A BMI equal or more than 25 is considered overweight, while a BMI of 30 and above is considered obese. The prevalence of overweight and obesity is increasing worldwide.¹,⁵
2013, the increase in weight reached epidemic levels globally, with more than two billion adults being either overweight or obese, and many medical societies including the American Medical Association and the American Heart Association view obesity as one of the most serious public health problems of the 21st century and classify it as a disease. The prevalence of overweight and obesity in Iraq is different from one city to another. According to the 2006 World Health Organization survey, 26% of Iraqi men and 38% of Iraqi women aged 25-65 years were obese. A study was done in 2018 at Kufa city among employees women, showed that the prevalence of overweight and obesity was 27.5% and 43.1%, respectively. In Basrah city, the prevalence of overweight was 31.3% and obesity was 23.8% according to Mansour et al study. The prevalence of overweight and obesity in Erbil city was 33.4% and 40.9%, respectively.

Obesity increases the likelihood of many diseases such as cardiovascular diseases, obstructive sleep apnea, certain types of cancer, and metabolic syndrome. Adipose tissue produces many proinflammatory cytokines such as Tumor Necrosis Factor (TNF), interleukins-1, and 6 (IL-1 and IL-6) which can regulate the synthesis of CRP in the liver. CRP is an acute-phase protein whose circulating levels increase in response to inflammation. There is growing evidence to link inflammation and many chronic health conditions including diabetes, metabolic syndrome, and cardiovascular disease. As a result, and to enhance the clinician’s ability to predict these diseases, many studies have demonstrated that biomarkers of inflammation, such as CRP, can improve risk prediction. A hs-CRP test measures low levels of CRP using laser nephelometry. The test gives results in 25 minutes with sensitivity down to 0.04 mg/L. The American Heart Association and U.S. Centers for Disease Control and Prevention have defined risk groups as follows: low: hs-CRP level under 1.0 mg/L, average: between 1.0 and 3.0 mg/L, and high: above 3.0 mg/L. It was mentioned that healthy individuals with elevated hs-CRP values were up to 4 times as likely to have coronary heart disease.

Although the association between high BMI and rising levels of hs-CRP were mentioned in previous studies (correlating this rise with aging and concomitant diseases like rheumatoid arthritis, diabetes, and other cardiovascular diseases), recent studies reported that an increase in hs-CRP levels was related to weight gain without the presence of other diseases. There is still a disagreement regarding this subject.

To the best of our knowledge, there was no previous study done in Erbil city regarding the same subject. Accordingly, this study aimed to correlate between hs-CRP levels and body mass index among apparently healthy adults.

**Materials and Methods**

This cross-sectional study was conducted in Nawroz primary health center and Rizgary Teaching Hospital in Erbil city, Iraq, from May 2019 to October 2019. A convenience sample of 200 healthy adults, aged ≥ 18, with variant BMI, who came for routine health check-up, was selected to participate in this study. The exclusion criteria for this study considered having any of the followings: hypertension, diabetes mellitus, kidney or liver disease, an endocrine disorder, Rheumatoid arthritis, Inflammatory bowel diseases, a history of recent infection or inflammation (for the last two weeks), pregnancy, using weight-loss drugs, vitamins, taking contraceptive pills, statins, or Non-Steroidal Anti Inflammatory Drugs (NSAIDs). The weight of the participants was measured in kilograms using a calibrated digital scale. The height was measured in centimeters using a portable tape measure which was fixed on the wall. Calculation of BMI was done using the formula: Weight (kg)/ Height (m)². The participants were classified into four groups based on BMI as Normal (BMI 18.5-24.9 kg/m²), Overweight (BMI 25-29.9 kg/m²), Obese I (BMI 30-34.9 kg/m²), and Obese II (BMI 35-39.9 kg/m²), according to WHO standards. Under aseptic precautions, a blood sample was drawn from all participants to estimate serum hs-CRP levels using
COBAS INTEGRA cardiac C - Reactive Protein High Sensitive (CRPHS) test, and to be analyzed using fully automated Cobas E601 clinical chemistry analyzer. The American Heart Association (AHA) recommends that for most women/men, an optimal level of hs-CRP is less than 0.5 mg/l.\textsuperscript{24} Hs-CRP is the analyte of choice for cardiovascular risk assessment, due to superior assay precision, accuracy, availability, and the existence of standards for proper calibration when compared to other acute-phase reactants.\textsuperscript{15} According to the Centers for Disease Control (CDC) and AHA, low risk for cardiovascular disease is defined as hs-CRP <1 mg/L, average risk as 1 to 3 mg/L, and high risk as >3 mg/L.\textsuperscript{15}

**Questionnaire and data collection:**

An interview was made to collect the data using a questionnaire that was designed by the researchers. The questionnaire consisted of many sections regarding sociodemographic data (like age, gender, occupation, marital status, parity, and educational status), risk factors’ data (like smoking and alcohol consumption), body fat anthropometric index like BMI, and finally the laboratory investigations (like serum hs-CRP level).

**Ethical considerations:**

Ethical approval was obtained from the Ethics Committee of the College of Medicine at Hawler Medical University, and a facilitation letter was obtained from Erbil Directorate of Health (DOH). This study was conducted by using informed verbal consent that was obtained from all patients before participation in the study. The purpose of the study was carefully explained to each patient, and none of them refused to participate.

**Statistical Analysis**

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 25). The Chi-square test of association was used to compare proportions. Fisher’s exact test was used when the expected count of more than 20\% of the cells of the table was less than 5. A scatter plot was made between the hs-CRP and BMI using the Microsoft Excel (2013) computer program. The correlation coefficient (r) was calculated to assess the strength of the correlation between hs-CRP and BMI. One way analysis of variance (ANOVA) was used to compare three means. A post hoc test (LSD) was used to compare the means of each two groups (after doing the ANOVA test). Multiple regression model was made where the dependent variable was the hs-CRP. A p-value of ≤ 0.05 was considered statistically significant.

**Results**

Two hundred persons participated in the study. Their mean age ± SD was 33.01 ± 9.91 years. The age range was 18 to 60 years, and the median was 31 years. Table 1 shows that the highest proportion of the sample (38\%) was aged 20-29 years, and the majority (73\%) were females.

| Table 1. Basic characteristics of the study population. |
|----------------|----------------|---|
| **Age**       | **No.**        | **(\%)** |
| < 20          | 11             | (5.5) |
| 20-29         | 76             | (38.0) |
| 30-39         | 59             | (29.5) |
| 40-49         | 39             | (19.5) |
| ≥ 50          | 15             | (7.5)  |
| Mean (±SD)    | 33.01          | (±9.91)|
| Gender        |                |       |
It is evident in Table 2 that the mean hs-CRP of the normal weight and overweight participants was 1.03 mg/dl and 1.37 mg/dl respectively (p = 0.725), then there were a marked increase to 3.75 mg/dl and 7.69 mg/dl among the obese I and obese II participants respectively (p < 0.001).

### Table 1. Basic characteristics of the study population.

<table>
<thead>
<tr>
<th>Marital status</th>
<th>Male</th>
<th>(27.0)</th>
<th>Female</th>
<th>(73.0)</th>
</tr>
</thead>
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<tr>
<td>Single</td>
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<td>(15.5)</td>
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<td>Married</td>
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<td>(83.0)</td>
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<td>Divorced</td>
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<td>(0.5)</td>
<td></td>
<td></td>
</tr>
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<td>Widowed</td>
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<td>(1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Non-smoker</td>
<td>122</td>
<td>(61.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>22</td>
<td>(11.0)</td>
<td></td>
<td></td>
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<tr>
<td>Ex-smoker</td>
<td>53</td>
<td>(26.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive smokers</td>
<td>3</td>
<td>(1.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
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<tr>
<td>No</td>
<td>196</td>
<td>(98.0)</td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>4</td>
<td>(2.0)</td>
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<td>Regular exercise</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>182</td>
<td>(91.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
<td>(9.0)</td>
<td></td>
<td></td>
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<tr>
<td>Socio-economic status</td>
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</tr>
<tr>
<td>Low</td>
<td>63</td>
<td>(31.5)</td>
<td></td>
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</tr>
<tr>
<td>Medium</td>
<td>106</td>
<td>(53.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>31</td>
<td>(15.5)</td>
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</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>(100.0)</td>
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<td></td>
</tr>
</tbody>
</table>

### Table 2. Means of Hs-CRP by BMI categories.

<table>
<thead>
<tr>
<th>BMI (Kg/m2) groups</th>
<th>N</th>
<th>Mean Hs-CRP</th>
<th>(±SD)</th>
<th>p by ANOVA</th>
<th>LSD groups</th>
<th>p (LSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) &lt; 25 (Normal)</td>
<td>56</td>
<td>1.03</td>
<td>(±1.35)</td>
<td>A X B</td>
<td>0.725</td>
<td></td>
</tr>
<tr>
<td>B) 25-29 (Overweight)</td>
<td>43</td>
<td>1.37</td>
<td>(±1.48)</td>
<td>A X C</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>C) 30-34 (Obese I)</td>
<td>57</td>
<td>3.75</td>
<td>(±3.33)</td>
<td>&lt; 0.001</td>
<td>A X D</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>D) ≥35 (Obese II)</td>
<td>44</td>
<td>7.69</td>
<td>(±9.00)</td>
<td>B X C</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>3.34</td>
<td>(±5.31)</td>
<td>B X D</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C X D</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>
Table 3 shows that the proportion of patients with high hs-CRP increased significantly (p < 0.001) from 8.9% among the normal-weight participants, to 81.8% among those with morbid obesity (obese II).

Table 3. Hs-CRP categories by BMI categories.

<table>
<thead>
<tr>
<th>Hs-CRP risk categories</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
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<tr>
<td>Normal</td>
<td>39</td>
<td>(69.6)</td>
<td>12</td>
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<tr>
<td>Over-weight</td>
<td>22</td>
<td>(51.2)</td>
<td>17</td>
</tr>
<tr>
<td>Obese I</td>
<td>3</td>
<td>(5.3)</td>
<td>30</td>
</tr>
<tr>
<td>Obese II</td>
<td>1</td>
<td>(2.3)</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>(32.5)</td>
<td>66</td>
</tr>
</tbody>
</table>

Figure 1. Correlation between Hs-CRP and BMI.

It is clear in Table 4 that the only factors that are associated significantly with the high hs-CRP were age (≥ 30 years) (p < 0.001), and not practicing regular exercise (p = 0.017). No significant association was detected between hs-CRP and the following variables: gender (p = 0.089), socio-economic status (p = 0.583), smoking (p = 0.902), and alcohol (p = 0.471).
Table 4. Association between Hs-CRP risk categories and the studied variables.

<table>
<thead>
<tr>
<th>Hs-CRP risk categories</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30</td>
<td>44</td>
<td>(50.6)</td>
<td>29</td>
<td>(33.3)</td>
</tr>
<tr>
<td>≥ 30</td>
<td>21</td>
<td>(18.6)</td>
<td>37</td>
<td>(32.7)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
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<tr>
<td>Male</td>
<td>24</td>
<td>(44.4)</td>
<td>15</td>
<td>(27.8)</td>
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<tr>
<td>Female</td>
<td>41</td>
<td>(28.1)</td>
<td>51</td>
<td>(34.9)</td>
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<td><strong>Socio-economic status</strong></td>
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<tr>
<td>Low</td>
<td>16</td>
<td>(25.4)</td>
<td>24</td>
<td>(38.1)</td>
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<tr>
<td>Medium</td>
<td>38</td>
<td>(35.8)</td>
<td>34</td>
<td>(32.1)</td>
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<tr>
<td>High</td>
<td>11</td>
<td>(35.5)</td>
<td>8</td>
<td>(25.8)</td>
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<td><strong>Smoking</strong></td>
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<tr>
<td>Non-smoker</td>
<td>39</td>
<td>(32.0)</td>
<td>41</td>
<td>(33.6)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>9</td>
<td>(40.9)</td>
<td>6</td>
<td>(27.3)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>17</td>
<td>(32.1)</td>
<td>17</td>
<td>(32.1)</td>
</tr>
<tr>
<td>Passive smokers</td>
<td>0</td>
<td>(0.0)</td>
<td>2</td>
<td>(66.7)</td>
</tr>
<tr>
<td><strong>Alcohol</strong></td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>63</td>
<td>(32.1)</td>
<td>66</td>
<td>(33.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>2</td>
<td>(50.0)</td>
<td>0</td>
<td>(0.0)</td>
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<tr>
<td><strong>Regular exercise</strong></td>
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</tr>
<tr>
<td>No</td>
<td>54</td>
<td>(29.7)</td>
<td>64</td>
<td>(35.2)</td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>(61.1)</td>
<td>2</td>
<td>(11.1)</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>(32.5)</td>
<td>66</td>
<td>(33.0)</td>
</tr>
</tbody>
</table>

*By Fisher’s exact test.
Table 5 shows that BMI is the only factor that was independently and significantly associated with Hs-CRP ($B = 0.357$, $p < 0.001$). So the more the BMI, the higher the hs-CRP level.

**Table 5. SPSS output for multiple regression analysis where the hs-CRP is the dependent variable.**

<table>
<thead>
<tr>
<th></th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>95.0% Confidence Interval for B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
</tr>
<tr>
<td>(Constant)</td>
<td>-8.725</td>
<td>2.005</td>
<td>-4.351</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>0.357</td>
<td>0.059</td>
<td>0.421</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.015</td>
<td>0.037</td>
<td>0.028</td>
</tr>
<tr>
<td>Exercise</td>
<td>1.025</td>
<td>1.189</td>
<td>0.055</td>
</tr>
</tbody>
</table>

**Discussion**

The present study found a strong positive correlation between BMI and hs-CRP. The mean hs-CRP level was increasing with weight gain. This result is in correspondence with many previous studies done by Klisic et al., Aronson et al., and Kao et al.

In the Kurdistan region, as in Iraq, the prevalence of overweight and obesity is increasing and becomes a major health problem. Obesity is considered an important risk factor for many chronic diseases like hypertension, stroke, diabetes mellitus, and other cardiovascular problems. Multiple pathophysiological mechanisms had been presumed to bind obesity to cardiovascular diseases. One of these mechanisms is inflammation, which recently has been postulated to be the cornerstone process in the initiation of CVDs. Overweight and obesity were found to be associated with the presence of low-grade systemic inflammation indicated by higher levels of acute-phase reactants like CRP, and this result is in agreement with our study which revealed a significant rise in the hs-CRP level with the increasing BMI from normal weight to obese participants. The association between obesity and increasing hs-CRP levels can be elucidated by the potential role of adipose tissue in the body, which produces many proinflammatory cytokines such as Tumor Necrosis Factor (TNF), interleukins-1 and 6 (IL-1 and IL-6) that can regulate the synthesis of C-reactive protein (CRP) in the liver, as mentioned above. Adipose tissue can approximately produce 25% of IL-6 in vivo. The release of IL-6 from adipose tissue may produce low-grade systemic inflammation in individuals with increase body fat. TNF α can also play a role in raising hs-CRP levels with increasing BMI by potentiating the effect of IL-6 in CRP production. Another finding in the present study is that the proportion of patients with elevated hs-CRP levels (a CRP concentration of more than 1 mg/dl) among the normal weight participants was nearly 30% which is considered high when compared to previous studies like the Third National Health and Nutrition Examination Survey (NHANES III). In this study, which included 16 616 adult US population from 1988-1994, clinically raised CRP levels (a CRP
concentration of more than 1 mg/dl) were present in 4.4% of men and 8.9% of women. In the same study, and with increasing BMI, the prevalence of clinically raised CRP levels increased to 7.7% in over-weight women, and 20.2% in obese women. These results are in agreement with our study, but the prevalences were much higher in our study. Accordingly, this will put our over-weight and obese participants at an increased risk for cardiovascular disease.

In the current study, we included healthy participants with no previous history of diseases or conditions that might affect the accuracy of the results and also reduce any chances or possible reasons for raising hs-CRP levels. However, in this study, two factors emerged to affect hs-CRP levels in addition to BMI and they were significantly associated with the high hs-CRP, and those were age of 30 years or higher, and not practicing regular exercise. But after performing the multiple regression analysis [including hs-CRP as a dependent variable and the above mentioned parameters (age, exercise, and BMI) as independent variables], BMI was found to be the only factor that was significantly correlated with hs-CRP. Similar results were observed by other studies. On this base, and taking into account the results of this study, we recommend that all necessary and required measures should be taken to produce changes in the diet, physical activity, and lifestyle so that the BMI will be maintained within the normal range and as a result, the incidence of cardiovascular and other chronic diseases will be reduced.

Conclusion

BMI was found to be the only factor that is significantly and positively correlated with hs-CRP. So irrespective of the other factors, we can predict the presence of high hs-CRP by knowing the BMI of the individual; hence we can prevent the incidence of CVDs.

Conflict of Interest: No conflict of interest was declared by the author.

Financial Disclosure: The author declared that this study has received no financial support.

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Effect of 410 nm Diode Laser Irradiation on Human Sperm Motility in Vitro

Sama E. Mohammed, Layla M.H. Al-ameri

1Researcher, 2Lecturer, Institute of Laser for Postgraduates Studies, University of Baghdad, Iraq

Abstract

Background: Infertility can be defined as an absence of pregnancy after 12 months of regular unprotected sexual intercourse, the most important of the sperm function disorders are reduced sperm motility (asthenospermia). Photobiomodulation have an effect on the decrease of the sperm disorder.

Materials and Methods: Diode laser with wavelength of 410nm was used to induce the sperm motility in an asthenozoospermia human seminal fluid in vitro, 20 fresh seminal fluids samples were used in this study and each sample was divided into two parts, one of them non-irradiated by laser light (control) and the other was exposed to laser irradiation with exposure time of 30 seconds of 410nm diode laser with 0.67 W/cm², power density.

Results: Progressive motility was significantly increased while non-progressive motility was non significantly increased and the immotile sperms were significantly decreased at 5 min following irradiation compared to non-irradiated samples, while at 15, 30, 45 min following irradiation begin to decreased. We found that low power laser could induce a short term bio stimulation effect on sperm motility.

Key words: asthenozoospermia, bio-stimulation, laser.

Introduction

Infertility can be defined as an absence of pregnancy after 12 months of regular unprotected sexual intercourse (1). Male infertility factor is a term that involves a variety of different sperm function disorders that can make fertilizing an egg under normal conditions difficult for sperm, problems with male fertility factor due to changes in the quality of the semen as evaluated by the semen analysis, the most important of these are reduced sperm motility (asthenospermia), defective sperm morphology (teratospermia), and low concentration of sperm (oligospermia), semen volume and other seminal markers of prostatic, epididymal and seminal vesicle function are less well associated with infertility (2). The sperm motility is one of the most essential parameters required to determine the reproductive capability of the semen sample because of male with complete lack of motile sperm is sterile since that immotile sperm either it dead or alive cannot penetrate the cervical mucus as well as the fertilizing potential can also be affected by the form of the movement therefore sperm swimming in closed circle cannot easily pass through the uterotubal junction and only sperm with straight motion can fertilize the ova (3), also, that in order to accomplish its biological function of delivering the male haploid DNA complement to the oocyte, the sperm cell must reach the fertilization site in the oviduct, therefore the sperm cell must be able to develop a motility pattern during maturation in the testes to reach the oviduct and retain this motility pattern throughout its migration via the reproductive tract of the female, and The ability of the sperm cell to exhibit motion in vivo as well as in vitro is greatly dependent on its capability to produce ATP that in turn is used by the contractile proteins of the flagellum as the dynein ATPase substrate to transform chemical energy into the mechanical function (4). Low sperm motility can be caused by genital infection, anti-sperm

Corresponding author:
Layla M.H. Al-ameri
E-mail: layla@ilps.uobaghdad.edu.iq
antibodies, inflammation of the accessory sex gland and sperm structural defect, Ultrastructural defects in the sperm midpiece or tail, a condition called immotile cilia syndrome or Kartagener’s syndrome can lead to a complete absence of motility (5). Photobiomodulation or low-level laser therapy is the application of light (usually LED or low power laser in the range of 1 mW to 500 mW) to pathology to facilitate tissue repair, decrease inflammation and reduce pain (6). And in the field of reproductive research, photobiomodulation can be used to improve viability, metabolism, and motility of the sperm cells because of its positive effect on mitochondria due to the activation of the mitochondrial respiratory chain, and the production of ATP, this therapy can certainly be helpful for preventing the use of certain chemicals in the culture medium of spermatozoa and also in promoting the survival and the motility of the sperm cells particularly the following thawing or in largely immotile sperm samples (7). Several studies used the laser to enhance the sperm motility, (8) found that 830 nm diode laser improve progressive motility depending on both post-exposure time and laser density. (9) study the diode laser effect on human sperm motility by comparing two different wavelengths red (635 nm) and infrared (830 nm) with each other and with no laser control group, all sperm motility parameters are significantly increased in both infrared and red with no statically significant difference between two laser group and slightly better results in the infrared group. (10) study the effect of He-Ne laser 632.8nm on the motility of sperm in asthenozoospermia samples in vitro, the results show that progressive motility in treated samples increased significantly while non-progressive motility is not significantly increased also the percentage of non-motile sperms are significantly decreased.

Aim of the Study

The main purpose of this study is to evaluate how exposure to continuous wave 410 nm diode laser effect the motility of sperm.

Materials and Method

This study was conducted at the laboratory in the institute of laser for postgraduate study at university of Baghdad during period from February to September 2020, the semen sample of twenty males with decreased sperm motility (Asthenozoospermia), and age between (20-43 years old) was selected then used after routine semen analysis. After a sexual abstinence time of (48-72) hours, all samples were obtained by masturbation of males into a wide-mouthed sterile specimen container. Samples were then incubated to be liquefied at 37°C for 30 min. After liquefaction, each sample was divided into two parts: one was called control and the second was exposed to the laser beam.

A Continuous-wave 410 nm diode laser with 100 mW output power and laser diameter of 0.15 cm² was used in this experiment. Each sample was divided into two portions one called control (non-irradiated) and the second was exposed to the laser beam for 30 seconds. 1 ml of each liquefied sample was put in the Eppendorf tube and the laser probe was positioned at 30 cm distance above the Eppendorf tube. Irradiation was performed from above that mean whole sample was evenly irradiated (the common method for irradiating liquid samples).

Computer Assisted Semen Analysis (Mira-9000 CASA) was used to evaluate the motility of the sperm, this system follows WHO (2010) strict criteria for motility patterns and morphometric assessment of human semen. The motility of the sperm was assessed after 5, 15, 30, 45 min after irradiation, after every measurement the sample was put in the incubator at 37°C to overcome the influence of temperature on the motility of the spermatozoa.

The statistical analysis was carried out by SPSS (v 20). ANOVA test was used to analyze repeated measure between irradiated sample and control. Data expressed as mean±SE. Values of p>0.05 were considered statically non-significant while p≤0.05 and <0.01,0.001 were considered significantly different, highly significantly different respectively. Estimate of correlation coefficient between different parameters in this study.


Table 1: Results of progressive motility

<table>
<thead>
<tr>
<th>Time following irradiation (min)</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.33 ± 1.69</td>
<td>15.38 ± 2.54</td>
<td>12.27 ± 1.58</td>
<td>11.32 ± 1.36</td>
</tr>
<tr>
<td>irradiated</td>
<td>25.06 ± 2.54</td>
<td>22.75 ± 1.36</td>
<td>19.63 ± 1.75</td>
<td>17.05 ± 1.45</td>
</tr>
<tr>
<td>Mean± SE</td>
<td>1.69 ± 2.54</td>
<td>1.58 ± 1.36</td>
<td>1.36 ± 1.75</td>
<td>1.34 ± 1.45</td>
</tr>
</tbody>
</table>

P value c vs T * 0.01 0.01 0.01 0.006

*c: control, T: irradiated

Table 2: Results of non-progressive motility

<table>
<thead>
<tr>
<th>Time following irradiation (min)</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.88 ± 1.19</td>
<td>12.93 ± 1.12</td>
<td>11.26 ± 0.99</td>
<td>10.67 ± 1.11</td>
</tr>
<tr>
<td>irradiated</td>
<td>16.84 ± 0.88</td>
<td>14.91 ± 1.12</td>
<td>14.63 ± 1.07</td>
<td>13.36 ± 0.94</td>
</tr>
<tr>
<td>Mean± SE</td>
<td>1.19 ± 0.88</td>
<td>1.12 ± 1.07</td>
<td>0.99 ± 1.07</td>
<td>0.94 ± 1.07</td>
</tr>
</tbody>
</table>

P value c vs T 0.19(NS) 0.23(NS) 0.02 0.07(NS*)

*NS: non-significant

Table 3: Results of immotile sperm

<table>
<thead>
<tr>
<th>Time following irradiation (min)</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>68.88 ± 2.7</td>
<td>71.34 ± 2.29</td>
<td>75.43 ± 1.88</td>
<td>75.35 ± 2.36</td>
</tr>
<tr>
<td>irradiated</td>
<td>58.22 ± 2.87</td>
<td>61.18 ± 2.61</td>
<td>66.76 ± 2.47</td>
<td>69.1 ± 2.23</td>
</tr>
<tr>
<td>Mean± SE</td>
<td>2.7 ± 2.87</td>
<td>2.29 ± 2.61</td>
<td>1.88 ± 2.47</td>
<td>2.36 ± 2.23</td>
</tr>
</tbody>
</table>

P value C vs T 0.01 0.006 0.008 0.06 NS
Table 4: Results of total motility (progressive and non-progressive motility)

<table>
<thead>
<tr>
<th>Time following irradiation (min)</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.66 ± 2.48</td>
<td>31.22 ± 2.98</td>
<td>24.59 ± 2.21</td>
<td>24.64 ± 2.41</td>
</tr>
<tr>
<td>Irradiated</td>
<td>42.02 ± 2.76</td>
<td>37.77 ± 2.45</td>
<td>33.91 ± 2.16</td>
<td>31.86 ± 2.57</td>
</tr>
</tbody>
</table>

P value
C vs T 0.01 0.09 (NS) 0.004 0.03

Results and Discussion

Diode laser with wavelength 410 nm was used to enhance the motility of the sperm. As shown in the table 1 the progressive motility was significantly increased after laser irradiation, the maximum influence of laser on the progressive motility was at 5 min following irradiation and the progressive motility of control samples was decreased by passing of time while non-progressive motility non-significantly increased after laser irradiation as shown in results in table 2, also the number of immotile sperm of irradiated samples was significantly decreased and total motility was significantly increased.

We supposed that the low power laser can enhance the mitochondrial respiratory chain components and increased the energy supply to spermatozoa and this study is the first study that use laser with wavelength of 410 nm to enhance the human sperm motility in vitro. One of the most important causes of reduced sperm motility is characterized by impaired integrity of the mitochondrial membrane and impaired function of its sheath because mitochondria provide apart of energy necessary for the motility of the sperm as light is applied to cells, the first sites of light absorption are mitochondria and the photoreceptors are assumed to be cytochromes, consequently photon absorption induces a sequence of reactions known as cellular signaling pathways which result in ATP synthesis as well as the production of ROS, mitochondria house the electron transfer chain which includes complex I, II, III, and IV and photon absorption by these complexes induce electrical excited states which can speed up electron transfer reactions and this result in increased ATP synthesis because of more electron transport necessitate for increased ATP production. Flavins and flavoproteins such as Flavin mononucleotide and Flavin dinucleotide are believed to be excited by blue light (400-500 nm), complex II, is a Flavin containing cytochrome (contain FADH2) that absorb blue light, as a result, it’s possible that blue light may influence mitochondrial function like red and NIR light.

Conclusion

We found that low power laser could induce a short term bio stimulation effect on the motility of the sperm.

Source of Funding – self

Conflict of Interest – Nil

Ethical Clearance – Before the study began, the institutional ethical and research committee gave their approval.

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Indonesian National Social Security Management Law Problematics with the Enabling of Law Number 40 of 2004 Concerning a National Social Security System

Nurrochmah Ihayani 1, Nurul Istiqomah S H 1, Sitti Nursanti 1, Budi Pramono 2
1 Master of Law Students at Hang Tuah University, Surabaya, Indonesia, 2 Lecturer, Faculty of Law, Hang Tuah University, Surabaya Indonesia

Abstract
This study discusses legal problems that occur in the management of Indonesia’s national social security with the enactment of Law number 40 of 2004. This research is a normative legal research with a conceptual and statutory approach. This study uses the theory of Laurence M. Friedman as a knife of analysis that the problem of managing national social security includes institutional structures, substance, the Healthcare and Social Security Agency and The National Social Security regulation and legal culture. It is hoped that the management of national social security will accelerate the transfer of management of the Savings Fund and Civil Servant Insurance Company and the Social Insurance of the Armed Forces of the Republic of Indonesia Company, so that they do not have to wait up to 25 years.

Keyword: Law Problematics, National Social Security System, Healthcare and Social Security Agency

Introduction
The right to obtain the fulfillment of social security for all Indonesian people is stipulated in Article 28 H paragraph (3) of the 1945 Constitution of the Republic of Indonesia, which states that “Everyone has the right to social security which enables his or her complete development as a dignified human.” This norm is then reinforced in Article 34 paragraph (2) of the 1945 Constitution of the Republic of Indonesia, which explains that “The state develops a social security system for all people and empowers people who are weak and underprivileged according to human dignity.” These articles constitute a material constitutional reason in the field of social security, which confirms that social security is a right not a privilege.

Corresponding author:
Budi Pramono
Lecturer, Faculty of Law, Hang Tuah University, Indonesia
Contact: Tel.+6281372479999
Email – budi.pramono@hangtuah.ac.id

The concept of social security for all Indonesian people is then accommodated in state life with the passing of Law Number 40 of 2004 concerning the National Social Security System, Law Number 11 of 2009 concerning Social Welfare, Law Number 24 of 2011 concerning Healthcare and Social Security Agency (BPJS), and Presidential Regulation Number 64 of 2020 concerning Second Amendment to Presidential Decree Number 82 of 2018 concerning Health Insurance.

With the enactment of this National Social Security System Regulation, there has been a change in legal norms, from which the government’s obligation to fulfill the right to health of the people becomes the obligation of the people to pay dues in order to fulfill their rights. Subsequent developments, in 2011 the Healthcare and Social Security Agency Regulation, was enacted which appointed a government-owned implementing agency that had the role of an insurance company.

This fact shows that the legal politics of the formation of National Social Security System Regulation...
and Healthcare and Social Security Agency Regulation have not shown a responsive-populist character that prioritizes the interests of the people. These two legal products have not fulfilled the rights to social welfare and health of the Indonesian people, which should be the responsibility of the government (state). Therefore, the President and the DPR together need to make changes to the two legal products so that they are in accordance with the mandate of the 1945 Constitution of the Republic of Indonesia. The purpose of this research is to knowing law problematics with the enabling of law number 40 of 2004 concerning a national social security system.

Methods

To answer the problems that have been formulated in this study, a normative legal research type is used with a statutory and conceptual approach.

Result and Analysis

Social security is part of the concept of social protection, where social protection is broader in nature. The difference between the two is that social security provides social protection for individuals with funds obtained from periodic contributions, whereas social protection usually involves many parties in providing protection either to individuals, families or communities from various unpredictable life risks such as economic crises, or natural disasters. Health issues are valuable things that the people yearn for, so that the fulfillment of the right to health is one of the basic needs of the people. Health is the basis of recognition of human dignity, an unhealthy individual will automatically reduce his right to life, he cannot exercise his right to work properly, he cannot use his right to congregate and express thoughts, he cannot use his right to education for his future, and so.

The following will explain the problems in the management of national social security which include aspects of its structure, substance and culture or legal culture.

1. Problems in the Institutional Structure of The National Social Security

The National Social Security institution is one of the elements of the National Social Security System with the function of carrying out the realization of social security objectives which have been formulated in accordance with the underlying principles. The National Social Security System administration is formed by two organs, namely the National Social Security Council (DJSN) and the Healthcare and Social Security Agency (BPJS).

The Healthcare and Social Security Agency (BPJS) is a legal entity established under The Healthcare and Social Security Agency Regulation to administer social security programs. Law Number 24 Year 2011 established two Healthcare and Social Security Agency, namely Healthcare and Social Security Agency that focused on healthcare (BPJS Kesehatan) which functions to organize health insurance programs and Healthcare and Social Security Agency that focused on Labor (BPJS Ketenagakerjaan) which functions to organize work accident insurance, death benefits, old age savings and pension benefits.

Since the enactment of the Healthcare and Social Security Agency regulation, the existing social security administering body is declared the Healthcare and Social Security Agency according to this law. This is in accordance with the norms of Article 5 of the National Social Security System regulation, the formulation is as follows:

(1) Healthcare and Social Security Agency (BPJS) must be established by law.

(2) Since the enactment of this Law, the existing Healthcare and Social Security Agency (BPJS) is declared a Healthcare and Social Security Agency according to this Law.

(3) The Healthcare and Social Security Agency as referred to in paragraph (1) are:

   a. Labor Social Security Company (Jamsostek);

   b. Savings Fund and Civil Servant Insurance Company (Taspen);
c. Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri); and

d. The Company (Persero) Indonesian Health Insurance (Askes).

(4) In the event that a Healthcare and Social Security Agency other than those referred to in paragraph (3) is required, a new one can be formed by law.

Article 52 National Social Security System Regulation have functions to fill the legal vacuum so that Social Security, Savings Fund and Civil Servant Insurance Company (Taspen), Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri), and The Company (Persero) Indonesian Health Insurance (Askes) are still valid as long as they have not been adjusted to this law. Based on the provisions of Article 52 paragraph (2), it is given 5 (five) years from the time of promulgation to comply with this law. The transfer mechanism of Social Security, Savings Fund and Civil Servant Insurance Company (Taspen), Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri), and The Company (Persero) Indonesian Health Insurance (Askes) from the Ministry of Indonesian State-Owned Enterprises (BUMN) to become under The Healthcare and Social Security Agency through a transformation program.

The following will explain the development and transformation process of Social Security, Savings Fund and Civil Servant Insurance Company (Taspen), Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri), and The Company (Persero) Indonesian Health Insurance (Askes) which were previously under the Ministry of the Indonesia State-Owned Enterprises to become under the authority of The Healthcare and Social Security Agency.


The preparation period for the transformation of The Company Indonesian Health Insurance (Askes) into the Healthcare and Social Security Agency (BPJS Kesehatan) for two years starting November 25, 2011 to December 31, 2013. During the preparation period, the Board of Commissioners and Directors of The Company Indonesian Health Insurance (Askes) were given the task of preparing the Healthcare and Social Security Agency (BPJS Kesehatan) operations, as well as preparing for asset transfers, and The Company Indonesian Health Insurance (Askes) liabilities, employees and rights and obligations to Healthcare and Social Security Agency (BPJS Kesehatan).

Preparation for the transfer of assets and liabilities, employees and rights and obligations of The Indonesian Health Insurance Company (Askes) to the Healthcare and Social Security Agency (BPJS Kesehatan) , including the appointment of a public accounting firm to audit: (1) the closing financial statements of The Indonesian Health Insurance Company (Askes); a report on the financial position of the opening Healthcare and Social Security Agency (BPJS Kesehatan); report on the financial position of the opening of the health insurance fund. When the Healthcare and Social Security Agency (BPJS Kesehatan) started operating on January 1, 2014, The Indonesian Health Insurance Company (Askes) was declared disbanded without liquidation. All assets and liabilities as well as legal rights and obligations of PT Askes (Persero) become assets and liabilities as well as legal rights and obligations of Healthcare and Social Security Agency (BPJS Kesehatan), and all employees of The Indonesian Health Insurance Company (Askes) become employees of Healthcare and Social Security Agency (BPJS Kesehatan).

At the same time, the Minister of Indonesian State-Owned Enterprises (BUMN) As General Meeting Of Shareholders ratified the closing financial position report of The Indonesian Health Insurance Company (Askes) after an audit of the public accounting firm. The Minister of Finance ratifies the opening financial position report for the Healthcare and Social Security Agency (BPJS Kesehatan) and the opening financial report for the health insurance fund. For the first time, the Board of Commissioners and Directors of The Indonesian Health Insurance Company (Askes) were
appointed as the Supervisory Board and the Board of Directors of the Healthcare and Social Security Agency (BPJS Kesehatan) for a maximum period of 2 (two) years since the Healthcare and Social Security Agency (BPJS Kesehatan) began operating. Starting January 1, 2014, the social health insurance program that has been implemented by the government has been transferred to the Healthcare and Social Security Agency (BPJS Kesehatan). The Ministry of Health no longer operates the Public Health Security (Jamkesmas) program. The Ministry of Defense, The Armed Forces and Police no longer carry out health service programs for their participants, except for certain health services related to their operational activities as determined by Government Regulations. The Labor Social Security Company (Jamsostek) no longer maintains an employee health insurance program.1

b. Transformation of The Labor Social Security Company (Jamsostek)

The transformation of the Labor Social Security Company (Jamsostek) is carried out in two stages, namely the first stage is the transition period of Labor Social Security Company (Jamsostek) to the Healthcare and Social Security Agency Employment (BPJS Tenaker) which lasts for 2 (two) years, from 25 November 2011 to 31 December 2013. The first stage ends with the establishment of the Healthcare and Social Security Agency Employment (BPJS Tenaker) on January 1 2014. the Healthcare and Social Security Agency Employment (BPJS Tenaker) will continue the implementation of three the Labor Social Security (Jamsostek) programs, namely the work accident program, the old age security program, and the death security program no later than 18 (eighteen) months later (1 January 2014–3 June 2015). Provisions for the implementation of these three programs are still based on Law Number 3 of 1992 concerning Social Security.1

The second stage is the preparation stage for the operationalization of the Healthcare and Social Security Agency Employment (BPJS Tenaker) for the implementation of work accident insurance, old-age savings, pension and death security programs. The second stage of preparation takes place no later than 30 June 2015 and ends with the operation of the Healthcare and Social Security Agency Employment (BPJS Tenaker) for the implementation of the four programs in accordance with the provisions of the National Social Security System regulation by 1 July 2015.

Preparation for the transfer of assets and liabilities, employees and the rights and obligations of the Labor Social Security Company (Jamsostek) to the Healthcare and Social Security Agency Employment (BPJS Tenaker) includes the appointment of a public accounting firm to audit the closing financial statements of the Labor Social Security Company (Jamsostek), report on the financial position of the opening of the Healthcare and Social Security Agency Employment (BPJS Tenaker), and reports on the financial position of opening funds. employment guarantee. As with the dissolution of Health Insurance Company (Askes) on January 1, 2014 the Labor Social Security Company (Jamsostek) was declared dissolved without liquidation and the Labor Social Security Company (Jamsostek) changed to the Healthcare and Social Security Agency Employment (BPJS Tenaker). Government Regulation Number 36 of 1995 concerning the Determination of the Manpower the Healthcare and Social Security Agency is revoked and declared no longer valid.

All assets and liabilities as well as legal rights and obligations of the Labor Social Security Company (Jamsostek) become assets and liabilities as well as legal rights and obligations of the Healthcare and Social Security Agency Employment (BPJS Tenaker). All employees of the Labor Social Security Company (Jamsostek) are employees of the Healthcare and Social Security Agency Employment (BPJS Tenaker). At the time of dissolution, the Minister of Indonesian State Owned Enterprises (BUMN) As General Meeting of Shareholders ratified the closing financial position report of the Labor Social Security Company (Jamsostek) after an audit was carried out by a public accounting firm. The Minister of Finance ratifies the position of the financial statement for the opening of the the Healthcare and
Social Security Agency Employment (BPJS Tenaker) and the financial position report for the opening of the employment guarantee fund. Since January 1, 2014 until no later than June 30, 2015. The Healthcare and Social Security Agency Employment (BPJS Tenaker) will continue the implementation of the three programs that have been organized by the Labor Social Security Company (Jamsostek), namely the work accident insurance, old age insurance and death insurance programs, including accepting new participants.

c. Transformation of the Savings Fund and Civil Servant Insurance Company (Taspen) and the Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri)

The Healthcare and Social Security Agency Regulation did not dissolve the Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri) and the Savings Fund and Civil Servant Insurance Company (Taspen), nor did they transfer the two Persero to become The Healthcare and Social Security Agency. The Healthcare and Social Security Agency Regulation does not regulate the dissolution of the agency, transfer of assets and liabilities, transfer of employees and the rights and obligations of the Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri) and the Savings Fund and Civil Servant Insurance Company (Taspen). The Healthcare and Social Security Agency Regulation only transfers the second function of the Company, namely the implementation of the old age protection program and pension payments organized by both of them to the Healthcare and Social Security Agency Employment (BPJS Tenaker) by 2029. The Healthcare and Social Security Agency Regulation delegates the arrangements for program transfer procedures that are carried out by the two of them to a Government Regulation. Article 65 paragraph (1) and paragraph (2) of Law no. 24 of 2011 concerning the Healthcare and Social Security Agency requires The Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri) and the Savings Fund and Civil Servant Insurance Company (Taspen), to compile a transformation roadmap no later than 2014. The transfer of the Savings Fund and Civil Servant Insurance Company (Taspen), and The Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri) from the Ministry of Indonesian State-Owned Enterprises (BUMN) to the Healthcare and Social Security Agency no later than 2029, which requires a period of 25 years is very unrealistic. The government, in this case the Indonesian State-Owned Enterprises (BUMN) Ministry, seems to be delaying in transferring its management to the Healthcare and Social Security Agency. The Litigation Director of the Ministry of Law and Human Rights explained that, regarding this transfer, the government represented by the Ministry of Law and Human Rights and the Ministry of Manpower have a different view. Director of Litigation for Laws and Regulations of the Ministry of Law and Defense, Ardiansyah, revealed that the transfer of the program from the Savings Fund and Civil Servant Insurance Company (Taspen) and The Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri) to Healthcare and Social Security Agency (BP Jamsostek) is an order of the National Social Security System-related laws that the government must realize, but it must be understood that the matter of transfer is actually the program, not the institution within the National Social Security System. He said, programs other than those implemented by Healthcare and Social Security Agency (BP Jamsostek) could still be held by the Savings Fund and Civil Servant Insurance Company (Taspen) in the National Social Security System regulations and institutions, while the Old Age Security (JHT) and Pension Security (JP) benefits programs were still held by Healthcare and Social Security Agency (BP Jamsostek).
The regulations are clear. Law Number 24 of 2011 concerning The Healthcare and Social Security Agency, Law Number 40 of 2004 concerning Social National Security System. The implementation is how program transfer will be derived through a Government Regulation (PP). Regarding the transfer of the program from the Savings Fund and Civil Servant Insurance Company (Taspen) and The Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri) to Healthcare and Social Security Agency (BP Jamsostek), he emphasized, it would not cause overlapping regulations. Because, Ardiansyah continued, from the start there had been cross-ministerial coordination and harmonization facilitated by the State Secretariat (Setneg). There was even an assignment for Ministry of State Apparatus Utilization and Bureaucratic Reform to follow up the Minister Regulation program transfer. Including the roadmap like what because it involves civil servants, said Ardiansyah.2

The Indonesian State-Owned Enterprises (BUMN) Ministry’s move is understandable because indeed one of the duties of the Indonesian State-Owned Enterprises (BUMN) ministry is to carry out a business that functions to increase non-tax state revenue. According to the author’s analysis, it is not very suitable if the Savings Fund and Civil Servant Insurance Company (Taspen), and The Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri) have social functions, but in their organizational structure under the Ministry of Indonesian State-Owned Enterprises (BUMN), whose main task is to carry out business (business) to increase non-tax state revenue, so it is more appropriate to accelerate the transfer. The Savings Fund and Civil Servant Insurance Company (Taspen), and The Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri) from the Ministry of Indonesian State-Owned Enterprises (BUMN) to The Healthcare and Social Security Agency.

2. The National Social Security System Substance Problems.

Problems related to the substance of the National Social Security System Regulation that can interfere with the implementation of the national social security system, the problem is that National Social Security System Regulation combines social security with social insurance. The formation of National Social Security System Regulation and the Healthcare and Social Security Agency Regulation which combine social security with social insurance has dragged legislators into the neo-liberalism scenario. Neo-liberal theory has its roots in classical political work by Thomas Hobbes, John Lock and John Stuart Mill which essentially argued that an essential component of a society is individual freedom. Broadly speaking, neo-liberal proponents argue that social security should be provided by self-help groups, religious institutions or by families. The role of the state is only as a residual agent or night guard who can only interfere when the above institutions are no longer able to carry out their duties.3

Social security is a system of giving money and or social services to protect a person from the risk of not having or losing income due to accident, disability, illness, unemployment, pregnancy, old age, and death. Paul Spicker (1995), provides limits and explanations for social security as follows:

The term social security is mainly now related to financial assistance, but the general sense of the term is much wider, and it is still used in many countries to refer to provisions for health care as well as income. Although the benefits of security are not themselves material, they do have monetary value; people in Britain, where there is a National Health Service, are receiving support which people in the US have to pay for through private insurance or a Health Maintenance Organisation.4

Social security can be provided through the social insurance system which is funded by insurance premiums or through social assistance whose funds are obtained from tax revenues. Social insurance is determined based on insurance expertise, the provision of insurance benefits is calculated based on insurance premiums, the government together with other public
Institutions are the providers of social insurance. Social insurance membership is compulsory (obligatory), the medical insurance system and pension insurance are two types of social insurance that are very widely known.

The definition of insurance in general is a system for a group of people to protect the risks that may occur to them. A number of people who are deemed to have a similar risk form a group, and each member of the group pays a premium as a precondition for obtaining benefits when facing an accident or risk in the future. Meanwhile, the definition of social insurance is social security provided to insurance participants based on the premiums they pay. The formation of the National Social Security System Regulation and the Healthcare and Social Security Agency Regulation has not been in accordance with the mandate of Article 28H paragraph (3) of the 1945 NRI Constitution, the formulation of which is as follows:

(1) Every person has the right to social security which enables his complete development as a dignified human being.

The formation of the National Social Security System Regulation and the Healthcare and Social Security Agency Regulation has changed the government’s obligation to fulfill the right to people’s health to become an obligation for the people to pay dues in order to fulfill their right to health. The right to health is a fundamental right and must be realized in accordance with the aspirations of the people as stated in Pancasila and the 1945 Constitution of the Republic of Indonesia, so that legal support is needed to realize the health status of all Indonesian people. Legal support aims to ensure that the government can fulfill the right to health of the Indonesian people. Legal support aims to ensure that the government can fulfill the right to health of the Indonesian people. Legal support aims to ensure that the government can fulfill the right to health of the Indonesian people. Legal support aims to ensure that the government can fulfill the right to health of the Indonesian people.

Health issues are valuable things that the Indonesian people crave, so that the national social security system must be able to protect the people in a certain way. Therefore, the fulfillment of the right to health is one of the basic needs of the people.

The establishment of the the Healthcare and Social Security Agency Regulation appointed a government-owned implementing agency to act like an insurance company, which could collect fees, manage funds, impose sanctions for those who do not pay premiums, and make investments. The role of the implementing agency like this has the potential to shift the role of the government-owned administering body to the role of the private-owned administering body. This concern began to appear when the Coordinating Minister for Maritime Affairs LB. Pandjaitan plans to invite China’s Ping An Insurance company to help the Social Security Administration Agency, which is currently in financial deficit. Concerns grew when the Coordinating Minister for Human Development and Culture confirmed the increase in dues starting January 1, 2020. The people had high hopes for the government to safeguard national sovereignty and interests. The government must be responsible for fulfilling the right to health of the Indonesian people, lest the fulfillment of the right to health of the Indonesian people be delegated to private-owned administering bodies. This consideration is based on the idea that the formation of laws relating to the fulfillment of the right to health of the people must prioritize national sovereignty and interests. The Indonesian government needs to take lessons from the Australian Government which is strong in its stance to protect and safeguard national sovereignty and interests.

In fact, the issuance of the National Social Security System Regulation and the Healthcare and Social Security Agency Regulation is not a complete and perfect legal product, so that some legal experts have criticized it. Jimly Ashhiddiqie suggested that the the Healthcare and Social Security Agency Regulation be revised immediately because many problems arise in the governance run by the the Healthcare and Social Security Agency. Jimly Ashhiddiqie’s suggestion needs to be responded to by the government and the member of Indonesia parliament, with the consideration that the fulfillment of the right to health is a form of basic rights as stipulated in the 1945 Constitution of the Republic of Indonesia. The President and the the member of Indonesia parliament need to make changes to the two legal products so that they are in accordance with the orders of the 1945 Constitution of the Republic.
of Indonesia.

3. Problems in the field of Legal Culture

According to Hilman Hadikusuma, legal culture is the same general response from certain societies to legal phenomena, this assumption is a unified view of legal values and behavior. So the legal culture shows a pattern of individual behavior as members of society that describes the same response (orientation) to the legal life lived by the community concerned. Legal culture is closely related to legal awareness and is manifested in the form of behavior as a reflection of legal compliance in society. In the legal culture, it can be seen that a tradition of daily community behavior is in line with and reflects the will of the law or legal signs that have been established which apply to all legal subjects in the life of the nation and state. In the legal culture of society, it can also be seen whether the community in its legal awareness has really upheld the law as a rule of the game in living together and as a basis for solving any problems that arise from the risk of living together.

The potential for fraud in health services is increasingly visible, emerges and widens due to pressure from the new financing system, opportunities due to lack of supervision, and there is justification for taking action. Shariari (2010) explains that the potential for fraud from clinical groups may arise due to several things, which are as follows:

1. low paid medical personnel,
2. there is an imbalance between the health service system and the burden of health services,
3. service providers do not provide adequate incentives,
4. a shortage of medical equipment supply,
5. inefficiencies in the system,
6. lack of transparency in health facilities, and
7. cultural factors.

From the questionnaire distributed to the blended learning participants with the topic of prevention, detection, and enforcement of hospital group health services fraud in 2015, the INA CBG’s rates which are considered low by clinicians and the high workload make them think of unreasonable efforts to defend themselves so as not to until you lose. The worse thing is that these clinicians sometimes share experiences in this self-rescue effort. They then apply these experiences in providing health services so that it becomes a culture. The basis for setting price list is also mysterious for most groups, causing dissatisfaction with the system.

This dissatisfaction also encourages doctors and hospitals to implement coping strategies as a step to cover up their shortcomings or at least to seek profit even from something illegal. This coping mechanism is present when the surveillance system is weak and is unable to cover opportunities for individuals to commit fraud. Certainly people will continue to commit this fraud as long as they can still enjoy the benefits with the opportunity that is always open to commit fraud. A systematic and ongoing effort is needed to control health service fraud. Activities in the anti-fraud system must be in the form of a cycle starting from awareness building, reporting, detection, investigation, imposing sanctions, and returning to awareness building. Awareness building is the key to preventing or spreading fraud in health services. Awareness building can be done in the form of socialization and education.

In the regulatory group, prior to the issuance of Minister of health regulations Number 36 of 2015, regulators such as district / city health offices assumed that the responsibility for eradicating fraud lies with the Indonesian Ministry of Health or only reaches the provincial level health office. Whereas at the regional level, the authority to build an anti-fraud system lies with the district / city health offices. They also thought that their authority was only to control fraud at the Primary Level Health Facility (FKTP) level and not to the Advanced Level Referral Health Facility (FKRTL). However, after being given socialization and education, the awareness of this regulatory group increased. Education and advocacy for regulatory groups (district
process of detecting potential fraud. Other challenges faced in the detection process include the limited human resources to process the available data. The Healthcare and Social Security Agency has a lot of claim data that can be used as a source of potential fraud detection. However, limited technology and human resources hinder this process. Furthermore, this large amount of data has not been optimally utilized to develop potential fraud detection technology. Investigations are carried out to confirm the alleged national health insurance (JKN) fraud, an explanation of what happened, and the background / reasons thereof. The challenge faced in Indonesia is that currently there are no special investigators for health service fraud. In contrast to the United States, which already has a special investigator profession for fraud cases in the health sector, which is a member of the Association of Healthcare Fraud Investigators (AHFI). After the investigation process, the investigator will provide recommendations for sanctions for the perpetrators who are proven to have committed fraud. Firm sanctions for perpetrators of fraud will have a deterrent effect. In Minister of health regulations Number 36 of 2015, administrative sanctions can be given to perpetrators of fraud. However, currently these sanctions have not yet been firmly applied.

There are legal problems related to the management of the National Social Security (JSN), which includes the structure, substance and culture of law. In the National Social Security (JSN) structure, the transfer of the Savings Fund and Civil Servant Insurance Company (Taspen) and the Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri) from the Ministry of the Indonesia State-Owned Enterprises to Healthcare and Social Security Agency in 2029 requires a period of 25 years, although juridically in accordance with statutory regulations, it is unrealistic. The formation of the National Social Security System Regulation and the Healthcare and Social Security Agency Regulation has changed the government’s obligation to fulfill the right to people’s health into an obligation of the people to pay contributions so that the right to health is fulfilled, which is not in accordance with the norms of Article 28.
Paragraph (3) of the 1945 Constitution. The problem with the legal culture that was found was that there was fraud that occurred in various fields, from the basic to the high ranking officials. The management of national social security is carried out by accelerating the transfer of management of the Savings Fund and Civil Servant Insurance Company (Taspen) and the Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri) from the Ministry of the Indonesia State-Owned Enterprises to Healthcare and Social Security Agency, then synchronizing several laws and regulations in the field of social security systems.

Conclusion

There are legal problems related to the management of the National Social Security (JSN), which includes the structure, substance and culture of law. In the National Social Security (JSN) structure, the transfer of the Savings Fund and Civil Servant Insurance Company (Taspen) and the Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri) from the Ministry of the Indonesia State-Owned Enterprises to Healthcare and Social Security Agency in 2029 requires a period of 25 years, although juridically in accordance with statutory regulations, it is unrealistic. The formation of the National Social Security System Regulation and the Healthcare and Social Security Agency Regulation has changed the government’s obligation to fulfill the right to people’s health into an obligation of the people to pay contributions so that the right to health is fulfilled, which is not in accordance with the norms of Article 28 H Paragraph (3) of the 1945 Constitution. The problem with the legal culture that was found was that there was fraud that occurred in various fields, from the basic to the high ranking officials. The management of national social security is carried out by accelerating the transfer of management of the Savings Fund and Civil Servant Insurance Company (Taspen) and the Social Insurance of the Armed Forces of the Republic of Indonesia Company (Asabri) from the Ministry of the Indonesia State-Owned Enterprises to Healthcare and Social Security Agency, then synchronizing several laws and regulations in the field of social security systems.

Ethical Clearance: Nil

Conflict of Interest: Nil

Source of Funding: Self-Funding

Acknowledgement: Nil

References


Effect of Using Smart Mobile Device on Child Prosocial and Difficult Behaviors in School Age; Parents’ Perception

Samar Salah Eldin Mohamed Diab
Assistant Professor of Pediatric Nursing Faculty of Nursing, Menoufia University, Egypt

Abstract

Background: The time that children spend using digital devices is increasing rapidly with the development of new portable and instantly accessible technology. Mobile devices are embedded in and dominate the daily lives of young children. Research Aims: assess the effect of using smart mobile device on child Prosocial and difficult behaviors in school age. Methodology: A cross sectional research design was utilized at August 2019 - January 2020. Convenience sample include the 400 school children. An online survey by using Google form, which contains three parts (characteristics of parents, children and Strengths and Difficulties Questionnaire). Results: revealed that 47.2% and 47.7% of studied children had abnormal emotional symptoms and conduct problems. In addition (34.5%) of studied children was normal related peer problems domain. Also, (51.2%) of them was abnormal related hyperactivity. While, (30%) of studied children had normal Prosocial behavior. Conclusions: the current study concluded that about half of studied children had abnormal Prosocial and difficult behaviors and less than one quarter of them had borderline Prosocial and difficult behaviors. While, less than one third of them had normal Prosocial and difficult behaviors.

Keywords: Smart mobile, Prosocial, difficult behaviors, parent perception

Introduction

Mobile devices (mobile phones, + iPads /tablets, iPods, computers, etc.) play an increasingly important role in today’s rapidly changing and developing the information technology sector. They are becoming the preferred media choice for children because of their mobility, broad content, and interactivity. Mobile devices are embedded in and dominate the daily lives of young children.

Smartphone are becoming an important part of life for people of all ages worldwide, and Smartphone use has begun to change daily activities, family relations, and social interactions. Because smart phones can be carried easily and accessed anywhere and at any time and allow use of a variety of applications, overuse of Smartphone by children and adolescents has emerged as a significant social issue.

The time that children spend using digital devices is increasing rapidly with the development of new portable and instantly accessible technology, such as Smartphone and digital tablets. Furthermore, with the dramatically rapid development of media games, learning packages, and educational applications for young children, opportunities for using mobile devices have been growing, children’s usage time has become increasingly longer, and child target users of mobile devices are becoming younger.

Research suggested that cell phone exposure could affect children’s behavior. The children in the study who were hyperactive or had emotional or behavioral problems, including trouble getting along with other kids, were much more likely to have mothers who used...
cell phones during pregnancy. Excessive screen time is associated with poor sleep and risk factors for metabolic and cardiovascular diseases such as high blood pressure, obesity, low HDL cholesterol, insulin resistance, and reduced bone density.

The current generation is the first generation of children growing up with mobile devices from birth; hence, no empirical longitudinal data exist on the long term effects of mobile device usage on children’s development. A review of the literature on both mobile devices and social interaction practices indicated that, although mobile communication is becoming more prominent, what is not known is the extent to which social competency is affected by the way mobile devices are being used.

Aims
The current study aimed to assess the effect of using smart mobile device on child Prosocial and difficult behaviors in school age.

Research questions:
- What is the effect of using smart mobile device on child Prosocial and difficult behaviors?
- What are factors affect child’s Prosocial and difficult behaviors?

Methods
Research design: A cross sectional research design was utilized at August 2019 - January 2020

Research Setting: The study was carried out at primary schools in Menoufia Governorate. This study was conducted at Al-Nasr Primary School (governmental school) Serce Ellian city, Menoufia governorate. The number of classes in the school was 12 semesters, two classes for each grade, the average number of students per class was 51 children.

Subjects: Convenience sample include the school children were available and enthusiastic to participate at the study regardless gender and graduate year, with inclusion criteria age of children 6 -12 year and their parents had computer skills to full online questionnaire. At our study 422 children’ parents participate at the study and excluding 22 subjects due to incomplete the questionnaires, so total subjects were 400.

The estimated sample size was 422 school children, at confidence level 95%, and the precision rate at 0.05 by using the equation devised by Thompson (2012) as the total number of available of school children was 35200.

P= 0.5, N= Total population, Z= Z value “1.96”, D= Standard Error, n= sample size

Related to the principles and rules of social distancing and limit the spread of COVID-19, we used an online survey and using email, Facebook, Whatsapp and telegram services to collect the data from the parents of children. Google form permits questionnaire design, collection of data, descriptive analysis of results, and download data through excel spreadsheet for extra analysis.

The instruments:
Study instrument was designed by the researchers based on literature review related effect of using mobile on children behavior and included three parts:

Part I: Characteristics of the parents such as age, educational level, average spending time of talking or playing with children, monthly income and number of children.

Part II: Characteristics of the children such as age, gender, academic year, mobile devices child used, purpose of mobile device use and period mobile device used / day.

Part III: The Strengths and Difficulties Questionnaire (SDQ) is a 25-item measure of parents’ perceptions of their children’s Prosocial and difficult behaviors. The measure is categorized into five subscales: conduct problems (five items), hyperactivity/inattention (five items), emotional symptoms (five items), peer problems (five items), and Prosocial behavior (five items). In the
present study, the conduct problems, hyperactivity/inattention, emotional symptoms, and peer problems subscales were used to assess children’s emotional and behavioral problems. Items were rated on a 3-point Likert scale ranging from 0 (Not true), 1 (somewhat true) and 2 (Certainly true). Normal if score from (0 – 18), Borderline if score (19 – 21) and Abnormal if score (22 – 50).

**Pilot Study:**

The pilot study was conducted with 42 students who represent 10% of total sample at the previously mentioned settings in order to test the applicability of the constructed tools and the clarity of the included tools. Also, to assess the reliability and validity of developing tool before using at the study. The pilot also served to estimate the time needed for each subject to fill in the questionnaire.

A group of experts in the pediatric and community nursing ascertained the content’s validity; their opinions were elicited regarding the format, layout, consistency, accuracy, and relevancy of the tools. Reliability testing was carried out to test the reliability in terms of Cranach’s Alpha for instrument was 0.886, after deleted five items to improve reliability of the scale.

Data collected from the studied sample was revised, coded, and entered using Personal Computer (PC). Computerized data entry and statistical analysis were fulfilled using the Statistical Package for Social Sciences (SPSS) version 24. Data were presented using descriptive statistics in the form of number and percent. Pearson correlation coefficient was used to measure of linear correlation between two sets of data. A linear regression model is a linear approach to modeling the relationship between a scalar response and one or more explanatory variables.

**Ethical Consideration:**

The research ethics committee revised and approved the study. The submission of the online answer to the questionnaire was considered as consent to take part in the study. Confidentiality of the study subjects’ data was sustained throughout the study by making the students’ data nameless.

**Results**

Table (1) showed that (39%) of the studied parents their age was 28 - <38 year with mean 40.38±6.75 year and (54%) of maternal educational level was bachelor. Regarding to parent educational level, (39.7%) of them had secondary level. Also, (77.3%) of them spending time of talking or playing with children <60 minutes. Moreover, (56%) of them had insufficient monthly income and (41%) of them had one child.

Table (2) revealed that (40%) of the studied children had age ranged from 8 < 10 year with mean 8.95±2.46 year and (56.2%) of them were female. Regarding to academic year, (25.8%) of them was at third level. Also, (53%) of them used own mobile. Moreover, (50.7%) of them used mobile 60 m or more per day and (78.7%) of them used mobile device for learning/using applications related to education.

Table (3) demonstrated that, 47.2% and 47.7% of studied children had abnormal emotional symptoms and conduct problems. In addition (34.5%) of studied children was normal related peer problems domain. Also, (51.2%) of them was abnormal related hyperactivity. While, (30%) of studied children had normal Prosocial behavior.

Figure (1) illustrated that, (49%) of studied children were abnormal related their total strengths and difficulties questionnaire scale. While, (30%) of them were normal and (21%) of them were borderline related their total strengths and difficulties questionnaire scale.

Table (4) showed that, there were highly statistically positive correlation between emotional symptoms domain and their conduct problems domain, hyperactivity domain and Prosocial behavior domain at (P= 0.01). While there were slight positive correlation between peer problems domain and their emotional symptoms domain, conduct problems domain and hyperactivity domain at (P= 0.05).
Table (5) stated that high significant model detected through F test value was 12.210 with p value .000. This model explain 46% of the variation in children’s strengths and difficulties questionnaire detected through R2 value 0.46. Also, explained that, academic year, mobile device child used “own mobile”, period mobile device used per day had high frequency positive effect on children’ strengths and difficulties questionnaire at p value <0.01**. While, male gender and increasing age of children had slight positive effect on children’ strengths and difficulties questionnaire with p value <0.05.

<table>
<thead>
<tr>
<th>Items</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 - &lt;38</td>
<td>156</td>
<td>39</td>
</tr>
<tr>
<td>38 - &lt;48</td>
<td>164</td>
<td>41</td>
</tr>
<tr>
<td>48 or more</td>
<td>80</td>
<td>20</td>
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<tr>
<td>Mean SD</td>
<td>40.38±6.75</td>
<td></td>
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<tr>
<td>Maternal educational level</td>
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<td>Preparatory</td>
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<tr>
<td>Secondary</td>
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<td>34.2</td>
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<tr>
<td>Bachelor</td>
<td>243</td>
<td>54</td>
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<tr>
<td>Post graduate</td>
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<tr>
<td>Parent educational level</td>
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<td>17.8</td>
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<tr>
<td>Secondary</td>
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<td>39.7</td>
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<tr>
<td>Bachelor</td>
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<td>39.5</td>
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<tr>
<td>Post graduate</td>
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<td>3</td>
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<tr>
<td>Average spending time of talking or playing with children</td>
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<tr>
<td>&lt;60 minutes</td>
<td>309</td>
<td>77.3</td>
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<tr>
<td>60 m or more</td>
<td>91</td>
<td>22.7</td>
</tr>
<tr>
<td>Monthly income</td>
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<td></td>
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<td>Sufficient</td>
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<td>44</td>
</tr>
<tr>
<td>Insufficient</td>
<td>224</td>
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<tr>
<td>Number of children</td>
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<tr>
<td>1</td>
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<td>41</td>
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<tr>
<td>2</td>
<td>107</td>
<td>26.8</td>
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<tr>
<td>3</td>
<td>105</td>
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</tr>
<tr>
<td>&gt;3</td>
<td>24</td>
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Table (2): Distribution of studied children according to their characteristics (N=400).

<table>
<thead>
<tr>
<th>Items</th>
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<td><strong>Age</strong></td>
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<tr>
<td>6 - &lt;8</td>
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<tr>
<td>8 &lt; 10</td>
<td>160</td>
<td>40</td>
</tr>
<tr>
<td>10 – 12</td>
<td>91</td>
<td>22.7</td>
</tr>
<tr>
<td>Mean SD 8.95±2.46</td>
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<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>175</td>
<td>43.8</td>
</tr>
<tr>
<td>Female</td>
<td>225</td>
<td>56.2</td>
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<tr>
<td><strong>Academic year</strong></td>
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<td></td>
</tr>
<tr>
<td>First</td>
<td>86</td>
<td>21.5</td>
</tr>
<tr>
<td>Second</td>
<td>76</td>
<td>19</td>
</tr>
<tr>
<td>Third</td>
<td>103</td>
<td>25.8</td>
</tr>
<tr>
<td>Fourth</td>
<td>75</td>
<td>18.7</td>
</tr>
<tr>
<td>fifth</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td><strong>Mobile devices child used</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Own mobile</td>
<td>212</td>
<td>53</td>
</tr>
<tr>
<td>Parents’ mobile</td>
<td>108</td>
<td>27</td>
</tr>
<tr>
<td>None</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td><strong>Period Mobile device used / day</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 minutes</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>&lt;60 minutes</td>
<td>117</td>
<td>29.3</td>
</tr>
<tr>
<td>60 m or more</td>
<td>203</td>
<td>50.7</td>
</tr>
<tr>
<td><strong>Purpose of mobile device use</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viewing videos</td>
<td>146</td>
<td>36.5</td>
</tr>
<tr>
<td>Playing games</td>
<td>195</td>
<td>48.7</td>
</tr>
<tr>
<td>Taking and sharing pictures, figures, or photos</td>
<td>96</td>
<td>24</td>
</tr>
<tr>
<td>Learning/using applications related to education</td>
<td>315</td>
<td>78.7</td>
</tr>
<tr>
<td>Using internet/searching for information</td>
<td>146</td>
<td>24.3</td>
</tr>
<tr>
<td>Social media</td>
<td>254</td>
<td>63.5</td>
</tr>
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</table>
Table (3) Distribution of studied children according domains of strengths and difficulties questionnaire scale (n=400).

<table>
<thead>
<tr>
<th>Domains</th>
<th>Normal</th>
<th>Borderline</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Emotional Symptoms domain</td>
<td>100</td>
<td>25</td>
<td>111</td>
</tr>
<tr>
<td>Conduct Problems domain</td>
<td>112</td>
<td>28</td>
<td>97</td>
</tr>
<tr>
<td>Hyperactivity domain</td>
<td>93</td>
<td>23.3</td>
<td>102</td>
</tr>
<tr>
<td>Peer Problems domain</td>
<td>138</td>
<td>34.5</td>
<td>85</td>
</tr>
<tr>
<td>Prosocial Behavior domain</td>
<td>120</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure (1) Distribution of studied children according to strengths and difficulties questionnaire scale (n=400).

Table (4) Correlation between domains of strengths and difficulties questionnaire scale (n=400).

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emotional Symptoms domain</td>
<td>R</td>
<td>P</td>
<td>0.602</td>
<td>&lt;0.01**</td>
<td>0.702</td>
</tr>
<tr>
<td>Conduct Problems domain</td>
<td>R</td>
<td>P</td>
<td>0.855</td>
<td>&lt;0.01**</td>
<td>0.246</td>
</tr>
<tr>
<td>Hyperactivity domain</td>
<td>R</td>
<td>P</td>
<td>0.265</td>
<td>&lt;0.05*</td>
<td>0.802</td>
</tr>
<tr>
<td>Peer Problems domain</td>
<td>R</td>
<td>P</td>
<td></td>
<td></td>
<td>0.290</td>
</tr>
<tr>
<td>Prosocial Behavior domain</td>
<td>R</td>
<td>P</td>
<td></td>
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</table>
Table (5): Multiple Linear regression model for children’ strengths and difficulties questionnaire

<table>
<thead>
<tr>
<th></th>
<th>Unstandardized Coefficients</th>
<th>Beta</th>
<th>T</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Beta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Academic year</td>
<td>.299</td>
<td>.216</td>
<td>5.908</td>
<td>.004**</td>
</tr>
<tr>
<td>Gender “Male”</td>
<td>.217</td>
<td>.153</td>
<td>2.706</td>
<td>.022*</td>
</tr>
<tr>
<td>Mobile devices child used “Own mobile”</td>
<td>.345</td>
<td>.267</td>
<td>6.557</td>
<td>.002**</td>
</tr>
<tr>
<td>Period Mobile device used / day</td>
<td>.477</td>
<td>.401</td>
<td>9.510</td>
<td>.000**</td>
</tr>
<tr>
<td>Age</td>
<td>.241</td>
<td>.198</td>
<td>2.968</td>
<td>.034*</td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th>Model</th>
<th>R2</th>
<th>F</th>
<th>P. value</th>
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</thead>
<tbody>
<tr>
<td>Regression</td>
<td>0.46</td>
<td>12.210</td>
<td>.000**</td>
</tr>
</tbody>
</table>

a. Dependent Variable: Strengths and Difficulties Questionnaire

b. Predictors: (constant): Academic year, Gender “Male, Mobile devices child used Own mobile”, Period Mobile device used / day and Age

Discussion

According to parents’ characteristics, the current study demonstrated that mean age was 40.38±6.75 year and more than half of maternal educational level was bachelor. Regarding to parent educational level, more than one third of them had secondary level. Also, more than three quarters of them spending time of talking or playing with children <60 minutes. Moreover, more than half of parents had insufficient monthly income and more than one third of them had one child. These results inconsistent with the study conducted by Chang et al.3, who revealed that about three quarters had high income and mean of their age was 35.41±4.2. Also, disagree with the study performed by Kiliç et al.9 who detected that the minority of studied mothers and fathers had university education; more than one third of maternal education was primary.

Related children characteristics, the present results mentioned that revealed that mean age of studied children was 8.95±2.46 year and more than half of them were female. Regarding to academic year, about one quarter of them was at third level. Also, more than half of them used own mobile. Moreover, about half of them used mobile 60 m or more per day and more than three quarters of them used mobile device for learning/using applications related to education. These results explained as related COVID-19 pandemic and to achieve social distancing, electronic education has been relied upon, so the child’s interaction with mobile phones has increased during that period. These results in cohort with the study by Domoff et al.5 who reported that more than half of studied children were male and less than two thirds of
them had age less than 6 year. While, supported with the study conducted by Lee & Kim\textsuperscript{13} who stated that half of them were female and more than half of them used Smartphone more than one hour.

According domains of strengths and difficulties questionnaire scale, the current study demonstrated that less than half of studied children had abnormal emotional symptoms and conduct problems. In addition about one third of studied children were normal related peer problems domain. Also, more than half of them were abnormal related hyperactivity. While, only more than one quarter of studied children had normal Prosocial behavior. Also, about half of studied children were abnormal related their total strengths and difficulties questionnaire scale. These results attributed to more than half of studied children used mobile device more than 60 minutes per day and more than half of them had own mobile. These results similar with the study performed by Hosokawa & Katsura\textsuperscript{8} who detected that regular use of mobile devices was significantly linked to conduct problems (IPTW-OR: 1.77, 95% CI: [1.03–3.04], \( p < .05 \)) and hyperactivity/inattention (IPTW-OR: 1.82, 95% CI: [1.15–2.87], \( p < .01 \)). Also, regular with the study by Bozzola et al.\textsuperscript{2} who stated that using mobile device had negative effect on children behavior. In addition, similar with the study done by Kwon et al.\textsuperscript{11} who revealed that attention-deficit hyperactivity disorder symptoms were positively correlated with Smartphone addiction (\( r = .424, p < .01 \)) and poor sleep quality (\( r = .313, p < .01 \)). Also, supported with the study by Topan & Kuzlu Ayyildiz\textsuperscript{22} who reported that technological device use negatively impact on children physical and emotional health with \( p \) value <0.05\textsuperscript{*}. In addition, agreement with Liu et al.\textsuperscript{15} who demonstrated that violence/pornography on mobile devices were more likely to have emotional and behavioral problems.

Regarding to correlation between domains of strengths and difficulties questionnaire scale, there were highly statistically positive correlation between emotional symptoms domain and their conduct problems domain, hyperactivity domain and Prosocial behavior domain at (\( P= < 0.01 \)). While there were slight positive correlation between peer problems domain and their emotional symptoms domain, conduct problems domain and hyperactivity domain at (\( P= < 0.05 \)). These results cohort with the study performed by Panagiotidi & Overton\textsuperscript{18} who stated that there was highly significant correlation between Hyperactivity domain, inattention domain and withdrawal domain with \( p \) value <0.01\textsuperscript{**}.

According to Multiple Linear regression model, the current study explained that, academic year, mobile device child used “own mobile”, increase period mobile device used per day had high frequency positive effect on children’ strengths and difficulties questionnaire at \( p \) value <0.01\textsuperscript{**}. While, male gender and increasing age of children had slight positive effect on children’ strengths and difficulties questionnaire with \( p \) value <0.05. These results similar with the study conducted by Xie et al.\textsuperscript{23} who detected that gender was found to moderate the indirect effect of on mobile phone addiction. These results consistent with the study by Srisinghasongkram et al.\textsuperscript{21} who stated that had high significant correlation between gender and conduct, hyperactive behavior of child. Also, cohort with the study performed by Domoff et al.\textsuperscript{5} who detected that there was association between excessive mobile device use and children’s physical health outcomes.

**Conclusion**

To conclude our study, about half of studied children had abnormal Prosocial and difficult behaviors and less than one quarter of them had borderline Prosocial and difficult behaviors. While, less than one third of them had normal Prosocial and difficult behaviors. Also, academic year, mobile device child used “own mobile”, period mobile device used per day had high frequency positive effect on children’ Prosocial and difficult behaviors at \( p \) value <0.01\textsuperscript{**}. While, male gender and increasing age of children had slight positive effect on children’ strengths and difficulties questionnaire with \( p \) value <0.05.

**Conflict of Interest:** Not present any conflict

**Funding:** Self-funding, without any external source.
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Nursing Intervention to Improve the Caregivers’ Practices toward Elderly Care at Geriatric Homes

Sanya Zakarya Mohammed1, Magda Abdelsattar Ahmad2, Nadia Hamed Farahat2
1Assistant Lecturer of Community Health Nursing, Faculty of Nursing, October 6 University, Cairo, Egypt; Studied at Faculty of Nursing, Ain Shams University, 2Professor of Community Health Nursing, Faculty of Nursing, Ain Shams University, Cairo, Egypt

Abstract

Population ageing is a global phenomenon with the number of persons 60 years and over estimated to reach two billion by the year 2050; living longer is joined to challenges of disability and being dependent on caregivers who should have the chance to perform their practices by professional way as an integral part of elderly care. Aim: To evaluate the nursing intervention program to improve the caregivers’ practices of elderly care at geriatric homes. Design: Quasi-experimental research design. Setting: In geriatric homes at Cairo governorate. Sample: A convenient sample of all elderly caregivers working in the previous mentioned settings. Tool: Was used an interviewing questionnaire to assess socio-demographic characteristics of elderly caregivers, their knowledge, their practices & factors causing stress to them & their coping with stress. Conclusion: The nursing intervention program implementation had statistically significant positive effect on elderly caregivers at geriatric homes regarding to their knowledge, practices & coping with stress.

Keywords: Elderly, Elderly Caregivers, Practice, Nursing intervention & Geriatric homes

Introduction

The trends of ageing in the Arab countries and the increase of life expectation at 60, is accompanied by an increase in disability rates for old age. In Egypt, in 2018, the number of elderly persons reached 6.410 million (3.418 million men, 2.992 million women) by the ratio of elderly, 6.7% (6.9% men, 6.4% women) of the total population, this number increased in 2031 to 11.5%. The total number of geriatric homes are 54 one, where there are 1774 elderly person & 639 caregiver.

As the numbers of elderly people requiring nursing or residential care increase, the professionalism of the elderly caregivers employed in this setting grows in importance. Caregiving is inevitably a demanding and stressful job in a complex organizational setting and it has been widely regarded as one of the most stressful occupations, associated with high levels of lacking of knowledge, skills & coping with work stress.

The role of the caregiver includes actions that are intended to assist the elderly people prevented physically or mentally from performing the practical tasks of activities of daily living and self-care. A caregiver is defined as being the person that is responsible for caring for a sick or dependent person, facilitating the performance of their daily activities, such as feeding, personal hygiene, providing routine medication and accompanying them to the health services, or carrying out other things required in their daily lives.

Geriatric homes provide a broad range of health-related services for people aged ≥ 60 years. The services provided include skilled nursing care, rehabilitation services, and dietary services. Disabling or burdensome disorders, most commonly dementia, incontinence, and immobility may
trigger consideration of geriatric home placement.\(^5\)

Nursing intervention through the community health nurse (CHN) is involved in primary, secondary, and tertiary prevention efforts for elderly and caregivers. The major areas in which CHN plays a vital role toward elderly are decrease of risk and maintenance of independence; supervision and encouragement of caregivers. CHN should become involved in exploring service options for elderly individuals and in educating the general public in the problems and needs of elderly individuals and their caregivers. These goals can be accomplished by designing and carrying an intervention that educate elderly caregivers and organizations or by advocating for legislation to assist elderly independence and elderly caregivers when possible.\(^6\)

### Aim of the Study

The aim of this study is to evaluate the nursing intervention program to improve the caregivers practices of elderly care at geriatric homes through:

1. Assessing caregivers’ knowledge about elderly care.
2. Assessing caregivers’ practices toward elderly care.
3. Assessing factors leading to stress to caregivers and influence the pattern of care provided to the elderly.
5. Designing and implementing a nursing intervention program to improve the caregivers’ practices toward elderly care at geriatric homes.
6. Evaluating the effectiveness of nursing intervention program to improve the caregivers’ practices of elderly at geriatric homes.

### Subjects and Methods

#### Research hypotheses:

The educational program will improve quality of life for adult client with a permanent pacemaker at home pre/post program.

#### Subject and methodology:

I- Technical design

II- Administrative and ethical design

III- Operational design

IV- Statistical design

#### I-Technical Design:

The technical design includes; the setting, sample & tools were used in the study.

#### B- Setting:

This study will be conducted to half number of districts in Cairo governorate, and select two geriatric homes of each district that more density in the following geriatric homes which located at Cairo governorate and these settings were chosen because they serve the community around Ain Shams University. Where those are; facility of mosenensamanelsheikh (Rod el farag), daranwaralmostafa & Dar mosenenalrahman (Almataria), botrosghaly for elderly (Alwaily), darmosenen and mosenat el hadialeslamy (Alsharabia), darMosenenalkhairiaaleslamia (alsaidazainab).

#### C- Sampling:

A convenient sample was used in this study. The total numbers of elderly caregivers who work in the previous mentioned setting were 73 person and pilot study it was chosen and carried out on 13 person whom & excluded later the study sample. The sample became 60 person for both genders (male & female), 10 persons from each geriatric home, elderly caregiver from 20 to 60 years old. Work at geriatric homes and accepting to participate in the study program.

#### C. Tools of data collection:

Four tools were used for data collection.

1. **Interview questionnaire form (Appendix I):**
   
The questionnaire sheet was designed by the investigator and written in simple Arabic language based on scientific
literature review, experts’ opinion and personal experience and filled. It is comprised of two parts:

**Part I:** It was used to assess demographic characteristics of elderly caregivers include age, gender, education, marital status, No of elderly people who are caring for and No of caregivers care for (preprogram)

**Part II:** It was used to assess knowledge about elderly care such as concept of aging, factors of elderly wellbeing, health problems related aging, human rights related elderly at geriatric home, normal changes related aging (physical, mental, social and psychological), daily care for elderly (physical exercises, healthy & balanced nutrition, sleeping disturbance, personal hygiene, bed sores, elderly falls) and first aids for common cases (bruises, fracture, fainting, diabetic coma, diarrhea and burn) (pre/ post program).

Scoring system for knowledge questions:

The questionnaire of the elderly’ caregivers related to knowledge (pre & post program application) was constructed in the form of Multiple Choice Questions. The right answers were scored one and those wrong were scored zero. These scores were summed-up and converted into a percent score and categorized into three levels as:

§ Poor: if the percent score was 70-100%
§ Fair: if the percent score was 50-69%
§ Good: if the percent score was 0-49%

**II. Reported practice through self-assessment tool (Appendix II):**

It was designed to assess the elderly’ caregivers practices and filled by investigator to meet their needs through asking questions regarding role of elderly caregiver to keep legal issues related elderly at geriatric home, meet elderly needs related aging (physically, mentally, socially and psychologically), perform daily care for elderly (physical activity, preparing healthy & balanced nutrition, provide safety during nutrition the elderly, defecating, sleeping & rest pattern), assist elderly according to his ability to do personal hygiene (mouth & teeth, hair, nail, foot care and bathing), provide safe environment for elderly, take care of unable elderly (mobilization in bed, ambulation (helps the elderly to get up and sit, helps the elderly to stand and walk), lifting from bed to chair & inverse, proper precautions to avoid bed sores, right steps for elderly bed lying bathing and first aids for common cases (nasal bleeding, bruises, fracture, fainting, diabetic coma, diarrhea and burn) (pre & post program application).

Scoring system for practices questions:

§ Score from 0 < 60 referred to not competent practices.
§ Score from 60 ≤ 100 referred to competent practices.

**III. Factors leading to stress to care givers (Appendix III):** The tool was adopted from Leonard & Shirley (1990) and adapted as translated; modified and filled by investigator to assess the factors causing stress to care givers (preprogram application) as follows:

A-Primary stressors of caregivers (that are based on the health, behavior and functional capabilities of the elderly):

1- Dependency in Activity of Daily Living.

It is used to detect dependency in performing activities of daily living (ADLs) and to plan care accordingly. In ADLs each function is scored at two-point scale (independent = 1 and dependent = zero). Summation of items scores yields a total score ranging from “zero to 6”. Where score 0 = low (very dependent elderly) and 6 = high (independent elderly).

2- Cognitive status of elderly: to measure the range and difficulty of caregiving activities and the ability of caregivers to manage their relationships with their impaired elderly grow out of the elderly’s memory loss, communication deficits, and recognition failures. The evaluation of cognitive status, although made by the caregiver, is based on standard tests typically used in the clinic. The questions asking about elderly’s cognitive status are 8 questions, presented in five scales placed in: can’t do at all = (4), very tough = (3), fairly tough = (2), just a little tough = (1) and not at all tough = (0). A score of 0 to 4
was given to each response, whereby higher scores indicated a stronger intention. The reliability (alpha) of this scale was 0.86.

3-Problematic Behavior: to measure the level of elderly’s awareness that must be maintained and the “damage control” that must be exercised to ensure that the elderly harms neither himself nor others constitute. It consists of 16 questions, presented in four scales placed in: 5/more days = (4), 3/4 days = (3), 1-2 days = (2) and no days = (1). A score of 1 to 4 was given to each response, whereby higher scores indicated a stronger intention. The reliability (alpha) of this scale was 79.

4-Overload (burnout felt by caregiver): the items constituting the measure reveal not only the level of fatigue felt by caregivers but also the relentlessness and rigid nature of its source. It was composed of 4 items and answers were coded according to the following: wholly = (4) score, quite a bit = (3), fairly = (2) and not at all = (1). By means of higher scores indicated a high stress because of overload.

B- Secondary stressors: Role strains and intrapsychic strains

5-Work colleagues conflict: to assess the elderly’ caregivers stress because of conflict with work colleague. Three dimensions of conflict was identified in issues of seriousness/safety of elderly, attitudes & actions toward elderly and attitudes and actions toward caregiver and composed of 9 questions. Where issues of seriousness/safety of elderly was included in 3 items (Q1-Q3), attitudes and actions toward elderly included was involved in 4 items (Q4-Q7), and attitudes and actions toward caregiver was comprised in 2 items (Q8-Q9). Answers were coded as the following: a lot of conflict = (4), some conflict = (3), just a little conflict = (2) and no conflict = (1). Through the higher scores indicated that care givers under high stress because of work colleagues’ conflict.

6- Personal Gain: the measure of personal gain or enrichment is proof to the fact that many people manage to findsome inner growth as they face the severe challenges of caregiving. Competence & gain were included partly out of interest in whether the enhancement of self is negatively related to stress outcomes, the deterioration of self to be positively related to stress. They were also included to determine if the enhancement of some elements of self-counter balanced or compensated for the deterioration of others. It was composed of 4 items and answers were coded according to response categories as follow: very much = (4), somewhat = (3), just a little = (2) and not at all = (1). By means of lower scores indicated a high stress.

7-Caregiving Competence: to measure the adequacy of performance as caregivers, (alpha = 0.74). Competence is measured by a four-item scale that essentially asks caregivers to rate the adequacy of their performance as caregivers and answers were coded as next: very much = (4), somewhat= (3), just a little = (2) and not at all = (1). By means of lower scores indicated a high stress as inability to be adequate at perform caregiving.

IV: Caregivers Coping with stress (Appendix IV): the tool was adopted from 7,8&9 and adapted as translated, modified and filled by investigator to assess caregivers coping with stress(pre & post program application) as follows; caregivers coping in response to elderly caregiving problems as having three possible functions: management of the situation giving rise to stress; management of the meaning of the situation such that its threat is reduced; and management of the stress symptoms that result from the situation. Where function of management of the situation was included in 6 items (Q1-Q6), management of the meaning of the situation was involved in 8 items (Q1-Q8), where this item involves three parts, one involves the reduction of expectations (Q1-Q3), a second the use of positive comparisons (Q4-Q5), and the third a search for a larger sense of the illness (Q6-Q8),and management of the stress symptoms was comprised in 6 items (Q1-Q6). Answers were coded according to the following: very often = (4), fairly often = (3), once = (2) and never = (1). Through the higher scores indicated that care givers can represent coping behaviors and practices to manage stressors that lead to from caregiving to the elderly at geriatric homes.

The Validity: it was tested through 5 experts, from community health nursing department, faculty of nursing, Ain Shames University.
The reliability was done by Cronbach’s Alpha coefficient test which revealed that each of the three tools consisted of relatively homogenous items as indicated by the moderate to high reliability of each tool, where the reliability of them was 0.831

III. Operation design:

The operational design for this study consisted of three phases, namely preparatory phase, pilot study, and fieldwork.

A. Preparatory Phase

This phase included reviewing of literature related to caregivers’ knowledge about elderly care at geriatric homes by using books, articles, journals, and internet, in order to get a clear picture of the research problem, as well as, to develop the study tools for data collection. During this phase, the investigator also visited the selected places to be familiar with the personnel and the study settings. Development of the tools was under supervisors’ guidance and experts’ opinions were considered.

B. Pilot Study

A pilot study was conducted on ten of the elderly caregivers in the pre-mentioned settings, in order to test the eligibility, including the suitability and feasibility, availability of the study population. Also, to determine the time required to conduct the constructed research tools. Modification of the tools was done based on the findings of the pilot study, some questions and items were omitted, added, or rephrased, and then the final form was developed. The subjects included in the pilot study were excluded from the study sample.

C. Fieldwork

After obtaining a permit the investigator started to interview the caregivers. The investigator started with introducing herself and explaining the aim of the study for the subjects. The investigator assured that the data collected will be confidential and would be only used to achieve the purpose of the study. Caregivers were asked to answer the pretest questions to fill the interviewing questionnaire. The investigator asked the question and recorded the answers and each interview lasted for approximately 30 minutes to answer these questions. The investigator collected the sheets and checks any missed item.

All tools were used to get a baseline assessment for the elderly caregivers prior to the development of the program, and also used after the program implementation, in order to compare between the findings results (pre & post) to determine the level of improvement.

Data collection for this study was carried out within a period of six months, started from the beginning of December 2019 till the end of May 2020. The investigator visited the selected settings (six geriatric homes) as follow; 2 geriatric homes for 2 months, 4 days per week (Friday, Saturday, Sunday and Monday) 2 days for each geriatric home from 9 am to 1 pm.

The investigator had identified an appointment from the subjects for meeting them at the geriatric home which include an assessment for caregivers’ knowledge & practices. Also, the assessment was done after program implementation.

The intervention aimed to increase elderly caregivers’ knowledge and skills through 8 weeks (two-hour per week). 60 caregivers divided into 6 groups where each geriatric home represents one group, the number of caregivers attended in each session was 10 elderly caregivers. At the end session of program implementation, the investigator evaluates the caregivers’ knowledge & practices to ensure exposure of all elderly caregivers to the same learning experience, each of them received the same program content, using the same teaching strategies, discussion, and correction on the spot and real objects.

Program construction:

The educational program was constructed by the investigator and consists of the following stages:

• Preparatory stage, which included selection of the specific program, related topics outline, objectives, knowledge which based on the identified needs of caregivers through the data obtained from pre-assessment tools. The investigator conducted advanced
search for relevant studies on local and global levels. Search process included libraries of local universities, regional electronic web sites especially those of Egypt, and global electronic web sites including PubMed, Cochrane library, Medscape, and Medline, and the items needed to be included in the educational program were identified. The constructed program was disseminated to a panel of experts to be evaluated. The investigator took all comments of experts into consideration and modified.

- **Planning stage**, which plans for a series of sessions and preparing the suitable media.

- **Implementation stage**, where the program was implemented through application of Communication techniques and teaching-learning strategies. Lecture, discussions and demonstrations of the practical skills were approached as teaching methods. Audiovisual methods such as PPT, Videos, Handouts and Poster were used to facilitate the process of education. The investigator planned to meet the caregivers at the selected geriatric homes. The program was practical in nature, to improve elderly caregivers’ practices, based on one-to-one caregiver assessments. Knowledge and practices for each group by the end of the teaching experience were revised. Time was opened for attendance to ask questions and to receive the corresponding answers as well as to express their feedback toward the teaching session.

**Program sessions:**

Time allowed: 16 hours has been allocated for health education sessions. Involve theory and practices training. Teaching sessions were conducted at any available hall at each geriatric home were student being 6 grouped and every group included (10) caregiver.

At the beginning of the first session, an orientation about the program and its purposes was given. It was agreed at the time of the break with the elderly caregivers. From the second session and so on each session started by a summary about what was given through the previous sessions and objectives of the new one.

By the end of each session a summary was made and time allocated for questions and answers & plan for next session were made. Except for the last session a termination of sessions through feedback was done.

Educational media will use the poster, laptop, guidance handout which includes instruction and information for caregivers as a reference during and after program implementation. Teaching material will use Arabic Handout and audiovisual materials.

- **Evaluation of the program**, this stage aimed to evaluate the level of improvement in elderly caregivers’ knowledge, practices and their coping with caregiving stress through implementation of program. This was done through giving posttest similar to the pretest, evaluation administered to study subjects after completion of the program in order to estimate the effect of program on elderly caregivers’ knowledge and practices related to elderly care.

**Program Handout:**

The educational handed was designed by the investigator, including the all content of the program and given to as an educational reference during program implementation and as self-learning reference after program implementation. Its aim was providing accurate knowledge & practices related guideline instructions about obesity, risk factors and its consequences.

**Ethical consideration:**

Verbal approval was obtained from the caregivers before inclusion in the study; a clear and simple explanation was given according to their level of understanding, physical and mental readiness. They secured that all the gathered data was confidential and used for research purpose only.

**The ethical research considerations include the following:**

§ The research approval was obtained from the faculty ethical committee before starting the study.

§ The investigator clarified the objectives and aim of the study to caregivers before starting
§ The investigator was assuring anonymity and confidentiality of subjects’ data included in the study.

§ The subjects was informed that they are allowed to participate or not in the study and they have the right to withdraw from the study at any time.

**Limitation of the Study:**

1. Some geriatric homes {Dar mosenatalkarma&Agaezaramelahkarma (Shobra), Mosenena and mosenatalkelmaaltaeba&alqswahba (Alsahel)} refused the conduction of any kind of research for fear of transfer of infection of COVID-19.

2. The geriatric homes {Facility of mosenatsaneaelkhairat (Rod el farag), Evergreen (Alwaily), Dar Mosenenyomalmostashfiat (alsaidazainab)} were excluded from the sample because there was lack of flow of elderly caregivers, where they terminated from work.

**III- Administrative design:** To carry out this study, a letter was issued to pre-mentioned settings from the dean of Faculty of Nursing, Ain Shams University explaining the aim of the study to obtain permission and help. The necessary approval letter was obtained from the head of geriatric homes in pre-mentioned settings. The title, objectives, study technique and tools were explained to gain their approval in data collection.

**IV. Statistical design:** Data were revised, coded, analyzed and tabulated using the number and percentage distribution and correlations and carried out in the computer. The statistical analysis was performed using the statistical package for social science (SPSS) program, version 20 for Windows Data Editor.

**The following statistical techniques were used:**

Percentage, the arithmetic mean (X), the standard deviation (SD), the chi-square test (X2), and proportion probability (P-value).

**Results**

Table (1): Distribution of the elderly caregivers according to their demographic characteristics (No= 60)

<table>
<thead>
<tr>
<th>Items</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (in years):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>§ 20- &lt; 30</td>
<td>8</td>
<td>13.3</td>
</tr>
<tr>
<td>§ 31- &lt; 40</td>
<td>12</td>
<td>20.0</td>
</tr>
<tr>
<td>§ 41- &lt; 50</td>
<td>24</td>
<td>40.0</td>
</tr>
<tr>
<td>§ 51- &lt; 60</td>
<td>16</td>
<td>26.7</td>
</tr>
<tr>
<td><strong>Mean ± SD</strong></td>
<td></td>
<td>38.53± 9.79</td>
</tr>
<tr>
<td><strong>Gender:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>§ Male</td>
<td>17</td>
<td>28.3</td>
</tr>
<tr>
<td>§ Female</td>
<td>43</td>
<td>71.7</td>
</tr>
<tr>
<td><strong>Education:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>§ Illiterate</td>
<td>9</td>
<td>15.0</td>
</tr>
<tr>
<td>§ Read and write</td>
<td>16</td>
<td>26.7</td>
</tr>
<tr>
<td>§ Basic education</td>
<td>35</td>
<td>58.3</td>
</tr>
</tbody>
</table>
Cont... Table (1): Distribution of the elderly caregivers according to their demographic characteristics (No= 60)

<table>
<thead>
<tr>
<th>Marital Status:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>§ Single</td>
<td>13</td>
<td>21.7</td>
</tr>
<tr>
<td>§ Married</td>
<td>33</td>
<td>55.0</td>
</tr>
<tr>
<td>§ Widow/widower</td>
<td>5</td>
<td>8.3</td>
</tr>
<tr>
<td>§ Divorced</td>
<td>9</td>
<td>15.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No of elderly people who are caring for:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>§ Three</td>
<td>16</td>
<td>26.7</td>
</tr>
<tr>
<td>§ More than three</td>
<td>44</td>
<td>73.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No of caregivers care for:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>§ An able elderly</td>
<td>10</td>
<td>16.7</td>
</tr>
<tr>
<td>§ An unable elderly</td>
<td>8</td>
<td>13.3</td>
</tr>
<tr>
<td>§ Both</td>
<td>42</td>
<td>70.0</td>
</tr>
</tbody>
</table>

Table 1 displays that the caregivers’ age ranged between 20 to 60 years old. 40% of them were between age 41 and 50 years. As regard gender, it was found that the 71.7% of caregivers were females. Regarding educational level 58.3% has basic education. Concerning marital status, the table shows that 55% of the sample was married. Regarding the number of elderly people who are caring for, it was found that, 73.3% was caring for more than three elderlies. In relation to the health condition of the elderly person who cares for him the table shows that, 70% of caregivers were caring for both able and unable elderlies.

Figure (1): Distribution of total knowledge of the elderly caregivers about elderly care at geriatric homes (n= 60).

X268.217 at P <0.001***

*** Very highly statistically significant
Figure 1, indicated that, 3.3% of the elderly caregivers had good knowledge preprogram implementation regarding the total knowledge about elderly care at geriatric home, improved to 75% post program implementation with very highly statistically significant differences at P value < 0.001***

\[
\chi^2 = 34.17 \text{ at } P < 0.001^{***}
\]

*** Very highly statistically significant

For the total practices of the elderly caregivers toward elderly care at geriatric home, the above figure showed that 25% of the elderly caregivers were competent preprogram, while they improved to 78.3% post program implementation with very highly statistical significance at P value <0.001***

Figure (3): Distribution of the elderly caregivers according to total factors leading to stress to them (pre-program) (n= 60)
The above figure indicates that, total factors leading to stress to the elderly care givers were 76.7% of them felt stress in caring of their elderlies at geriatric home.

**Figure (4): Distribution of the elderly caregivers according to their total coping with stress (pre / post program) (n= 60).**

X234.17 at P<0.001***

*** Very highly statistically significant

The above figure, pronounced that total elderly caregivers’ coping with stress regarding being confronted were 25% preprogram changed to 78.3% post program application, there was a very highly significant difference between caregivers’ coping with stress regarding being confronted pre, post program application at P<0.001***

**Discussion**

Population ageing is a global phenomenon with the number of persons 60 years and over estimated to reach two billion by the year 2050; for some persons, living longer is accompanied by challenges of disability and being dependent on others to assist with everyday tasks. Already, 101 million people 60 years and over worldwide are estimated to be care-dependent.

Elderly caregivers should have the opportunity to perform their practices by professional way on a day-to-day basis as an integral part of elderly care. That is why the current study was carried out.

According to demographic characteristics of the elderly caregivers, the present study found that, elderly caregivers’ age ranged between 20 to 60 years old with two fifths of them age was ranged from 41 to less than 50 years old with the mean age was 38.53 ± 9.79. (Table 1). The findings of the present study are consistence with Van Houtven et al.10 who studied essential long-term care workers commonly hold second jobs and double or triple duty caregiving roles in northeastern United States who stated that, the mean age of long-term care workers was 41 years.

Bilal et al.11 disagree with the current results, where the previous study about elderly care in the time of coronavirus: perceptions and experiences of care home staff in Pakistan found that, the majority of elderly caregivers were less than 40 years. In this regards Shi et al.12 who studied Perceived stress and social
support influence anxiety symptoms of Chinese family caregivers of community dwelling older adults: a cross-sectional study illustrated that 39.83% of caregivers were less than 40 years.

Concerning gender, the current study demonstrated that, the dominance of females than males, who were in common married. this finding is in agreement with that of Moholt et al.13 who studied the factors affecting the use of home-based services and out-of-home respite care services: A survey of family caregivers for older persons with dementia in Northern Norway who reported that the more than three fifth of elderly caregivers were females. As well, Fagerström et al.14 who found in their study about the analyzing the situation of older family caregivers with a focus on health-related quality of life and pain: cross-sectional cohorts study in Sweden that, the great majority of their studied sample married.

This trend was opposite to what was found in a study in China by Shi et al.12 who found that, more than half of the studied sample were married men, in this regards James et al.3 revealed in their study about socio-demographic, health and functional status correlates of caregiver burden among care recipients age 60 Years and older in Jamaica that, more than half of the studied sample were single female. In Egypt, caregiving is a female occupation, and this give a reason why the majority of the study sample were more females than males.

In relation to the level of education, this study revealed that, more than half of them had basic education. This explains why they are not responding easy to the knowledge given to them so most of them feel stress in caring of their elderlies at geriatric home. This finding is in agreement with that of Ekström et al.15 who studied high burden among older family caregivers is associated with high prevalence of symptoms: data from the Swedish Study “Good Aging in Skane (GAS)”. Where they concluded that, more than one third of caregivers were with low educational level. Contradicting with the previous findings, Kehoe et al.16 found in their study about quality of life of caregivers of older patients with advanced cancer in United States that, more than half of the studied caregivers obtained some college or above.

As regard to total knowledge of the elderly caregivers about elderly care at geriatric homes pre/post program (Figure 1) the present study revealed that, only 3.3% of them had good knowledge preprogram implementation, while changed to three quarters of them post program implementation with very highly statistically significant differences pre/post program for elderly caregivers, this finding agree with a study about training of formal caregivers dealing with Alzheimer diseased patients at Helwan City Cairo Egypt by El-Kattan et al.17 who find that the pre/posttests among formal caregivers revealed that overall knowledge, attitude and practices improved with highly statistically significant differences at p < 0.001 that approved the hypothesis of implementing training program for formal caregivers.

Concerning total practice of the elderly caregivers about elderly care at geriatric homes pre/post program (Figure 4) the present study shown that, one quarter of them were competent preprogram implementation, while improved to more than three quarters of them post program implementation with very highly statistically significant differences pre/post program for elderly caregivers, this finding was in concordance with previous study about the effectiveness of a training program in developing professional performance of social workers with elderly groups in Egypt by 18 who indicated that the results of the research training program succeeded to develop this professional performance.

In relation to the total factors causing stress to caregivers preprogram (Figure 5). The current finding denoted that, more than three quarters of them felt stress in caring of their elderlies at geriatric home. These results agreed with those of Rachel and Francesco19 Who studied factors associated with and impact of burnout in nursing and residential home care workers for the elderly which conducted across a range of academic databases and suggested that nursing care aids working within nursing homes have high levels of job efficacy.
(a facet of burnout) but also hold a moderate risk for burnout domains of emotional exhaustion and cynicism. This would suggest that although care home workers place value and importance in the work they do, they also have a risk for developing a distrustful attitude and levels of emotional exhaustion. It is concerning to note that studies have reported how time worked at a nursing home negatively predicts personal accomplishment, a burnout factor. That is, the longer that staff worked at a nursing home, the lower their level of satisfaction and accomplishment around their work.

Considering total coping with stress pre/post program (Figure 6) from the data gathered, it appears that, one quarter of them was confronted pre-program implementation, while enhanced to more than three quarters of them post program implementation with very highly statistically significant differences pre/post program for elderly caregivers. This finding goes in the same line with the study about effect of counseling on burden and coping among caregivers of cancer patients with terminal illness in Kanyakumari District in Asia by Sam et al. who showed that, the pretest mean coping score was 27.46 ± 8.02 and the post test score was 92.66 ± 7.15 and statistically significant. And the counseling program is effective in reducing the level of burden and improving the coping among primary caregivers of terminally ill cancer patients.

**Conclusion**

The educational program implementation had statistically significant positive effect on elderly caregivers at geriatric home regarding their knowledge, practices & coping with work stress.

**Recommendation:** Continues of geriatric home program to all elderly caregivers to improve their knowledge and practices regarding elderly care. A further research on a large sample is recommended to achieve more generalization.

**Ethical Clearance:** Taken from ethical committee from faculty of nursing, October 6 University

**Source of Funding:** Self

**Conflict of Interest:** Nil

**References**


Assessment of Knowledge and Awareness about Medico Legal Issues among Interns and Post Graduate Students in Rajahmundry, Andhra Pradesh

Saptarishi Bose\(^1\), Naresh Jyothula\(^1\), U. Vinod Venkata Kumar Andey\(^1\), Ganapathi Swamy Chintada\(^2\), Paromita Roy\(^3\)

\(^1\)Assistant Professor, \(^2\)Associate Professor, Dept. of Community Medicine, \(^3\)Senior Lecturer in Dept. of Oral Medicine & Radiology at GSL Dental College, Rajahmundry, Andhra Pradesh

Abstract

In recent times there has been increased number of complaints and lawsuits against doctors. Therefore having knowledge about Medico Legal issues is as important to the practice of medicine as clinical skills. The present study was conducted to evaluate Awareness and Knowledge about Medico Legal issues among Interns and Post Graduate Students. Total 140 interns and 110 Post Graduate students participated in the study. Post graduate students had more knowledge about Medico Legal issues and consent than interns but there was deficit of knowledge about Injury and Death certificates. About Consumers protection act (COPRA) there is little awareness but regarding Human organ transplantation Act (HOTA) and Hospital indemnity Insurance awareness should be increased. Our study conveys that action should be taken to cover the lack of awareness and knowledge for doctors as well as patient safety.

Key Words: Medico Legal issues, Consent, Consumers protection act, Interns, Post Graduate students Rajahmundry, Andhra Pradesh

Introduction

It is important to have a good knowledge of all the branches of medical and ancillary science taught to a medical student during the course of studies for an expert medical witness\(^1\). The trainee period is a critical time for foresting ethical reasoning\(^2,3\). Good medical practice requires that medical graduates can demonstrate in practice knowledge and understanding of the law\(^4\).

Universal trends of medico legal issues are slowly gaining the attention of the public and complaints against physicians looks to be going up in developing countries. This has brought to the fore need for a high sense of professionalism among health care practitioners. This professionalism depends on knowledge and application of medical ethics in daily practice of the practitioners\(^5\). The knowledge of Medico Legal issues are essential for maintaining the patient doctor relationship and prevent the commercialization of the profession\(^6,7\). Lack of understanding of legal aspects of medicine practice is important matter that needs to be dealt promptly\(^8\).

However, it is not possible for medical schools to give ample time to teaching of ethics, confidentiality and medico legal issues. The curriculum on medico legal issues may be inadequate or impractical enough to permit the medical students to address all ethical difficulties likely to be faced in practice\(^8\). Widespread deficiencies in knowledge and understanding of legal rules has been reported by students and practicing doctors.

Training period is the crucial time for developing the ethical views and awareness in young doctors\(^2,7\). Doctors get acquainted with the regulations and laws that deal with in their practice. Doctors deal with many ethical moral and legal obligations in their duties.
It is therefore very important that every doctor understands the nature of these obligations and then fulfils these obligations to the best of their ability\textsuperscript{9,10}. They should improve the quality of patient care by identifying, analyzing and attempting to resolve the ethical problems that arise in practice\textsuperscript{2}.

Medical practitioners must be aware of legal and ethical implications of clinical practice\textsuperscript{11}. Law and Medicine go hand in hand. The recent trends towards codifying the individual rights and freedom has filtered down to the relationship between physicians and patients\textsuperscript{12}. Future doctors must have a good grasp of the law and the confidence to apply the understanding if they are to uphold and advocate effectively for the legal rights of patients\textsuperscript{4}.

By keeping these things in mind we carried out our present study to assess Knowledge and Awareness about Medico Legal issues among Interns and Post Graduate Students (PG Students).

Objective:

To assess knowledge and knowledge about Medico Legal Issues among Interns and Post Graduate Students.

Material and Methods

A questionnaire based cross sectional study was conducted in GSL Medical College and General Hospital, Rajahmundry, Andhra Pradesh, India, in month of October to November 2020. The study was carried out in and 140 Interns and 110 Post graduate Students. Aim of the study was explained to the participants and written informed consent was obtained from them. Confidentiality about the result was ensured to participants so as to get reliable answers. Those who not willing to participate were excluded from the study. A structured self administered questionnaire related to medico legal cases(MLCs), Consent taking, Consumers protection act(COPRA), Human organ transplantation Act(HOTA), Hospital indemnity Insurance containing 14 questions were designed. The data was collected, tabulated and analysed statistically for numbers and percentage.

**Questionnaire :**

1. Do you know what is the Medico Legal case(MLC)?
2. Do you know your responsibilities while handling Medico Legal case?
3. Which is the best type of consent in MLC?
4. For how many years Hospital is legally bound to maintain the Medical records?
5. Do you know steps in management of poisoning case?
6. Do you know which preservatives are used for gastric lavage in poisoning cases?
7. Do you know Rule of nine?
8. What evidence should be collected in case of Sexual Assault cases?
9. Do you know how to give Injury certificate?
10. Do you know how to give WHO Death certificate?
11. Which cases require Medico legal Autopsy?
12. Do you know what is COPRA(Consumers Protection Act)?
13. Do you know what is HOTA(Human organ transplantation Act)?
14. Do you know about Hospital indemnity Insurance?

**Results:**

Total 140 interns and 110 PG students were included in our study, out of which it was observed that 53 interns and 71 PG students can identify the MLC and know about their medico legal responsibilities while handling the case.
Table 1: Various Medico legal Issues Related Questions

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Questions</th>
<th>Interns (140)</th>
<th>PG students (110)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Know what is the Medico Legal Case?</td>
<td>74(53%)</td>
<td>97(88%)</td>
</tr>
<tr>
<td>02</td>
<td>Know responsibilities while handling MLC.</td>
<td>73(53%)</td>
<td>98(88%)</td>
</tr>
<tr>
<td>03</td>
<td>Type of consent in MLC.</td>
<td>87(62%)</td>
<td>102(93%)</td>
</tr>
<tr>
<td>04</td>
<td>Preservation of hospital medical record.</td>
<td>64(46%)</td>
<td>88(80%)</td>
</tr>
<tr>
<td>05</td>
<td>Know steps in management of poisoning cases.</td>
<td>34(24%)</td>
<td>69(63%)</td>
</tr>
<tr>
<td>06</td>
<td>Know the preservatives used for analysis of gastric lavage.</td>
<td>38(24%)</td>
<td>72(65%)</td>
</tr>
<tr>
<td>07</td>
<td>Know the Rule of nine.</td>
<td>91(65%)</td>
<td>77(70%)</td>
</tr>
<tr>
<td>08</td>
<td>Know about the evidence to be collected in case of Sexual Assault</td>
<td>29(21%)</td>
<td>43(47%)</td>
</tr>
<tr>
<td>09</td>
<td>Know how to write Injury certificate.</td>
<td>28(20%)</td>
<td>33(30%)</td>
</tr>
<tr>
<td>10</td>
<td>Know WHO Death certificate.</td>
<td>20(14%)</td>
<td>28(25%)</td>
</tr>
<tr>
<td>11</td>
<td>Need of Medico legal Autopsy.</td>
<td>67(48%)</td>
<td>84(76%)</td>
</tr>
<tr>
<td>12</td>
<td>Know about COPRA.</td>
<td>63(45%)</td>
<td>97(88%)</td>
</tr>
<tr>
<td>13</td>
<td>Know about HOTA.</td>
<td>17(12%)</td>
<td>44(40%)</td>
</tr>
<tr>
<td>14</td>
<td>Know about Hospital indemnity Insurance.</td>
<td>15(11%)</td>
<td>47(43%)</td>
</tr>
</tbody>
</table>

62% interns and 93% of PG students were aware that Written and Informed consent is the best type of consent in MLC. 46% interns and 80% PG students knew about Medical record keeping in Hospital.

24% interns and 63% PG students knew about the steps in management of poisoning cases and the preservatives used for analysis of gastric lavage. Rule of nine which is utilized to calculate and manage burn cases was known to 65% interns and 70% PG students, but very few i.e. 21% interns and 47% PG students knew about evidence to be collected in Sexual assault cases.

20% interns and 30% PG students were knew the proper way to write Injury certificate, but only 14% interns and 25% PG students were aware about Death certificate designed by WHO (World Health Organization). 48% interns and 76% PG students were aware of the cases which needed Medico legal Autopsy.

45% interns and 88% PG students know about Consumers protection act (COPRA) but only 12% interns and 40% PG students know about Human organ transplantation Act (HOTA). Hospital indemnity Insurance which holds big importance for Private Practitioners was known to 11% intern and 43% PG students.

Discussion

An MLC is defined as case of injury/illness where the attending doctor, after taking proper history and examination of patient, has the impression that some investigation by law enforcement agencies is essential to establish and fix responsibility for the case in accordance with the law of the land. Many doctors
are apprehensive in handling such cases may be because of fear, unwarranted laws and regulations, attending the court, harassment by the lawyers and questions by police personnel\(^{14}\).

With the increase in use of internet and social media, there is an increase in awareness among public on subject of ethical conduct of medical practitioners. Hence there are more cases against doctors. This issue is of immediate concern to medical fraternity. Hence all medical practitioners must be aware of legal and ethical implications of clinical practice.

We carried out a study to determine the knowledge and awareness in interns and PG students in our institution. In our study of 140 interns and 110 PG students we noticed that PG students were more aware about MLCs than that of interns may be because they are exposed to more MLCs during their post graduation. This is in accordance with the study by Dash S.K. in 2010\(^{9}\).

Most Interns and PG students had good knowledge about written informed and valid consent but less were aware about medico legal record keeping in hospital. There is no certain time limit after which Medico Legal reports can be destroyed; so they have to be preserved carefully. In view of the multitude of cases against the doctors under the Consumer Protection Act, it is advisable to preserve all the inpatient records for at least 5 years and outpatient department records for 3 years\(^{13}\). This was known to less participants. This finding is similar to study conducted by Rai JJ, et al among interns and postgraduates about medical law and negligence in Vadodara in 2016\(^{2}\).

PG students can manage the poisoning cases and know how to preserve the gastric lavage, but both interns and PG students don’t know exactly the matter and manner of injury certificate and WHO death certificate i.e. How to write immediate, antecedent and other cause of death in death certificate. Our findings are in agreement with study to assess the need of Medico legal Education in interns and residents by Pratibha Mardikar and Arti Kasulkar in 2015\(^{15}\). Medical council of India (MCI) has recommended that it is compulsory to know about injury report, collection of biological material and all aspects of medico legal cases for MBBS graduates\(^{13}\).

Interns and PG students are aware about COPRA but very less is known about HOTA and Hospital indemnity insurance. Awareness about human organ donation among people is more due to social media, doctors should also make themselves aware about laws related to the same. Due to increase in medical negligence cases by doctors should have their Hospital indemnity insurance. Workshops related to various medical acts should be conducted regularly so as to update the knowledge.

**Conclusion**

This study was an attempt to evaluate the Knowledge and Awareness of Medico Legal issues in Interns and Post Graduate Students. Majority of the interns have lack of knowledge about Medico Legal issues while average PG students are aware about ML cases and can handle them independently. But both have poor knowledge about certificates, laws and hospital indemnity insurance. One month compulsory posting in Forensic Medicine Department should be introduced in their training period to increase the knowledge and awareness about Medico Legal issues. Also Medical associations should try to organize seminars, case discussions and CME’s for interns and post graduates to increase awareness and to update them about Medico legal issues in medical practice.

**Ethical Clearance** – Taken From Institutional Committee

**Source of Funding** – Self

**Conflict of Interest** - Nil

**References**

1. Modi’s Medical jurisprudence and toxicology.


Human Fatalities from Wild Elephant Attacks: A Fiveyear Autopsy-Based Study

Saptarshi Chatterjee¹, Ranjan Biswas², Tanmay Sardar¹, Shobhan Roy³

¹Associate Professor, Department of Forensic Medicine and Toxicology, ²Assistant Professor, Department of Psychiatry, Bankura Sammilani Medical College, Bankura, West Bengal, ³Assistant Professor, Department of Forensic Medicine and Toxicology, Deben Mahata Government Medical College, Purulia, West Bengal

Abstract

Background: Human-Elephant Conflict may take many forms, from crop raiding and infrastructural damage, to injury or death of humans or elephants. Conflicts or co-existence between human and wildlife is mostly attributed to the loss, degradation, and fragmentation of wildlife habitats through human activities like logging, animal husbandry, agricultural expansion, and development projects. The present study is conducted to study the prevalence of fatal wild elephant attacks, the socio-demographic profiles of the victims along with their nature of injuries and the risk factors associated with these conflicts.

Methods: This is a cross-sectional descriptive study, which was conducted by analysing 41 cases of fatal Human-Elephant Conflicts, which attended to B.S. Medical College Police Mortuary between 2015 to 2019.

Conclusion: Authors feel that the blockage of elephant migratory routes, coupled with cultivation of elephant preferred crops and scarcity of food in the forest in some season, inevitably attracts more human-elephant conflicts.

Keywords: Human-Elephant Conflict, Musth, Crop raiding

Introduction

Expansion of human settlements and agricultural fields across Asia and Africa has resulted in widespread loss of elephant habitat, degraded forage, reduced landscape connectivity and a significant decline in elephant populations relative to their historical size and overall range ⁴. As their habitats shrink, elephants are progressively forced into closer contact with people, resulting into more frequent and severe conflicts over space and resources with consequences ranging from crop raiding to reciprocal loss of life. Fatalities due to animal attack are rare in forensic medical work, but have been known to pose problems due to their potential to mimic homicide ².

Human-elephant conflicts (HEC) have been a common phenomenon, which accounts for 1,713 human and 373 elephant deaths by unnatural causes, including electrocution and poaching ³. The conflict occurs when the needs and behaviour of wildlife impact negatively on the goals of humans, or when the goals of humans negatively impact the needs of wildlife ⁴. Conflicts or co-existence between human and wildlife is mostly attributed to the loss, degradation, and fragmentation of wildlife habitats through human activities like logging, animal husbandry, agricultural expansion, and development projects ⁵.

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Corresponding author:
Dr. Shobhan Roy
Assistant Professor, Department of Forensic Medicine and Toxicology, Deben Mahata Government Medical College, Purulia, West Bengal
Though human deaths from wild elephant attack is common in various parts of the country, including West Bengal, reports on autopsy-based studies are scarce in literature. The most recent assessment in West Bengal on the number of human injuries and fatalities from wild elephants was conducted about 10 years ago. In this backdrop, the present study is conducted to study the prevalence of fatal wild elephant attacks, the socio-demographic profiles of the victims along with their nature of injuries, who fell prey to these wild elephants. Our study also aims to assess the common risk factors contributing to these HEC, and hence to suggest preventive strategies to be taken to protect the vulnerable population from the wild elephant attack.

Aims and Objectives

1. To study the prevalence of deaths due to wild elephant attacks from 2015 to 2019.

2. To study the socio-demographic profiles of the victims of elephant attacks.

3. To analyse the nature of injuries on the victims.

4. To assess the risk factors of human-elephant conflicts.

Materials and Methods

a. Place of study: Bankura Sammilani Medical College Police Mortuary, Bankura, West Bengal, India.

b. Period of study: 1st January – 30th June, 2020

c. Study population: All the patients sent for autopsy examination at BS Medical College Police Mortuary between 2015 to 2019 (From the Post-Mortem register)

d. Sample size: All the victims of fatal elephant attacks, attended to BS Medical College Police Mortuary for autopsy examination between 2015 to 2019.

· Exclusion criteria:
  i. Victims of fatal animal attacks, other than elephants
  ii. Cases with incomplete or inadequate history

e. Study design: Cross-sectional descriptive study

f. Source of data:
  i. Post-Mortem register of Bankura Sammilani Medical College Police Mortuary

g. Statistical analysis: Details regarding the cases were obtained from the inquest report, interviewing the eyewitnesses and the family members of the deceased. All the data were manually checked and edited for completeness in a pre-determined format and were then coded for computer entry. Collected data was recorded in Microsoft Excel worksheet and SPSS IBM 19. The data was collected, tabulated and statistically analyzed by applying student’s t-test. The p <0.05 was considered as statistically significant.

Results

1. Prevalence of Fatal Human elephant conflicts

The number of victims due to HEC was maximum in 2015, with a gradual decline over years [Figure 1].

2. Distribution according to age

Majority of the victims of fatal HEC belong to the age group of 30 to 70 years [Figure 2].

3. Distribution according to gender

Of all the victims of fatal HEC, males constituted 63.4% and the rest 36.6% were females.

4. Distribution according to residence

In our study, 38 out of total 41 victims (92.68%), belonged from places situated within 6 kilometres from the jungle.

5. Place of occurrence
28 victims (60.9%) were attacked in the crop fields, 5 victims (12.2%) each in jungles and roads, and the remaining 3 victims (7.3%) in their respective houses.

6. Time of attack

46.3% of the victims were attacked in between 8.00 pm and 12.00 midnight, followed by 28.8% of the victims between 4.00 pm and 8.00 pm. The rest 26.83% of the victims fell prey in between 12.00 at midnight to 8.00 in the morning.

7. Situations leading to Human-Elephant conflicts

As stated by the eye witnesses during interaction and inquiry, 41% of the victims were drunk and were chasing elephants in the crop fields and near households, 27% were returning home after dark from work, 11% had gone collecting fuelwood from the forests and 10% were defecating in the open at night. 3 victims succumbed to the injuries in their sleep, when their mud house’s wall accidentally caved in to an elephant’s rampage. One woman was attacked by a tusker, as she tried to offer prayers to the elephant in the name of a Hindu god.

8. Distribution according to months

Maximum number of deaths due to HEC was recorded between February to May [Table 1].

9. Causes of death of the victims

In our study, 37 victims (90.2%) died due to trampling of vital organs and three persons died due to traumatic asphyxia as a result of house collapse in their sleep. The body of the woman, who went to offer prayers, exhibited one fatal perforated wound in the abdomen produced by the tusk.

10. Elephants attacking in herd or single

In all the cases, the aggressors were lone tuskers, except three cases where the offender was a female elephant in herd.

**Discussion**

Elephants are usually peaceful animals. Females may, however, become aggressive when they are excessively irritated by the villagers and when they apprehend dangers to their young. Bulls can be exceptionally aggressive during musth, when they usually venture away from their home range in search of mating opportunities. All elephants may become aggressive when sick, injured or harassed.

According to the Union Environment Ministry, 2,398 people have died since 2014 up to March 31, 2019 due to Human-Elephant conflicts, with West Bengal recording the maximum 403 deaths in last five years. However, in our study, a total of 41 people died due to HEC from 2015 to 2019, with a steady decline in the mortality rate from 2016 onwards. A combination of early warning to detect elephants before they enter the crop fields and the employment of elephant driving squads, coupled with a front line communal guarding strategy proved most successful. Consistent to another study from the same region, the majority of the victims were between 30 to 70 years, with males predominating in all the age groups. Farmers and labourers are mostly males and often go alone in the forest path or work in agricultural lands near forest, and thus, they are most vulnerable to these human-animal conflicts.

Previous studies concluded that elephants do not discontinue using the high-risk human settlements, which were once part of their original home range and instead modify their activities by travelling faster or being nocturnal within such areas. Human mortality and morbidity in population residing around forest areas are primarily due to continued harassment and taunting of elephants while being driven back to forests from crop fields or by getting too close or getting involved in provoking activities to already injured, traumatized, harassed mammals or males in musth or females with young calves. The same findings are being reflected in our study, where more than 90% of the victims recorded their residence within 6 kilometres from the jungle, making themselves more vulnerable to the fatal HEC.

Human-elephant conflicts can be largely attributed to colonialism, with the people taking over the elephants’
indigenous culture, as they are pushed into smaller places and killed outright. Elephants need a large space to roam, with lots of vegetation and abundant water supplies to help them digest all that roughage. When those areas are taken up with human crops, elephants are happy to adjust eating corn or other plants meant for people. This very fact is amplified in our study, where the majority of the deceased were attacked in their crop fields. Chattopadhyay and Das reported that most of the incidents were noted between 4.00 am to 6.00 am. Contrary to this finding, critical time of major HEC in our study was between 8.00 pm to 12.00 at midnight, where almost 3/4th of the victims got affected while chasing elephants under intoxication or during late homecomings from workplaces. The growth of industries in the last ten years with increased employment and increased cash flow, adding to a steep rise of substance abuse and other intoxications, coupled with poor transportation may explain this difference in findings. This tilt in time frame may also be due to increased habits of indoor latrines amongst people of this area, compared to the ones in 2007-2009, when people usually visited outdoors for defaecation.

In order to understand the seasonal patterns of damage by elephants, the attractiveness of crops in relation to wild forage in a particular season, the extent of availability of crops, and the presence of elephants near cultivated areas should be considered. When the attractiveness of crops in relation to wild forage crosses certain threshold, high encounters can be expected, like we observed in our study between the months of February to May. The abundance of rains from June to October determined local agricultural practices and by January-February most of the seasonal crops become ripe, attracting the elephants. Coupled to this, the dry months of March to May, with scarce water and food resources, compel these mammals to descend to low elevated lands from the Dalma Hills in Jharkhand for their steady supplies.

In an attempt to kill, elephants usually first grab the victim with their trunk and then crush them under their feet. Sometimes they throw them from high lifting position on the ground, followed by trampling. Females and older males are less likely to put up much resistance. In concordance to this, 90.2% of the victims of our study died due to trampling of vital organs. The death of the three persons due to traumatic asphyxia as a result of house collapse, can be attributed to accidental encounters with these mammals.

Elephant males tend to move alone or in small temporal groups, whereas females move in family based larger groups. Studies on behavioural strategies between adult males and female-led herds indicate that the solitary tuskers are more ready to assume the risks of crop raiding than females. It has been suggested that females might become obligate crop raiders when living in highly fragmented habitats, where resources are more limited and scattered in human dominated landscapes. Reports in various newspapers also suggest that high number of human deaths occurs in the months of March to May due to sudden unprovoked attacks by lone tuskers. This is also considered to be the mating season of elephants in this region, where many adult bulls are in their musth state. During this musth phase, tuskers become violent and aggressive toward all other animals, even to its own kind. The same results are reflected in our study, where 38 out of 41 victims succumbed to their injuries inflicted by lone tuskers.

Human-Elephant Conflict may take many forms, from crop raiding and infrastructural damage, through disturbances of normal activities such as travel to work or school, to injury or death of humans or elephants. HEC is a problem that poses serious challenges to wildlife managers, local communities and elephants alike.

In the present study, majority of the victims belonged to the age groups of 30 to 70 years, with male preponderance in all the ages. Most of the victims of HEC were affected between 8.00 pm to 12.00 at midnight, in their act of either chasing the mammals under intoxication or while returning home from workplaces in the late hours. Though crop fields were the usual places of encounters, majority of the incidences occurred between February to May. Trampling of vital organs by
lone tuskers remain the usual finding in victims of HEC. Blockage of elephant migratory routes due to various anthropogenic activities coupled with cultivation of elephant preferred crops in large extent along the forest ranges and lack of food in the forest in some season inevitably attracts more human-elephant conflicts. However, understandings of how people living in or near conflict prone areas use natural resources, and how they make decisions about current and future resource use, remains key to addressing the underlying drivers of Human-Elephant conflict and their spatial variation. Without this knowledge, the task of resolving Human-Elephant Conflict and finding a means for these species to coexist in the Anthropocene is Sisyphean.

Acknowledgements: The authors are grateful to authors, editors and publishers of all those articles, journals and books from where the literature of this article has been reviewed and discussed.

Conflict of Interest: No conflict of interest is associated with this work.

Other Interest: The same article was sent to another Journal for consideration. However, even after receiving the acceptance letter from the editor, the authors refrained themselves from paying the processing charges and publication in the said journal, after finding the Journal not indexed as per The Gazette of India (Board of Governors) dated the 12th of February, 2020.

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Ethical Clearance: Taken

References


The Unrevealed Truth about the Tongue in Forensic Identification


1MPH Candidate, Temple University College of Public Health, Department of Epidemiology and Biostatistics, Ritter Annex, 9th floor, 1301 Cecil B Moore Ave, Philadelphia, 2Professor; 3Professor & HOD; Department of Oral and Maxillofacial Pathology and Microbiology, D.Y. Patil University School of Dentistry, Sector 7, Nerul, Navi Mumbai, Maharashtra, India, 3Professor & HOD; Department of Oral Medicine and Radiology, 5Associate Professor; Department of Oral and Maxillofacial Pathology and Microbiology, 6Assistant Professor; Department of Oral and Maxillofacial Pathology and Microbiology, D.Y. Patil University School of Dentistry, Sector 7, Nerul, Navi Mumbai, Maharashtra, India, 7Analyst, Department of Population Health, New York University Grossman School of Medicine, New York, NY

Abstract

Background: Forensic odontology, a branch of dentistry includes identification of individuals in various crime scenes, natural calamities, and mass disasters. The identification is possible because every individual body is unique and so is our tongue due to its morphological variations. The primary objective of the study was to assess the morphological features of the tongue and its use in sex determination.

Methods: The study included a sample size of 100 individuals (50 males and 50 females) in the age range of 20-50 years old. Photographs were taken of front and side view of the tongue; visual inspection was done and lastly impressions of the tongue were made with help of alginate and then poured with the help of dental stone. IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data. Microsoft word and Excel were used to generate graphs, tables etc. Females presented with triangular shape, presence of shallow fissures more commonly and a sharp lingual apex of tongue. Males presented with rectangular shape, presence of deep fissure/absence of fissures more commonly and septate/sharp lingual apex of the tongue.

Conclusion: Tongue exhibits various unique characteristics and can be used in sex determination.

Keywords: forensic odontology, morphological characteristics, sex determination, tongue impressions

Introduction

Forensic odontology, a branch of dentistry comprises of various aspects dealing with diagnostic and therapeutic evaluation and examination of various parts of oral cavity, identification of individuals and evaluation of bite marks in criminal investigations, sexual assaults, child abuse cases, mass disasters and in personal defence circumstances\(^{(1)}\). Various components of the oral cavity like teeth, saliva, pulp, dentin, lips etc. can be used in forensic odontology for age estimation, sex determination, DNA analysis and identification of the criminal.

The human tongue is a muscular organ covered by a thin mucous membrane which lies partly in the oral cavity and partly in the oropharynx. The tongue is well protected from the external environment and has its own skeletal muscles, nerve supply and blood vessels\(^{(2)}\).
The human tongue serves many purposes like being the organ of taste, articulation of speech, mastication, and intimacy. It is an internal organ which can be used for inspection thereby offering proof of life.

According to the Traditional Chinese Medicine, the tongue is a unique vital organ, and its vitality is well scored as, ‘Tongue of Life’ in the same (3). This muscular organ plays a very significant role in forensic identification due to its unveiling portion encompassing information with diverse visible changes from one individual to the other called as lingual impression thereby making it unique (2).

It possesses numerous characteristics presenting both geometric shape and physiological texture information which indeed can be useful in identity verification applications (4). The shape of tongue of an individual is unswerving and the physiological texture of tongue is immutable (2). In respect of shape and surface textures, everyone has unique pattern of tongue (5). The lingual morphological aspects are scrutinized with the help of alginate moulding technique which is the most reliable method for recording minute details of tongue; this in turn helps the forensic investigator to identify the person (2).

**Materials and Methods**

The present study was conducted in DY Patil Dental College and Hospital, Oral Pathology and Microbiology Department on June 17 for over 2 months. The study was carried out on 100 individuals (50 males and 50 females) in the age range of 20-50 years of age exclusively of Navi Mumbai population.

The inclusion criterion for the study is:

1. Individuals who are clinically healthy.
2. Individuals with clinically healthy tongue.

The exclusion criterion for the study is:

1. Individuals possessing any medical illnesses like HIV, diabetes, thyroid problems, hypertension, Down syndrome etc.
2. Individuals possessing any developmental anomaly or pathology of the tongue.

Institutional Ethics Research committee approval for the study and a written consent from each study participant was obtained prior beginning the study.

![Fig-1 shows a disposal tongue cleaner.](image)

Before the examination of the tongue, the patient was requested to clean his tongue with a disposable tongue cleaner and rinse his oral cavity [Fig 1]. The examination process of the present study was divided into three categories: 1) Photographs, 2) Visual inspection and 3) Impressions. The study participants were requested to protract their tongue in a relaxed position to prevent any contraction of the striated lingual muscles which would thus alter various characteristic features.
Fig-2a fig-2b shows the front and the side view of the tongue.

Fig-3a fig-3b shows making of impression, the alginate impression, and the cast of the tongue.

Photographs of the front and side view of tongue were then taken in the same setting using DSLR camera [Fig-2a and fig-2b]. Later, visual inspection was done i.e., clinically all the characteristics of the tongue such as the shape, colour, surface texture, type of lingual apex etc. that were visible were noted down on the performa. Lastly impressions of the tongue were made with the help of chromatic alginate impression material (Tropicalgin) [Fig-3a]. Chromatic Alginate was chosen as the choice of impression material as it has got an excellent property of duplicating minute details and it can be easily removed. To prevent the regurgitation reflex, impression was made from the level of the oral commissures up to the lingual tip of tongue and was then poured with the help of dental stone [Fig-3b]. This helped us obtain a relevant positive image for identification.

At the end of the examination, all the characteristic features of the tongue that were obtained using the above three methods were cross checked and evaluated.

**Statistical Analysis**

Descriptive and inferential statistical analyses were carried out in the present study. Results on categorical measurement were presented in number (%). Level of significance was fixed at $p=0.05$ and any value less than or equal to 0.05 was statistically significant.

Chi square analysis was used to find the significance of study parameters on categorical scale. The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data and Microsoft word and Excel were used to generate graphs, tables etc.
**Results and Discussion:**

In the current study, various features of tongue like shape, fissures and type of lingual apex were included.

Based on the study participants, the shape of tongue was categorised into 1) Triangular, 2) Ovoid, 3) Rectangular and 4) Square. Fissures of tongue was categorised into 1) Absence of fissures, 2) Shallow fissures and 3) Deep fissures. Lastly, the lingual apex was categorised into 1) Septate and 2) Sharp.

<table>
<thead>
<tr>
<th>Table 1: Comparison of tongue shape among males and females using chi square test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Chi square value: 88.395 p value: <0.001**

(p< 0.05 - Significant*, p < 0.001 - Highly significant**)

Table 1 shows comparison of tongue shape among males and females. There was a significant difference seen in the tongue shape of males and females. 98% of females presented with triangular shape of tongue and only 2% females presented with rectangular shape of tongue. In comparison, 96% of males presented with rectangular shape of tongue and only 4% of males presented with triangular shape of tongue. When these results were statistically analysed using chi square test, highly significant results were obtained.

<table>
<thead>
<tr>
<th>Table 2: Comparison of fissures among males and females using chi square test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Chi square value: 81.818 p value: <0.001**

(p< 0.05 - Significant*, p < 0.001 - Highly significant**)
Table 2 shows comparison of fissures among males and females. There was a significant difference seen in the fissures of tongue among males and females. 60% of males presented with deep fissures, 40% of males presented with absence of fissures. In comparison, 6% of females presented with deep fissures, 90% of females presented with shallow fissures and 4% of females presented with absence of fissures. When these results were statistically analysed using chi square test, highly significant results were obtained.

Table 3: Comparison of lingual apex among males and females using chi square test

<table>
<thead>
<tr>
<th>Group</th>
<th>Lingual apex</th>
<th>Total N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sharp n (%)</td>
<td>Septate n (%)</td>
</tr>
<tr>
<td>Female</td>
<td>50 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Male</td>
<td>43 (86)</td>
<td>7 (14)</td>
</tr>
<tr>
<td>Total</td>
<td>93 (93)</td>
<td>7 (7)</td>
</tr>
</tbody>
</table>

Chi square value: 7.527 p value: 0.006*

(p< 0.05 - Significant*, p < 0.001 - Highly significant**)

Table 3 shows comparison of lingual apex among males and females. There was a significant difference seen in lingual apex among males and females. 100% of females presented with sharp lingual apex. In comparison, 86% males presented with sharp lingual apex and 14% of males presented with septate lingual apex. When these results were analysed statistically analysed using chi square test, significant results were obtained.

Forensic odontology is gaining great interest in today’s world due to presence of innovative and efficient identification systems. The purpose of introducing the use of tongue prints in forensic odontology is due to the uniqueness of the tongue. In the present study, females presented with triangular shape of tongue and presence of shallow fissures more commonly than deep or absence of fissures. While males presented with rectangular shape of the tongue and presence of deep fissures more commonly than absence of fissures. As far as lingual apex was considered, females presented with sharp lingual apex while men presented with sharp as well as septate lingual apex. The current study shows diversification in various morphological aspects of tongue with respect to males and females.

On the contrary, there are also similar kinds of studies conducted. One of the studies includes shape and texture of the tongue as criteria, which showed results based on the age groups. The other study includes criteria like shape of tongue and presence of fissures on the tongue. In this study, females presented with deep fissures while the males presented with shallow fissures. Because of the uniqueness of the tongue, several studies have been undertaken to prove the use tongue prints in biometric authentication along with fingerprints and iris.

**Conclusion**

The field of forensic odontology scrutinizes various dental evidence, and it confirms that individuals have some unique characteristics which can be used for evidence purpose. The dorsal surface of the tongue...
holds prominent morphological features, thereby difficult to forge and can therefore be used in forensic odontology for sex determination\(^2,\,5\). A photographic image of the lingual aspect along with the lingual impression, together can aid in forensic identification along with other proved methods\(^2\). Every dentist should therefore comprehend the forensic values and its connection with dental practice\(^3\).

Financial support and sponsorship: Nil

Conflicts of Interest: There are no conflicts of interest.

References

A Study to Evaluate Effectiveness of Kangaroo Mother Care
on Physiological Parameters of Premature Babies in Vadodara City

Sejal Solanki1, Prachi Kachhiya1, Kamali Patel1, Vrunda Patel1, Trushali Baria1, Kinnari Jatva1, Sanjana Bhatia2
1Final Year B.Sc Nursing Student, 2Assistant Professor, Dinsha Patel College of Nursing, Nadiad, Gujarat, India

Abstract

According to WHO, mostly babies are die in their neonatal period. India, current infant mortality rate is about 32 infants per 1000 live birth. A Quasi Experimental study was conducted to evaluate the effectiveness of Kangaroo Mother care on physiological parameters of premature neonate of experimental group and control group in selected hospital in Vadodara city. Sample size for the present study was consist of 60 babies. (30 experimental and 30 control group) The instrument used for data collection are Kangaroo Mother Care Assessment Flow Sheet (KMCAFS). The data analysis was done by using descriptive and inferential statistics. The result of the present study was that most of the premature babies after receiving KMC Care, there was a significant incensement in the weight. Here the paired t-test value of temperature and weight are(t<0.050)not significant and pulse and respiration are (>0.05) significant. The study concluded that KMC is a simple and acceptable method for the mother can be continued at home and there by improves the infant growth and reduce morbidity.

Keyword: Evaluate, Effectiveness, Kangaroo Mother Care, Physiological parameter, Premature Babies.

Introduction

Preterm birth, also known as preterm birth, is the birth of a baby at less than 37 weeks gestational age. Preterm babies are at greater risk for cerebral palsy, delays in development, hearing problems, and problems seeing. These risks are greater the earlier a baby is born. Preterm birth is the most common direct cause of newborn mortality. Preterm birth and being small for gestational age (SGA), which are the reasons for low-birth-weight (LBW), are also important indirect causes of neonatal deaths. LBW contributes to 60% to 80% of all neonatal deaths.

According to WHO, mostly babies are die in their neonatal period. India, current infant mortality rate is about 32 infants per 1000 live birth. And preterm birth is considered to be the largest cause of neonatal mortality, there are many studies that highlight the importance of KMC in maintaining the premature health and improving the condition. Indeed, kangaroo mother care has positive effects on premature babies and mothers.

Need For the Study: A study has estimated that, at birth 95% of underweight infants are born in developing countries. without access to appropriate infant care the KMC may be the only alternative for this children not only for survival but to achieve a better quality of life.

Objectives:

1) To evaluate the effectiveness of Kangaroo Mother Care on Physiological parameters of premature neonates of experimental group and control group in selected hospital in Vadodara city.

2) To find out the association between the
physiological parameters of premature infants with the selected demographic variables in selected hospital in Vadodara city.

**Hypothesis**

H1: There will be a significant difference between mean post-test physiological parameters outcome score and mean pre-test physiological parameters outcome score among experimental and control group at the 0.05 level of significance.

H2: There will be a significant association between physiological parameters outcome scores and selected demographic variables of preterm baby among experimental group during KMC care at the 0.05 level of significance.

**Research Methodology**

Research Approach: Quantitative research approach is used for this present study

Research Design: The research design used for the present is quasi-experimental pre-test and post-test design with control group.

Population: patient admitted in hospital in KMC ward

Sampling Technique: Non probability purposive sampling technique.

Sample size: The sample size 60 (30 experimental group and 30 control group)

Descriptive Tool:

**Section 1:** comprised of demographics data

**Section 2:** comprised of kangaroo mother care assessment flow shit.

**Result**

The result of the present study was that most of the premature babies After receiving kangaroo mother care, there was a significant incensement in the weight.

Percentage Distribution of Preterm babies in Experimental Group According their characteristics.
1. Findings related to Mean, Mean difference, standard deviation and ‘t’ test

The paired t-test value of temperature and weight are (t<0.05) not significant and pulse and respiration are (t>0.05) significant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre test</th>
<th>Post test</th>
<th>Paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Temperature</td>
<td>97.6</td>
<td>0.938</td>
<td>97.7</td>
</tr>
<tr>
<td>Pulse</td>
<td>136.26</td>
<td>3.841</td>
<td>139.8</td>
</tr>
<tr>
<td>Respiration</td>
<td>37.73</td>
<td>5.09</td>
<td>40.3</td>
</tr>
<tr>
<td>Weight</td>
<td>1.72</td>
<td>0.18</td>
<td>1.79</td>
</tr>
</tbody>
</table>

2. Findings related to association between selected demographic variable and physiological parameters outcome of preterm babies.

1. In temperature among age, gender, length in hospital stay, gestational age, mother education, type of delivery value (p<0.05) not significant, and birth weight value is (p>0.05) significant.

2. In pulse among all demographic variables are (p<0.05) not significant.

3. In respiration among age, gender, birth weight and education of mother are (p<0.05) not significant and gestational age, length in hospital stay and type of delivery are (p>0.05) significant. The value.

4. in weight among age, gender, birth weight and education of mother are (p<0.05) not significant and gestational age, length in hospital stay and type of delivery are(p>0.05) significant.

Conclusion

The main conclusion drawn from the present study was that most of the premature babies after receiving kangaroo mother care there was a significant incensement in the weight. Samples became familiar and found themselves comfortable and also expressed satisfaction. It is concluded that the kangaroo mother care is effective and a simple strategy to balance physiological parameters.

Ethical Clearance- Taken from Institutional Ethical committee

Source of Funding- Self (Management of Institute)

Conflict of Interest - Nil

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The Regularity of Antenatal Care through Knowledge of Pregnant Women and Support from Husbands

Sendy Ayu Mitra Uktutias¹, ⁵, Ade Ayu Mitra Ramadita Daluas¹, Sri Iswati², Cholichul Hadi³

¹Postgraduate School, Department of Human Resource Development, ²Faculty of Economics and Business, Department of Economics and Business, ³Faculty of Psychology, Department of Psychology, Universitas Airlangga, Surabaya, Indonesia, ⁵Stikes Yayasan Dr. Soetomo, Surabaya, Indonesia

Abstract

Pregnant women’s knowledge and husband’s support are factors that influence antenatal care regularity. This study aims to analyze the effect of knowledge of pregnant women and husband’s support on antenatal care regularity. This study a cross-sectional design and was conducted on 38 pregnant women as respondents using linear regression statistical tests. The result showed that there was an effect of knowledge of pregnant women and the husband’s support on the regularity of antenatal care. The better the knowledge of pregnant women about their pregnancy and the higher the support of their husbands will have an impact on the regularity of antenatal care. The implication of this research is for midwives who are at the forefront of empowering cadres and providing health education to pregnant women and husbands about the importance of regular antenatal care. Also, midwives need to improve competence through midwifery training efforts.

Keywords: knowledge, husband’s support, antenatal care, pregnant women

Introduction

Antenatal care is a health service by professionals for pregnant women during their pregnancy which is carried out in accordance with established antenatal service standards. Antenatal care is an important way to monitor and support the health of pregnant women. It is advisable to visit a midwife or doctor as early as possible so that early detection of risk factors for pregnancy can be carried out. According to the World Health Organization (WHO), antenatal care during pregnancy to detect early on the occurrence of high risks to pregnancy and childbirth can also reduce maternal mortality and monitor the condition of the fetus. Visit for pregnant women to health services are recommended once in the first trimester, 1 time in the second trimester, and at least 2 times in the third trimester.² The indicators used to assess maternal health services during pregnancy are K1 and K4. Saipudin explained that antenatal care is said to be regular if pregnant women do antenatal care >4 visits, less regular if two to 3 visits, and irregular if pregnant women only do antenatal care less than 2 visits.³

In Indonesia, the importance of antenatal care visits has not become a top priority for some pregnant women regarding their pregnancy. The result of basic health research (Riskesdas) conducted by the ministry of health shows that data on antenatal care coverage in Indonesia during the last 3 years in 2013-2015, namely in 2013 amounted to 92.7% and in 2015 amounts to 95.2%. The first antenatal care coverage in the first trimester during the last 3 years in 2013-2015 namely in 2013 amounted to 92.7% and in 2015 amounts to 95.2%. The first antenatal care coverage in the first trimester during the last 3 years in 2013-2015 namely in 2013 amounted to 92.7% and in 2015 amounts to 95.2%.
Several researchers have conducted research to determine the factors that can affect maternal Antenatal care visits during pregnancy. Factors that influence a person’s behavior in utilizing health services based on the theory of Lawrence W. Green are derived from behavioral factors and factors outside of behavior (non-behavior causes). Meanwhile, the division according to the concept and behavior of a person as suggested by Green includes predisposition factors, enabling factors, and reinforcing factors. Predisposing factors include age, level of education, employment status, parity of pregnant women, pregnancy distance, knowledge of pregnant women, and attitudes of pregnant women. Meanwhile, the enabling factors are the distance of residence, family income, and information media. Strengthening factors include husbands’ support, family support, and health worker factors. In line with it explains that maternal knowledge is a problem that often arises and results in low antenatal care coverage. Not only knowledge, research conducted, states that husband support is a very influential factor in decision making, including antenatal care.

This study focuses more on the factors of knowledge of pregnant women and husband’s support for regular antenatal care visits, that pregnant women miss antenatal care visits during their pregnancy. The purpose of this study was to analyze the effect of knowledge of pregnant women and husband’s support on the regularity of antenatal care visits.

Methods

Type and design

This study is analytical research with a cross-sectional design. This study to know the effect of the regularity of antenatal care through knowledge of pregnant women and support from husband’s.

Population and sample

The population of all pregnant women at Waru Health Care Centre of Pamekasan District with 38 respondent pregnant women with a proportional random sampling technique. This study has inclusion criteria, namely only third-trimester pregnant women or gestational age >32 weeks and willing to be respondents who will be sampled.

Results

The result of the study of research that has been conducted with 38 respondents of pregnant women is as follows.

<table>
<thead>
<tr>
<th>No.</th>
<th>Characteristics</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>17 – 25 years</td>
<td>15</td>
<td>39.5</td>
</tr>
<tr>
<td></td>
<td>26 – 35 years</td>
<td>17</td>
<td>44.7</td>
</tr>
<tr>
<td></td>
<td>36 – 45 years</td>
<td>6</td>
<td>15.8</td>
</tr>
<tr>
<td>2.</td>
<td>Gravida</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Primi</td>
<td>12</td>
<td>31.6</td>
</tr>
<tr>
<td></td>
<td>Multi</td>
<td>20</td>
<td>52.6</td>
</tr>
<tr>
<td></td>
<td>Grandemulti</td>
<td>6</td>
<td>15.8</td>
</tr>
<tr>
<td>3.</td>
<td>Education Level</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elementary School</td>
<td>2</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>Junior High School</td>
<td>15</td>
<td>39.5</td>
</tr>
<tr>
<td></td>
<td>Senior High School</td>
<td>12</td>
<td>31.6</td>
</tr>
<tr>
<td></td>
<td>Academy/Univesity</td>
<td>9</td>
<td>23.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>4.</td>
<td>Mother’s Job</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>House wife</td>
<td>26</td>
<td>68.4</td>
</tr>
<tr>
<td></td>
<td>Civil worker</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Private Sector Worker</td>
<td>5</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>Entrepreneur</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Etc (Farmers)</td>
<td>6</td>
<td>15.8</td>
</tr>
</tbody>
</table>

Tabel 1. Characteristics of Pregnant Women at Waru Health Care Center of Pamekasan District
Based on the characteristics of pregnant women in the Waru Health Care Center of Pamekasan District, Table 1, showed that almost half of pregnant women were 26-35 years old and multigravida with 1-2 children still living and having an educational background, pregnant women are primary school and work as housewives.

**Tabel 2. Knowledge Women’s and Antenatal Care (ANC) visits at Waru Health Care Center of Pamekasan District**

<table>
<thead>
<tr>
<th>No.</th>
<th>Knowledge of Pregnant Women’s</th>
<th>Antenatal Care (ANC) visits</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Irregular</td>
<td>Regular</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
<td>Percentage</td>
<td>Frequency</td>
</tr>
<tr>
<td>1.</td>
<td>Low</td>
<td>15</td>
<td>100,0</td>
</tr>
<tr>
<td>2.</td>
<td>Average</td>
<td>1</td>
<td>7,7</td>
</tr>
<tr>
<td>3.</td>
<td>High</td>
<td>0</td>
<td>0,0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>16</td>
<td>42,1</td>
</tr>
</tbody>
</table>

Based on the knowledge of pregnant women and pregnancy check-ups for pregnant women in Table 2, it shows that many pregnant women who have low knowledge do not regularly perform antenatal care. Based on the knowledge of pregnant women and pregnancy check-ups for pregnant women in Table 2. It shows that many pregnant women who have low knowledge do not regularly perform antenatal care. Roger’s (1974) in Notoadmodjo. Soekidjo (2003), a theoretical review states that knowledge is a very important domain for shaping one’s action. Burhaeni S (2013) in her scientific work, explained that the aspect of knowledge is very important for the use of Antenatal Care (ANC).

**Tabel 3. Husband’s Support and Antenatal Care (ANC) visits at Waru Health Care Center of Pamekasan District**

<table>
<thead>
<tr>
<th>No.</th>
<th>Husband’s Support</th>
<th>Antenatal Care (ANC) visits</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Irregular</td>
<td>Regular</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
<td>Percentage</td>
<td>Frequency</td>
</tr>
<tr>
<td>1.</td>
<td>Not support</td>
<td>17</td>
<td>73,9</td>
</tr>
<tr>
<td>2.</td>
<td>Support</td>
<td>5</td>
<td>33,3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>22</td>
<td>57,9</td>
</tr>
</tbody>
</table>
Based on the regularity of visits to antenatal care with the support of husbands and antenatal care in Table 3, it is known that the husband’s support affects the regularity of pregnancy examinations for pregnant women.

**Tabel 4. Linear regression knowledge pregnant women and husband’s support for Antenatal Care (ANC) visits at Waru Health Care Center of Pamekasan District**

<table>
<thead>
<tr>
<th>No.</th>
<th>Variable</th>
<th>B</th>
<th>B</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Knowledge pregnant women</td>
<td>0.071</td>
<td>0.325</td>
<td>0.030</td>
</tr>
<tr>
<td>2.</td>
<td>Husband’s support</td>
<td>0.067</td>
<td>0.596</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Based on the result of linear regression in Table 4, knowledge of pregnant women and husband’s support affect the regularity of antenatal care visits with a p-value of 0.030 for knowledge pregnant women and a p-value of 0.000 for husband’s support.

**Discussion**

Antenatal Care (ANC) needs to be done because it has benefits to improve maternal health during pregnancy and childbirth. Knowledge of pregnant women regarding Antenatal Care (ANC) is still lacking. The reason for the lack of knowledge of pregnant women about Antenatal Care (ANC) at Waru Health Center Care of Pamekasan District has an educational background, namely elementary school graduates (Table 1). The theoretical review explains that highly educated people will give a more rational response to the information that comes, will think to what extent they will benefit from the idea. So, it’s undeniable, the higher a person’s education, the easier they will receive information so that in the end a lot of knowledge they have. It’s necessary to increase the knowledge of pregnant women about Antenatal Care (ANC) and risk in pregnancy to maintain a healthy pregnancy. If a risk is found, it will be detected early for immediate treatment. This research shows, If pregnant women are based on a good knowledge of health, then that pregnant woman will understand about maintaining health and have the motivation to apply good health patterns in life. According to the results of this study, which shows the higher the knowledge of pregnant women, the more regular they are in carrying out Antenatal Care (ANC). There is another factor, the husband’s support is important in implementing Antenatal Care (ANC). Tabel 3. Show that the more the husband is supportive, the more regularly he implements Antenatal Care (ANC). This research shows that the husband’s support is important in Antenatal Care (ANC) for pregnant women. Pregnant women feel comfortable and safe if their husbands.

Husband’s support is not only limited to taking women to health services to check their pregnancy. According to Friedman, there are four criteria for the support that needs to be provided by a husband, namely informational support, assessment/appreciation support, instrumental support, and emotional support. Examples of informational support are the husband gives information about the danger signs in pregnancy or the husband encourages his wife to read a book KIA. Support ratings/awards such as the husband are always asking for an explanation of examination results to the midwife. Instrumental support as husband preparing vitamin or milk for pregnant women. And emotional support as the husband soothe worried mother before delivery.

Her husband’s non-support for Antenatal Care (ANC) visits was explained in a study conducted by Evayanti because the husband didn’t know the purpose, benefits, and time of the ANC so that antenatal care visits became irregular. Low husband’s knowledge is manifested in the actions of the husband doesn’t provide
motivation to the mother to conduct regular Antenatal Care (ANC) visits by not sending the mother to do Antenatal Care (ANS), don’t look for information on the benefits of Antenatal Care (ANC), not give praise if you make regular visits. Emotionally, a husband supporting a pregnant woman will motivate the mother to go for Antenatal Care (ANC). The same study, states that family support, especially husband, plays an important role in realizing positive things and regular Antenatal Care (ANC) visits. Increased education for husbands so that the need for pregnant women to carry out Antenatal Care (ANC) visits properly and completely will be achieved.\textsuperscript{11}

Research Dinarohmayanti, Keintjem, and Losu, (2014) support this study which states that the support and role of family/husband during pregnancy can increase the readiness of pregnant women in the face of pregnancy and childbirth. The husband’s involvement during pregnancy will support the health of the mother and the baby to be born, also prevent the risks of unwanted risks. Pregnant women who receive support from the family and are followed by family income and parity will determine the caregivers during pregnancy. Thus, not only the two research variables were tested for their influence, but there were other factors such as income and parity for further research.\textsuperscript{12}

Knowledge of pregnant women can be increased by providing information through various information media that can be accepted by the community (pamphlets or leaflets). Agus and Horiuchi,\textsuperscript{13} explain in their findings that education is one of the factors influencing Antenatal Care (ANC) services for pregnant women. Research provides an explanation of the implementation of health education through counseling is one appropriate solution to increase the knowledge of pregnant women, so as to optimize the examination of pregnancy to health workers.\textsuperscript{14} Through the Pregnant Mothers Class Program, pregnant women can come face to face to study together, with the aim of increasing their knowledge and skills about Antenatal Care (ANC). Researcher, revealed that knowledge of pregnant women can be increased by empowering cadres. Health cadres are people who are known and part of the community, so it can easily provide information to pregnant women about the importance of Antenatal Care (ANC) visits, pregnancy until breastfeeding and pregnant women can receive information about it.\textsuperscript{15}

The role of the midwife is important in increasing the husband’s knowledge and support so that Antenatal Care (ANC) visits become regular. Midwives must also have the ability to communicate interactively with pregnant women and husbands during the Antenatal Care (ANC) service process. Research by Ernawati\textsuperscript{16}, states that poor communication skills of midwives with pregnant women with low educational backgrounds are one of the factors for low maternal visits. Therefore, training or improvement efforts to enhance the communication skills of midwives are needed.

\textbf{Conclusion}

Pregnant women’s knowledge and husband’s support are proven to have a significant effect on the regularity of antenatal care visits at Waru Health Care Center of Pamekasan. The implication of this research is to prioritize midwives and health workers at Waru Health Care Center of Pamekasan to promote health by providing health education about the importance of antenatal care (ANC) which is not only for pregnant women but for husbands. Midwives can empower cadres to collaborate. Midwives as front liners in reducing maternal mortality should improve competence through training, one of which is midwifery training to achieve good standards and status of maternal and child health

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\textbf{Fund}: Self

\textbf{Conflict of Interest}: Nil

\textbf{References}

Delictological Approach to the Classification of Offenses: Theoretical and Legal Analysis Using the Example of Ukrainian Legislation

Sergey V. Petkov, Veronika N. Shkabaro, Oleh H. Bodnarchuk, Igor E. Ivanov, Oleksandr D. Kolomoiets

Abstract

The article analyses the problem of the concepts of crime and offense definitions, considers legal issues of their set of facts; based on the analysis of constitutional, administrative and criminal legislation, the criteria to differentiate offenses from homogeneous crimes are proposed; scientific views of lawyers are analysed; legislation improvement of the post-Soviet republics in delictology are proposed.

Key words: delict, law violation, offense, crime, qualification.

Introduction

Legal liability is a type of social responsibility that occurs for violation of legal norms. To deal with such a complex phenomenon as legal liability for committing crimes and offenses, first of all, one should analyse the theory of law, as the essence of knowledge about law, the regulator of social and economic relations.

In different countries, the complex of issues related to legal liability for offense is resolved in different ways. One and the same act may become the subject of consideration in different countries by different authorities in a different procedural order. Even jurisdiction will fall under different branches of government: executive or judicial. Execution of documents, procedure performance, etc. also differ. In many US states and other countries of the world, in accordance with traditions and well-established standards of behavior, there are different approaches to responsibility for offenses.

Therefore, the mechanical transfer of certain elements or short stories or whole mechanisms, algorithms and rules without a comprehensive study and analysis of the national law and legal consciousness of citizens on national basis is impractical, and sometimes can result in negative consequences.

Criminology is an integral part of delictology, which, in turn, is a part of sociology (the science, studying social phenomena).

Methods

The article applies methods of information search, analysis, synthesis, comparative legal and systemic structural methods.

Results and Discussion

It is well known, that basic legal principles were laid down in ancient times, but the greatest contribution to the
formation of legal axioms, presumptions and principles was made by Roman legal experts. It is a generally recognized axiom that legal liability is incurred for the committed offense.

The rule of law in a modern democratic state, legal culture and legal consciousness in society are ensured by improvement of the already existing theoretical and methodological foundations of legal liability in all spheres of public life in general, as well as the institution of state responsibility in the person of its bodies and officials to civil society and individuals, in particular.

The essence and content of the state responsibility to a person are defined in the Constitution of Ukraine, in particular in Articles 3, 8, 19, 22. The provisions of Part 1 of Article 92 of the Constitution of Ukraine set forth a constitutional approach to the classification of offenses and legal liability.

The generally accepted paradigm is a scientifically grounded and practice-proven approach that public delicts are offenses, or as they are often called delicts, according to the social significance of the committed unlawful act, they are divided into crimes and offenses. This horizontal division is the main one relative to illegal actions in the public sphere of law. In Roman law, offenses in the public interest sphere were also called “crimen” (crime), they emphasized the social danger of these the most dangerous delicts.

The most successful distinction between crime and offense was described in the Penal Code of France of 1795. In particular, it singled out: a) crimes - acts for which corporal punishment was provided; b) offenses - acts for which punishment of a corrective nature was provided.

In post-Soviet republics, the “delict” concept is not officially applied in legislation. But it is widely used in scientific and legal literature and practice. By its content and essence, a delict is a direct violation of rules of law. In the legal encyclopedia edited by academician Yu.S. Shemchushenko, offenses are defined as a socially dangerous or harmful act (act or failure to act), violating the rule of law. Its characteristic feature is the unlawfulness of actions or failure to act of the subject of social relations. We mean the violation of the established order, failure to fulfil the duties assigned to individuals and legal entities. Professor V.P. Pastukhov notes that an offense is a socially harmful, unlawful act, its implementation provides for legal liability. Socially useful actions are consistent with rules of law, socially harmful actions are considered as violation of legal norms.

As A.M. Kolodiy notes in his work “Principles of Law of Ukraine”, the public law sphere shall be considered as vertical relations (managerial), subordination relations, power - subordination. Their regulation shall be based on imperativeness principle, the rights and obligations of subjects shall be directly and exhaustively formulated in the law.

Certain steps to improve anti-delict legislation were performed during the entire period of state development after the Soviet Union decline. In particular, Ukraine has adopted a new progressive Criminal Code (2001), the Customs Code of Ukraine (2002) and a number of other regulations. Even the Criminal Procedure Code of Ukraine (2013), on the whole, can be considered as complying with European standards. But the inertia of the Soviet and even the imperial period is still present. For example, over 10 years, after the Customs Code adoption, customs regulations have undergone such changes that in 2012 a new Customs Code of Ukraine was adopted.

In the context of delict analysis as a social phenomenon, it should be noted that “legal chaos” in the legal liability sphere is unacceptable. Moreover, it is a threat to national security. In this situation, for the national legislation of Ukraine, as well as for other countries, where Soviet law was in force, it is necessary to introduce a comprehensive reform in the legal liability sphere for commission of delicts.

The constitutional basis for legal regulation of the classification of offenses and legal liability are expressed in regulations of the Constitution of Ukraine,
paragraph 22, part 1, Article 92, providing for the following: “The following are determined exclusively by the laws of Ukraine: ... civil liability basics; acts, considered as crimes, administrative or disciplinary offenses and resulting responsibility”1. According to V. B. Averyanov, very often outwardly identical actions, depending on the degree of their social danger, can serve as a basis for both administrative and criminal liability10.

Parts 2 and 3 of Article 22 of the Constitution of Ukraine clearly define that: “Constitutional rights and freedoms are guaranteed and cannot be cancelled. When adopting new laws or making changes to existing laws, it is not allowed to confine the content and scope of existing rights and freedoms”11.

Thus, in the theory of law, a delict (offense) is divided into crimes and offenses according to the public danger degree. Let us note that we mean the vertical separation of offenses. It is the public danger degree that is the cornerstone of their division into crimes and offenses. Offenses are characterized by the act harmfulness. That is, these are actions that harm public morality, the environment, and health. For instance, smoking in public places adversely affects the formation of adolescents moral qualities, harms the environment and health of other people.

In the Soviet period, offenses, depending on the branch of government that made the decision to punish them, began to be called “administrative offenses”, which is erroneous from the point of view of the theory of law. The normative understanding of an administrative violation (offense) is given by Article 9 of the Code of Ukraine on Administrative Offenses11. The normative understanding of an administrative violation (offense) is provided by Article 9 of the Code of Ukraine on Administrative Offenses11.

In European continental law countries, crime and offense are clearly differentiated. In the countries, where the rule of law prevails, there is also a clear gradation between crime and offense. In its modern interpretation, present in the legislation of the USA (and until recently - in Great Britain, Australia, Ireland and Canada), felony means a crime, more serious than a misdemeanor. In particular, high treason is considered a felony, some offenses, for example, non-payment of taxes, depending on the gravity degree, are qualified either as felony or as misdemeanor. In the countries with developed democracies, the punishment, even for offenses, is considered on a commission basis12.

But Soviet law defacto established a three-level division of offenses into “crimes”, “administrative offenses” and “disciplinary offenses”. Although scientists understand the falsity of this Soviet model, basic principles, laid down in Soviet law, are still preserved and cultivated by followers of the totalitarian command state.

In fact, illegal acts, committed by employees of institutions and departments, the responsibility for the commission of which is stipulated by the articles of the Labor Code of Ukraine is just one of the types of offenses. That is, “disciplinary offenses” are offenses under labor or military law. Here we deal with labor or military offenses, for the commission of which a person will be subject to an appropriate, but insignificant, punishment. In case of a military commission - Section XIX “Crimes against the established procedure for military service (war crimes)” of the Criminal Code of Ukraine, or a labor crime - Article 172 “Gross violation of labor legislation”, Article 271 “Violation of the requirements of labor protection legislation”, etc. In case of violations, provided for by the Criminal Code of Ukraine, the punishment will be applied under the criminal law.

A.P. Zakalyuk “Course of modern Ukrainian criminology: theory and practice” assumes that crime is a phenomenon of social life in the form of mass, relatively stable, variously conditioned criminal activity of a part of the members of this society, unacceptable and dangerous for society. In the history of criminal law, the concept of a crime has been defined differently. Moreover, the “crime”, as a socially dangerous act, is a rather dynamic category. Even in the same society, within a short period of time, approaches to understanding the criminal and
the non-criminal change significantly\textsuperscript{13}.

Crimes are characterized by the danger of the act. That is, these are acts that present a public danger and, depending on gravity, are divided into crimes of low gravity, medium gravity, grave and particularly grave. In the legislation of Ukraine, a crime (criminal offense) is a socially dangerous culpable act (act or failure to act), committed by the subject of a crime (Part 1, Article 11 of the Criminal Code of Ukraine). The act is defined as a crime based on a combination of four characteristics: public danger, guilt, wrongfulness, liability to punishment. At the same time, it shall be considered that an act or failure to act is not a crime, although formally it contains signs of any act provided for by the criminal law, but due to its insignificance does not pose a public danger, that is, it did not cause and could not cause significant harm to physical or legal a person, society or state (Part 2, Article 11 of the Criminal Code of Ukraine)\textsuperscript{7}.

The terms “criminal offense” and “offense” are synonymous. According to Article 12 “Classification of Crimes” of the Criminal Code of Ukraine, until 2020, “depending on the crime gravity, they are divided into crimes of low gravity, medium gravity, grave and particularly grave”. The classification of crimes is understood as their distribution into separate types by certain criteria. Crimes are subdivided: a) by guilt: into intentional and negligent; b) according to the degree of criminal activity completeness - to completed and uncompleted (Article 13 of the Criminal Code of Ukraine); c) their gravity - for crimes of small gravity, medium gravity, grave and especially grave (Article 12 of the Criminal Code of Ukraine)\textsuperscript{7}.

Classification of crimes under Article 12 of the Criminal Code of Ukraine, as well as the category of offense, is perceived by society in different ways. In a democratic society, this is a minor wrongful act, resulting in public responsibility, mainly in the form of a fine, or community service. At the same time, in totalitarian societies, it is precisely the understanding of responsibility for committing a misdemeanor that is distorted, as responsibility before administrative bodies, for violation of established rules of management, etc.

In particular, Ukrainian society experienced such a distortion of the matter of law in the thirties of the 20th century, when for minor misdemeanors, citizens, even minors, could be severely punished and even shot, in accordance with the Decree of the Central Executive Committee and the Council of People’s Commissars of the USSR “On the protection of property of state enterprises, collective farms and cooperation and the strengthening of public (socialist) property” dated August 7, 1932\textsuperscript{14}.

In accordance with the legislation of Ukraine (Article 9 of the Code of Administrative Offenses), a misdemeanor (administrative offense) is considered an unlawful, guilty (intentional or negligent) action or omission that infringes on public order, property, rights and freedoms of citizens, on the established management procedure and for which by law administrative liability is envisaged if this offense by its nature does not entail, according to the current legislation, criminal liability\textsuperscript{11}. As you can see, the division of illegal act into offense and crime depends on the public danger degree.

A crime is an act (offense), characterized by a danger to society, an offense is an act (misdemeanor), characterized by harm to society. An offense, like a crime, is determined by a combination of four signs: social danger, guilt, wrongfulness, liability to punishment.

Let us emphasize that the concept of “administrative offense”, as a category, in no case can be equal to “misdemeanor”. Administrative means managerial. It means that an administrative offense can only be committed by a person endowed with power - a government body official. An administrative offense by public danger degree is divided into an administrative (official) crime and an administrative (official) offense.

Classification of offenses and their characteristics shall consider fundamental constitutional provisions: recognition of the highest social value of a person, his life and health, honor and dignity of inviolability and
security, determination of the content and direction of the state activities to guarantee human rights and freedoms, the state responsibility to a person for its activity, approval and ensuring of human rights and freedoms as the main duty of the state (Article 3 of the Constitution of Ukraine); recognition and operation of the principle of the rule of law in Ukraine, the highest legal force of the Constitution of Ukraine, direct action of the norms of the Constitution of Ukraine (Article 8 of the Constitution of Ukraine); building the legal order in Ukraine on the principles of inadmissibility of coercion to do what is not provided for by legislation (Part 1, Article 19 of the Constitution of Ukraine); obliging public authorities and local self-government bodies, their officials to act only on the basis, within the powers and in the ways provided for by the Constitution and laws of Ukraine (Part 2, Article 19 of the Constitution of Ukraine); an inexhaustible list of human and civil rights and freedoms, the inadmissibility of the abolition of constitutional rights and freedoms, as well as the inadmissibility of narrowing the content and scope of existing human and civil rights and freedoms (Article 22 of the Constitution of Ukraine).

Currently, it is recommended to perform a gradual codification of the administrative legislation norms in certain areas and institutions of administrative and legal regulation. This codification process can be conditionally divided into certain stages. Certainly, it is impossible to determine a clear distinction, but we note that corresponding processes began even before the start of the administrative reform in 199815. Within legislation, it is necessary to transfer the norms of Code of Administrative Offenses to basic codes: the Land Code, Natural Resources Code, etc., as was performed in the customs legislation preparation.

**Conclusion.** The main error of national science was that the main division of delicts (offenses) according to the public danger degree was put on a par with other classifications. This misunderstanding resulted in many conflicts in legislation. Therefore, it is necessary to form a clear distinction between crimes and misdemeanors is urgent, and prevent the confusion of these concepts in one regulatory act. Legal liability for delict commission shall also be regulated. The punishment for these different, diametrically opposed actions shall be differentiated. In particular, as a punishment for crimes, it is required to apply punishment in the form of imprisonment and confiscation. For offense commission, such measures are educational measures as a warning, a fine and correctional labor are applied.

The doctrinal model development for the classification of offenses in the theory of law shall consider the needs of practice to regulate relevant sphere of public relations.

The implementation of steps to bring the legislation of Ukraine to world standards shall be comprehensive and coordinated, not create additional conflicts in the country’s legal system. The openness of this process shall be accompanied by a broad public discussion, consultations with scientists and popularization of lawmaking actions of the authorities among population.

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Child Trafficking & Human Trafficking: Legal Aspects and Role of a Doctor

Shagun Thakur\textsuperscript{1}, Vikas Gurbani\textsuperscript{2}

\textsuperscript{1}Asst. Prof., \textsuperscript{2}Assoc. Prof., Dept. of F.M.T., Kalinga Institute of Medical Sciences (KIMS) & PBMH, Bhubaneswar

Abstract

Counter-trafficking measures are to be implemented at various levels and by various organisations. The medical professionals also play an important role in this area as in many cases, the victims are first identified when they require some form of medical attention.

A child victim of trafficking is any person younger than 18 years against whom the act of trafficking has been committed. The definition of child trafficking differs from that of trafficking in humans in the respect that children are considered being vulnerable not only because of their level of maturity and age but also other factors.

This article highlights the various legal aspects of child and human trafficking in India. The victim of trafficking may not himself disclose what he is going through, so there are certain points on history and examination which should arise the suspicion in the mind of the health professional. These signs and symptoms are mentioned in this article which are collected from various resources. Emergency medicine department has an important role in screening of these victims, although all the departments contribute in identifying the victims.

The article also mentions where India stands compared to its global counterparts in anti-trafficking measures.

Keywords: trafficking, child trafficking, human trafficking, doctor in trafficking, trafficking healthcare, trafficking medicine, trafficking in persons

Introduction

Child trafficking and Human Trafficking is not just a national but an international problem. Counter-trafficking measures are to be implemented at various levels and by various organisations. The medical professionals also play an important role in this area as in many cases, the victims are first identified when they require some form of medical attention. A medical professional should be aware of the signs and signals which point towards a victim of child trafficking.

India has adopted the United Nations PALERMO Protocol for Trafficking which states that trafficking in persons “shall mean the recruitment, transportation, transfer, harbouring or receipt of persons, by means of the threat or use of force or other forms of coercion, of abduction, of fraud, of deception, of the abuse of power or of a position of vulnerability or of the giving or receiving of payments or benefits to achieve the consent of a person having control over another person, for the purpose of exploitation. Exploitation shall include, at a minimum, the exploitation of the prostitution of others or other forms of sexual exploitation, forced labour or services, slavery or practices similar to slavery, servitude or the removal of organs.” \cite{1}
Exploitation shall include sexual exploitation, forced labour, slavery or the removal of organs

**Human Trafficking Vs Child Trafficking**

A child victim of trafficking is any person younger than 18 years against whom the act of trafficking has been committed. If the age of the person is unknown and there are reasons to presume that the person is a child, the person shall be considered under the definition of child, until his/her age is established. The definition of child trafficking differs from that of trafficking in humans in the respect that children are considered being vulnerable not only because of their level of maturity and age but also other factors.

The following is the data from National Crime Records Bureau of India showing the number of victims trafficked during the year 2019.²

<table>
<thead>
<tr>
<th>Victims Trafficked - 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 18 years</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Total (All India)</td>
</tr>
</tbody>
</table>

During the same year (2019), a total number of 6571 victims were rescued. From these rescued victims, the following data stating the purpose of trafficking was deduced.

<table>
<thead>
<tr>
<th>NCRBs Statistics (2019) - Purpose of Trafficking²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose of Trafficking (2019)</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Forced Labour</td>
</tr>
<tr>
<td>Sexual Exploitation for Prostitution</td>
</tr>
<tr>
<td>Other forms of Sexual Exploitation</td>
</tr>
<tr>
<td>Domestic Servitude</td>
</tr>
<tr>
<td>Forced Marriage</td>
</tr>
<tr>
<td>Petty Crimes</td>
</tr>
<tr>
<td>Child pornography</td>
</tr>
<tr>
<td>Begging</td>
</tr>
<tr>
<td>Drug peddling</td>
</tr>
<tr>
<td>Removal of organs</td>
</tr>
<tr>
<td>Other reasons</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
LAWS IN RELATION TO CHILD TRAFFICKING IN INDIA [3]

- **Article 23 of the Constitution** guarantees right against exploitation, prohibits trafficking human beings and forced labour and makes their practice punishable under law

- **Article 24 of the Constitution** prohibits employment of children below 14 years of age in factories, mines or other hazardous employment

- **Indian Penal Code:**
  
  S. 366A – Procuration of a minor girl from one part of the country to another is punishable

  S.372 – Selling of girls for prostitution

  S.373 – Buying of girls for prostitution

  S.374 – Provides for punishment for compelling any person to labour against his will

**Human Trafficking (S.370 and S.370A)** after enactment in 2013 into the Criminal Law, the Bureau has started collecting data under this section

**Major Acts in relation to trafficking in India**

- The Suppression of Immoral Trafficking in Women and Girls Act, 1956 (SITA)
- Child Labour (Protection and Regulation) Act, 1986
- Immoral Traffic (Prevention) Act, 1956 (ITPA)
- Information Technology Act, 2000
- Juvenile Justice (Care and Protection of Children) Act, 2000
- Prevention of Children against Sexual Offences Act, 2012

**Other Important Acts:**

- Indecent Representation of Women (Prohibition) Act, 1986
- The Child Marriage Restraint Act, 1929
- The Narcotic Drugs and Psychotropic Substances (NDPS) Act, 1985
- The Transplantation of Human Organ Act, 1994

**3Ps of Anti-Trafficking** [4]

The 3P Index assess the effectiveness of governmental policies against human trafficking in three main areas: Prosecution, Protection and Prevention (3Ps). The ranking of country and policy score are published every year.

**PROSECUTION (P)**

<table>
<thead>
<tr>
<th>SCORE</th>
<th>CRITERIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Country has legislative measure specifically prohibiting trafficking and the law is fully enforced in the form of investigations, prosecutions, convictions and punishment of such offenders</td>
</tr>
<tr>
<td>4</td>
<td>Has legislative measure prohibiting trafficking but law is not fully enforced</td>
</tr>
<tr>
<td>3</td>
<td>Does NOT have a legislative measure specific for trafficking: But has other relevant laws (Rape, slavery, exploitation, Human right violation etc) and law is fully enforced</td>
</tr>
<tr>
<td>2</td>
<td>Does NOT have legislative measure: But applies certain other laws and the law is NOT fully enforced</td>
</tr>
<tr>
<td>1</td>
<td>Does NOT have legislative measures: No other law is applied and there is no evidence of punishment for such a crime at all</td>
</tr>
</tbody>
</table>
PROTECTION ($P_1$)

<table>
<thead>
<tr>
<th>SCORE</th>
<th>CRITERIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>The country does not punish victims of trafficking for acts related to the situation being trafficked; does not impose the self-identification of victims; and exerts STRONG efforts to give victims information on, and assistance for, relevant court and administrative proceeding, as well as support for the physical, psychological and social recovery of victims such as housing (shelter), medical assistance, job training, (temporal) residence permit, and other assistance for rehabilitation and repatriation</td>
</tr>
<tr>
<td>4</td>
<td>Does not punish victims of trafficking, does not impose self-identification and exerts MODERATE efforts to give victims information and assistance…</td>
</tr>
<tr>
<td>3</td>
<td>Does not punish victims of trafficking, does not impose self-identification and exerts LIMITED efforts</td>
</tr>
<tr>
<td>2</td>
<td>The country fails to ensure that victims are not punished for acts related to trafficking and there is limited assistance and support</td>
</tr>
<tr>
<td>1</td>
<td>The country punished victims of trafficking and does not provide any assistance and support</td>
</tr>
</tbody>
</table>

PREVENTION ($P_2$)

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>The country demonstrates VERY STRONG efforts preventing trafficking in persons, such as implementing public and media campaigns for anti-trafficking awareness; training government and military officials (including peace keepers); facilitating information exchange among relevant authorities; monitoring borders, train station, airport etc; adopting national action plan for combating trafficking in persons; promoting co-operation with NGOs and international organizations in the country; and facilitating bilateral and/or multilateral co-operation with other governments</td>
</tr>
<tr>
<td>4</td>
<td>demonstrates STRONG efforts…</td>
</tr>
<tr>
<td>3</td>
<td>demonstrates MODEST efforts…</td>
</tr>
<tr>
<td>2</td>
<td>demonstrates LIMITED efforts…</td>
</tr>
<tr>
<td>1</td>
<td>demonstrates NO efforts</td>
</tr>
</tbody>
</table>

4th P – Partnership

Partnerships among governmental, nongovernmental and other civil society organisations, among academia, politics and industry, and among professionals from different fields of expertise are vital for comprehensive counter-trafficking efforts.

THE ROLE OF HEALTHCARE PERSONNEL IN TRAFFICKING

SCREENING PROTOCOL\textsuperscript{[5,6,8]}

The victim of trafficking may not himself disclose what he is going through, so there are certain points on history and examination which should arise the suspicion in the mind of the health professional.
Questions to Ask:

- Is anyone forcing you to do anything that you do not want to do?

- Is anyone forcing you to work or have sex against your will?

- Where do you work and what type of work do you do? Have you ever been lied to about your type of job?

- Are you allowed to freely leave your house/work?

- Has anyone threatened to hurt you/ your family or threatened to report you to the police?

- Does anyone hold your identification documents (i.e., passport or driver’s license)? Could you get it back if you wanted to do so?

- Is anyone restricting you from seeing your family and friends or tracking your movements? When was the last time you had contact with your family?

RED FLAGS (What to look for):

- Patient has no identification documents or documentation is in possession of an accompanying party

- Accompanying party insists on answering/interpreting for patient. Accompanying male is much older than young female in OB/GYN exam

- Patient is reluctant to explain his/her injuries

- Patient is unaware of his/her location

- Patient exhibits fear, anxiety, depression, submission, tension or nervousness and avoids eye contact

- Patient is under 18 years of age and engaging in commercial sex or trading sex for something of value

- Patient works and sleeps in the same place

- Patient has no money or control over money. Accompanying party pays with a lot of cash

- Patient is a runaway/ throwaway youth

PHYSICAL SIGNS:

- Frequent or recurrent UTIs

- Frequent treatment for STIs: Gonorrhoea, Chlamydia, and HIV/AIDS

- High number of sexual partners

- Multiple pregnancies/ abortions

- Maltreated previous injuries

- Burns from battery acid, hot iron or cigarettes, exposure to toxic chemicals

- Bruises, including evidence of being slapped or receiving rough treatment

- Shows of physical restraint or torture

- Branding - Tattoos or markings of ownership (ask meaning of tattoo & circumstances from which it was obtained)

- Presence of internal cotton cosmetic sponges to stop bleeding from cycle or abortion

The Philadelphia Anti-Trafficking Coalition has also suggested a guideline which can be followed in emergency department for screening of victims.[5,7] The summary of the guidelines is as follows : Red flags identified à Treat Medical Concerns à Human Trafficking Assessment à Patient in perceived danger à With the consent of the patient, call helpline or if he refuses consent, then guide him how to take help (In case of minor, follow mandatory reporting procedure.

Police – 100

Childline - 1098
ANTI-TRAFFICKING POLICY INDEX \(^3\)

TIRE-wise Placement of Countries

Department of State, USA places each country onto one of four tires, as mandated by Trafficking Victims Protection Act (TVPA). This placement is based more on the extend of government action to combat trafficking.

<table>
<thead>
<tr>
<th>TIER</th>
<th>CRITERIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tier 1</td>
<td>Countries whose government fully meet TVPA’s minimum standard (Prohibit trafficking, punishment sex traffickers, serious and sustain effort to eliminate trafficking)</td>
</tr>
<tr>
<td>Tier 2</td>
<td>Countries whose government do not fully meet TVPA’s minimum standard but are making significant efforts to meet those standard</td>
</tr>
<tr>
<td>Tier 2 Watchlist</td>
<td>Do not fully meet TVPA’s minimum standard but are making significant efforts to meet those standards AND</td>
</tr>
<tr>
<td></td>
<td>i) Number of victims is significant</td>
</tr>
<tr>
<td></td>
<td>ii) Failure to provide increasing efforts to combat severe forms of trafficking</td>
</tr>
<tr>
<td></td>
<td>iii) Determination based on commitments by the country to take additional future steps over the next year</td>
</tr>
<tr>
<td>Tier 3</td>
<td>Countries whose governments do not fully meet the minimum standard and are not making significant efforts to do so.</td>
</tr>
</tbody>
</table>

India is on TIRE 2 according to USA’s TVPA standards

<table>
<thead>
<tr>
<th>TIER 2</th>
<th>P</th>
<th>P1</th>
<th>P2</th>
<th>3P</th>
</tr>
</thead>
<tbody>
<tr>
<td>INDIA</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

As per the year 2019, USA has retained India on the Tier 2 list of countries in its Annual report on human trafficking, arguing that the country does not fully meet the minimum standards for its elimination even as it is making progress.

Conclusion

- The reported cases of child trafficking are only the tip of the iceberg.
- In measures to combat trafficking, the goal should be also to prevent re-trafficking.
- The healthcare personnel, mainly the emergency department personnel have an important role in identification of victims of trafficking.
- A physician equipped with skills, experience and expertise to contribute to various aspects of the counter-trafficking action will go a long way in helping with the identification of victims (and perpetrators) and also collection of evidence

Important Helpline Numbers in India:

1098 – CHILDLINE

CHILDLINE India is a project of Ministry of Women and Child Development. Childline India
foundation is a non-government organisation (NGO) in India that operates a telephone helpline called Childline, for children in distress. It was India’s first 24-hour, toll free, phone outreach service for children.

- 011-23478200 – National Commission for Protection of Child Rights
- 0674-2394041 – State Commission for Protection of Child Rights (Odisha)

Conflict of Interest : None

Source of Funding : None/ Self

Ethical Clearance : The content of this article are personal views and comments of the authors. There are no ethical issues involved.

References
Homesickness, Anxiety and Depression among Pakistani International Students in Indonesia during Covid-19 Outbreak

Shahzad Shoukat1, Cyuzuzo Callixte1, Theresia Indah Budhy2, Jusak Nugraha3, Tuyishimire Irene4
1Post-Graduate, Department of Immunology, Postgraduate School, 2Professor, Faculty of Dental Medicine, 3Professor, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, 4Post-Graduate Department of Horticulture, College of Agriculture, Animal Science and Veterinary Medicine, University of Rwanda, Rwanda

Abstract

Introduction: International students are exposed to multiple mental health crisis due to social, environmental and culture shocks in foreign lands but it gets worse in presence of life-threatening disease outbreak. This research was conducted to evaluate the homesickness, anxiety and depression among Pakistani international students in Indonesia during Covid-19 Outbreak. Methods: This study was conducted on 86 random students that are pursuing their studies in different public and private universities in Indonesia. The data were collected by distributing google forms via email and social media groups and the responses from the respondents were recorded and analyzed statistically. Results: The study findings revealed that there is a correlation between Covid-19 pandemic and the development of various mental health crisis where 33 students (38.37%) developed homesickness, 11 students (12.79%) developed anxiety and 2 students (2.33%) manifested low-grade depression. Homesickness was outstandingly observed in females (72.72%) whereas anxiety was highly reported in males (90.90%). The study findings demonstrated that there is a strong negative correlation between student financial statuses and the development of homesickness (r: -0.977, P: 0.023) and anxiety (r: -0.944, P: 0.056). Conclusion: All in all, the fear to contract coronavirus, lockdowns, financial instability, death toll of citizens and medical professionals, run out of medical facilities and social media hoaxes are significant risk factors of mental health crisis among Pakistani international students in Indonesia.

Keywords: Covid-19, Pakistani International Students, Mental Health Crisis, Indonesia

Introduction

The global pandemic which is currently threatening human life and causing both social and economic difficulties in both developed and developing countries is attributed to the new type of coronavirus. The coronavirus causes diverse clinical manifestations such as pneumonia, high body temperature and respiratory impairments including difficulty in breathing and infection of the lungs(1). The majority of these viruses are known to highly affect both domestic and wild animals but some of these viruses were also reported to infect humans and cause the disease called zoonotic diseases. On 29 December 2019, the World Health Organization (WHO) has temporarily named it 2019 novel coronavirus as the coronavirus that started affecting the lower respiratory tract of different patients in Wuhan, China (2). Later on, WHO officially named it severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which is the causative agent of Covid-19(3).

The lockdown caused by coronavirus disease outbreak is tremendously inconveniencing global citizens in various ways such mental health crisis, fear resulting from the deadliness of the new virus, financial instability, quarantine, contradicting information from different officials in high positions and misinformation.
from social media. All these factors contribute to the development of depression, homesickness, anxiety and other mental health crises that manifest incitizens but mostly in people who reside far away from their families like students who traveled abroad for further studies(4).

As global citizens are exposed to the consequences of coronavirus outbreak, mental health stability is considered as the most crucial feature of community health and wellbeing. From that perspective, depression is the highly prevalent mental disorder which doesn’t have limit to the time, place, and individual but also it involves all races and ethnicities of people from different demographic regions(5).

In response to the outbreak, the Pakistani students living in Indonesia are very scared about the virus incubation period and questioning themselves if the asymptomatic people could still buildout the infection. The serious quarantine measures in different major cities of Indonesia and their home countries which confined residents to their homes also aggravate the student’s condition by adding mental pressures. Apart from that, the reported insufficient medical protective equipments, few and unexperienced medical staff because SARS-CoV-2 is a new virus, shortage of hospital appliances and deaths of medical professionals in Pakistan causes the enormous concerns. In addition to the recent outbreak, the Pakistani students in Indonesia are exposed to psychological tension from the ongoing online classes with massive academic workload, living hand to mouth and financial instability due to the delay of their living allowances from the universities. From the above highlighted reasons, the big number of students are exposed to many environmental and academic pressures that could run them the development of various mental health crisis such as depression of various types(6).

One of the main challenges that Pakistani students experience during their stays in Indonesia is homesickness. It is explained as defined as a motivational and emotional situation which is characterized by the excessive desire and preoccupation of mind with thoughts of going back to the native countries and eventually lead to the absence of reassurance and increase the probability of developing depression and anxiety(7). Depression is a disorder that negatively affects the someone’s feeling and characterized by lethargy and the loss of appetite with increased sense of bad thinking and reduced concentration in diverse daily activities, cognitive performances and the development of insomnia(8). The study findings of published researches disclosed that the higher people get exposed to hard-pressing conditions, the lower his/her happiness will be. This was documented as a potential threat which affect people’s mental health and lead to homesickness, anxiety and depression of different degrees(7). By giving a great consideration to various published research works from different parts of the world, there are no published findings discussing about the pervasiveness of homesickness, anxiety and depression among Pakistani international students in Indonesia during Covid-19 outbreak. So, this current study is intended to light its beams on that research interest and provide primary data which will highly contribute to the continuous enrichment of the academic literatures and help the professionals to find out the effective solutions.

**Materials and Methods**

**Research design and participants**

This descriptive-analytical research was conducted in the duration of two months (from April to May 2020) on 86 (59 males and 27 females) Pakistani international students pursuing their studies in different public and private universities in Indonesia. Stratified randomization was used as a mean of sampling and collection. In this evaluation, the inclusion criteria were related to the active Pakistani international students in Indonesia who are enrolled in undergraduate, postgraduate and doctoral programs. The participants who reside in university hostels and off campus accommodations were all included in the study. As the study was carried with much consideration of the ethical clearance for research, whereby the exclusion criteria were the absence of students’ consent to be a part of this study.
Questionnaires

The distributed google forms via email and social media groups consisted of both closed-ended and open-ended questions. The forms were intentionally filled by the participants and those who filled in the forms were considered as the research participants through their intentional consents of participation. Questionnaires were employed to collect demographic data from the consented students including their personal identifications such as name, age, gender, marital status and other information like field of study, semester, level of education, living place, number of siblings and their rankings, financial and health statuses, physical exercise experience at home, hobby of reading novels, watching TV habits, the use of internet and laptop-based game playing during the day.

The questionnaires consisted of other supplementary points which examine the repeatedness of homesickness experience and the severity of homesickness. The questionnaire used to assess the depression among Pakistani international students in Indonesia consists of different scales which are lethargy, fear of contract corona, social media pressures with a lot of hoaxes, cognitive-emotion and academic motivation. Students were also asked about their health status to figure out the symptoms of anxiety like fatigue, irritability, trouble of sleeping or staying awake, panic attacks, excessive worrying and restlessness. All the responses from the respondents on each form were evaluated where the low scales indicated the normal states while from the medium to high scores in terms of symptoms were the perfect indications of homesickness, anxiety and depression of different stages.

Data Analysis

SPSS version 23.0 (SPSS Inc., Chicago, IL, USA) was employed to analyze the obtained data whereby nonparametric test was employed to test the normality and Pearson correlation was used to evaluate the correlation between the variables.

Results

The findings of the current research disclosed that there is a strong positive correlation between Covid-19 pandemic and the development of various mental health crisis among Pakistani students where the analysis of obtained data demonstrated that 40 students (46.51%) were free of any mental health problem, 33 students (38.37 %) developed homesickness, 11 students (12.79%) developed anxiety and 2 students (2.33 %) manifested low-grade depression. Of all 33 students who manifested homesickness, 24 (72.72 %) were females whereas the big number of anxious students were males and they occupied 90.90 % of all research subjects who presented anxiety.

As demonstrated by the results presented in table 1, the high prevalence of homesickness was seen among female participants whilst the anxiety was appeared in males and the equal number of low-grade depressions was observed in both sexes (1:1). In reference to the study findings presented in table 2, the research subjects that are less than 25 years of age were highly susceptible to anxiety, 9 (81.81%) whereas the informants that are aged between 25 and 30 years were vulnerable to homesickness,19 (57.57%) and the respondents above 30 years of age showed a resistance to anxiety and depression. As shown by the findings recorded in table 3, the students with financial instability (weak) manifested the symptoms of homesickness and anxiety compared to others. The development of mental health crisis in relation to the use of internet and TV watching habits as described in table 4, was highly observed among the students who frequently use internet and watch TV. Of all 33 students who developed homesickness, 18 (54.54%) of them use internet and 12(36.36%) use both internet and watch TV.
| Table I. The manifestation of mental health crisis in relation to gender |
|--------------------------|----------------|----------------|----------------|----------------|
|                         | Negative       | Homesickness   | Anxiety        | Depression     | Total |
| Males                   | 39(66.10%)     | 9(15.25%)      | 10(16.94%)     | 1(1.69%)       | 59    |
| Females                 | 1(3.70%)       | 24(88.88%)     | 1(3.70%)       | 1(3.70%)       | 27    |
| Total                   | 40(46.51)      | 33(38.37%)     | 11(12.79%)     | 2(2.32%)       | 86    |
| r                       | -1             | 1              | -1             | -1             |       |

| Table II. The manifestation of mental health crisis in relation to age |
|--------------------------|----------------|----------------|----------------|----------------|
|                         | Negative       | Homesickness   | Anxiety        | Depression     | Total |
| <25 years               | 22(50%)        | 12(27.27%)     | 9(20.45%)      | 1(2.27%)       | 44    |
| 25-30 years             | 10(31.25%)     | 19(59.37%)     | 2(6.25%)       | 1(3.12%)       | 32    |
| >30 years               | 8(80%)         | 2(20%)         | 0(0.00%)       | 0(0.00%)       | 10    |
| Total                   | 40(46.51)      | 33(38.37%)     | 11(12.79%)     | 2(2.32%)       | 86    |
| r                       | -0.924         | -0.585         | -0.952         | -0.866         |       |
| P value                 | 0.249          | 0.602          | 0.198          | 0.333          |       |

| Table III. The manifestation of mental health crisis in relation to financial statuses |
|--------------------------------|----------------|----------------|----------------|----------------|
|                                | Negative       | Homesickness   | Anxiety        | Depression     | Total |
| High                          | 3(21.42%)      | 9(64.28%)      | 2(14.28%)      | 0(0.00%)       | 14    |
| Good                         | 7(43.75%)      | 6(37.50%)      | 2(12.50%)      | 1(6.25%)       | 16    |
| Medium                       | 12(60%)        | 5(25%)         | 3(15%)         | 0(0.00%)       | 20    |
| Weak                         | 18(50%)        | 13(36.11%)     | 4(11.11%)      | 1(2.77%)       | 36    |
| Total                        | 40(46.51)      | 33(38.37%)     | 11(12.79%)     | 2(2.32%)       | 86    |
| r                            | 0.996          | -0.977         | -0.944         | 0.447          |       |
| P value                      | 0.004          | 0.023          | 0.056          | 0.533          |       |
Table IV. The manifestation of mental health crisis in relation to the use of internet and TV watching habits

<table>
<thead>
<tr>
<th></th>
<th>Negative</th>
<th>Homesickness</th>
<th>Anxiety</th>
<th>Depression</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both</td>
<td>16(48.48%)</td>
<td>12(36.36%)</td>
<td>4(12.12%)</td>
<td>1(3.03%)</td>
<td>33</td>
</tr>
<tr>
<td>Internet</td>
<td>20(45.45%)</td>
<td>18(40.90%)</td>
<td>5(11.36%)</td>
<td>1(2.27%)</td>
<td>44</td>
</tr>
<tr>
<td>TV</td>
<td>4(44.44%)</td>
<td>3(33.33%)</td>
<td>2(22.22%)</td>
<td>0(0.00%)</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>40(46.51)</td>
<td>33(38.37%)</td>
<td>11(12.79%)</td>
<td>2(2.32%)</td>
<td>86</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both</td>
<td>-0.721</td>
<td>0.488</td>
</tr>
<tr>
<td>Internet</td>
<td>-0.596</td>
<td>0.593</td>
</tr>
<tr>
<td>TV</td>
<td>-0.655</td>
<td>0.546</td>
</tr>
<tr>
<td>Total</td>
<td>-0.866</td>
<td>0.333</td>
</tr>
</tbody>
</table>

Discussion

The core results of this evaluation demonstrated that Covid-19 outbreak is highly attributed to the development of various mental health crisis in foreign students due to different environmental pressures such as the increase in number of Covid-19 cases, strict lockdown measures such as strict stay at home directives including home confinement, quarantine, and isolation, conflicting messages from authorities, social media news and other psychological tensions.

The study findings revealed great number of homesickness in studied population. This result is in agreement with the research results of Moeini who reported that homesickness is common in foreign students due to the fact that they have a desire to return in their home countries and nostalgia for their respective intimate friends and siblings. The reported homesickness should also be attributed to the loneliness during lockdowns as well as the incompatibility with the new environments. This result is also supported by the study findings published by Terry which showed the presence of homesickness in non-native students due to diversity of cultures and societal health instability.

The high prevalence of homesickness observed in females is consistent with past studies that found the vulnerability of females to homesickness in females than males. This statement could be assisted by the results obtained by other researchers who reported the high prevalence of homesickness in girls than boys. But, the result of current evaluation is also compromising with the findings of Uchenna who did not find significant gender difference in homesickness.

The observed anxiety in this evaluation is in conformity with the study results reported by a Chinese researcher from the study that was conducted on Chinese medical staffs. It should be defined by the fact that Covid-19 pandemic has become a potential stressor, particularly as this is a new viral infection which doesn’t have a vaccine or a specific treatment, fear of being infected and the big number of reported deaths globally.

In this evaluation, the prevalence of depression was equal between males and females. The observed depression in boys is in tandem with the research findings published in previous studies emphasizing that boys due to their concerns about employment and their future that could be more likely to induce depression.

In regard to the obtained results, student’s age could be considered as a major predictor of depression. This is in line with the findings from the study of Zaid and
friends which disclosed that older people should have better mental health statuses, ability to calm themselves during hard conditions and are not highly developing mental health problems due to self-counselling\textsuperscript{(15)}. Apart from the fact that every individual responds to stress in different ways, the Covid-19 pandemic and the lockdowns has caused a lot of worries, hunger, uncertainty, pending death, confusion and chaos which could drive students crazy and highly inconvenience their psychological stress and mental health\textsuperscript{(8)}.

By emphasizing on the observed findings, the correlation between the habits of using both internet and TV watching contributed to the development of mental health crisis. These results are in conformity with the study results published by Hökby that the duration spent on the Internet, web based content, the use of social platforms and watching TV programs or news highly contribute to depression and anxiety\textsuperscript{(16)}. The large negative correlation observed between mental disturbance and the use of internet and TV watching is attributed to the fact that social media and various TV stations are broadcasting depressive news and the conflicting messages between government officials and health professionals\textsuperscript{(17)}.

**Conclusion**

The conclusion that can be drawn from the results of this research is that the fear to contract coronavirus, lockdowns, financial instability, death toll of citizens and medical professionals, run out of medical facilities and social media hoaxes are significant factors in mental health crisis of Pakistani international students in Indonesia during Covid-19 pandemic. As mental health crises should be mitigated by having positive opinions and strong feelings of efficacy. From that standpoint, it is recommended to embrace the talking cure with their families, physical exercises and positive thinking to avoid unintended and irreparable consequences.

**Conflict of Interest:** We declare that there is no conflict of interest.

**Source of Funding:** None

**Ethical Clearance:** This research was approved by the ethical committee from RSUD Dr. Soetomo General Hospital, Surabaya, Indonesia with reference number 070/1013/CRU/IX/2020.

**References**

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Determination the Relationship between (KLF14 rs972283) Genotype and Type 2 Diabetes in a Sample of Iraqi Patients

Shaimaa Abbas Dawood¹, Mohammed Ibrahim Nader²

¹ Scholar Researcher Laboratories of Al Shahed Mohammad Baqer Alhakim Hospital, Ministry of Health, Iraq,
² Scholar Researcher, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq

Abstract

This study was aimed to detect KLF14 gene polymorphism in Iraqi type 2 diabetes mellitus patients (T2DM) to found the correlation between the SNP (rs972283) polymorphism in KLF14 gene and lipid metabolism and impact on the incidence of type 2 diabetes mellitus (T2DM). The result show high Significant difference was observed in FBS level (P<0.001) in patient group (172.9 ± 79.73) and in control (81.16 ± 7.18). Cholesterol, LDL-Cholesterol and VLDL-Cholesterol mean level value in diabetic patients was significantly higher than those of control group (p<0.0001), serum HDL-Cholesterol mean value was significantly difference (P<0.05) in serum mean level of HDL between T2DM patients and healthy controls. Real time PCR (HRM) RT-PCR were used to detect SNP (rs972283) in KLR14 gene (G>A) by using specific primers, as a related with SNP (rs972283) G>A in KLF14 gene, genotypes and alleles frequencies, odds ratios, 95% confidence intervals and P values for the KLF14 gene In this study, statistical analyses of genotypic frequencies for the KLF14 (rs972283) revealed significant difference between T2DM patients and controls in the examined population. GG genotype was significantly (P = 0.0006) more frequent in the patient group. The observed G/G, A/G, and A/A genotype frequencies were 50%, 42%, and 8%, respectively. The A (wild-type) and G (variant) allele frequencies were 29% and 71%, respectively in patient group while the observed G/G, A/G, and A/A genotype frequencies were 22%, 48%, and 30, respectively and the A (wild-type) , G (variant) allele frequencies were 54% and 46%, respectively in control group (P <0.0003). GG genotype was significantly (P = 0.0006) more frequent in the patient group, Conclusion: There was a relationship between polymorphism of KLF14 gene SNP (rs972283) and the incidence of T2DM in sample of Iraqi patients.

Keywords: KLF14 rs972283, Genotype, Type 2 Diabetes

Introduction

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces, Hyperglycemia, or increase in blood sugar, is a common effect of uncontrolled diabetes and over time it leads to serious damage to the body system, especially nerves and blood vessel (¹).

The global epidemic of diabetes is a major public health problem, and the number of cases has increased four times in the past 30 years (²). Incredibly, 1 in 11 adults suffered diabetes globally. It is estimated that about 463 million adults were living with diabetes mellitus worldwide in 2019, and most of them had type 2 diabetes mellitus (T2DM). Moreover, this number is expected to increase to 642 million by 2040 (³). Considering its high prevalence and rapid increasing speed, there are increasing numbers of investigations focusing on risk factors and susceptibilities for T2DM. However, the underlying etiology of T2DM remains unclear. Therefore, we conducted this meta-analysis to
further demonstrate whether genetic factors play a vital role in the pathogenesis of T2DM \(^{(4)}\) or not.

Lines of evidence suggest that genetic polymorphism plays a pivotal role in the pathogenesis of a wide range of human disorders including cancers, diabetes, cardiovascular disorders, kidney diseases, and neurodegenerative diseases \(^{(5)}\).

Several polymorphisms in candidate Kruppel-like factors that may influence susceptibility to T2DM. The transcription factor \(KLF14\), is a master trans-regulator of multiple genes that are associated with metabolic phenotypes in adipose tissue, \(KLF14\), is located on chromosome 7q32.3. Variations in these genes are associated with T2DM \(^{(4)}\).

### Materials and Methods

This study conducted during the period from 1 November 2020 until the end of March at University of Baghdad / Institute of Genetic Engineering and Biotechnology for post Graduate Studies. The study consisted of 50 patient with type 2 diabetic were selected from those attended Al Shahed mohammed Baqer alhakim Hospital. Their ages ranged between (25-65) years.

Fifty healthy controls with normal fasting blood glucose (80–110 mg/dl). And age range between (25-65) years. They were randomly selected from the people who attend the clinics for checkup also from relatives and colleagues. Questionnaire that includes information about age, sex, family history, BMI for all subjects had measured. Both groups were classified according to BMI, age, gender and family history.

### Samples collection

Amount of five ml of venous blood was withdrawn from each subject under aseptic conditions. Two ml of blood was placed in EDTA tube (1.5 mg/ml) and kept at -20 °C to be used in molecular study. The remaining 3 ml venous blood was placed into clot activator and gel serum separation tubes (5 ml) and left to stand at room temperature (18-22°C). Then, the serum separated by centrifugation at 3000 rpm for 15 minutes. Later, it was divided into three aliquots in microcentrifuge tubes for biochemical test.

#### Genomic DNA extraction:

Genomic DNA was automatedly extracted from the whole blood samples of all subjects by using Blood DNA Extraction Kit 200 (MagPurix/Taiwan).

The MagPurix technology is a state of the art platform that uses magnetic beads to extract nucleic acids from samples. The platform commits a truly walk-away automation in nucleic acid purification from samples to results. The purification process contains steps of lysis, binding, washing and elution. After genomic DNA was extracted, agarose gel electrophoresis was adopted to confirm the presence and integrity of the extracted DNA.

Fasting blood sugar FBS, Total cholesterol TC, high density lipids HDL, low density lipids LDL and Triglycride were measured using kits supplied by (Spainreact, Spain), while glycosylated hemoglobin HbA1C measured using kit supplied by (Nycocard ,Norway).

#### Genotyping:

Genotyping was carried out For SNP rs972283of \(KLF14\) gene polymorphism analysis, DNA was amplified using the forward primer 5'-GCTATTGAACCATCATTGT -3’ and Reverse primer 5’-AGCAATAACAGTCTTTAGTAATATG -3’.

The qRT-PCR-HRMwas performed in a 20 μl total volume, Primer forward 0.75 μl, Primer reverse 0.75 μl , DNA Template 3.5 μl, PCR Re Mix (Ready to use) EVA Green 10 μl and D.W. 5 μl. A total of 40 PCR cycles with denaturation at 95 °C for 15 sec., annealing for 40 Sec at 60 °C and extension at 72 °C for 20 Sec.

### Result and Discussion

All serum lipid and lipoproteins were significantly higher in diabetic patients compared to healthy control group. In Figure (1), T. Cholesterol mean level value in diabetic patients was significantly (p<0.0001) higher
than control group, this increase may be due to an increasing in the plasma concentration of VLDL and LDL, which may be due to the increase in hepatic production of VLDL or decrease in the removal of VLDL and LDL from the circulation. (Ganong, 2003).

In Figure (2) mean value of triglycerides in diabetic patients was significantly (P>0.05) increased compared to mean of control group. The elevated triglyceride levels can arise from two abnormalities, The impaired lipolysis of triglycerides and over production of VLDL and patients with type 2 diabetes have an over production of triglyceride-rich VLDL level, which is a result of high free fatty acid levels, hyperglycemia, obesity, and
insulin resistance\(^7\).

In Figure (3) LDL-Cholesterol mean value in diabetic patients was statistically significant\((p<0.0001)\) higher than the mean value of control group. The increased level of LDL in diabetic patients is due to insulin increases the number of LDL receptor, so chronic insulin deficiency might be associated with a diminished level of LDL receptor. This causes the increase in LDL particles and results in the increase in LDL-cholesterol value in diabetes mellitus\(^8\).

In Figure (4) Serum HDL-Cholesterol mean value was significantly \((p=0.0036)\). Difference in serum mean level of HDL between T2DM patients and healthy controls.
Figure (5): Comparison between control and patients in VLDL.

In Figure (5) VLDL-Cholesterol mean value in diabetic patients was significantly (P=0.00385) increased compared to mean of control group. Higher level of VLDL was the consequence of insulin resistance in which the skeletal-muscle system stimulates the conversion of energy from consumed carbohydrate to raise liver triglyceride synthesis. As a result, it will produce atherogenic TGs-rich lipoprotein units, like VLDL \((9)\).

Quantitative Real-Time Polymerase Chain Reaction - High Resolution Melting

Real-time PCR (RT-PCR) is also called quantitative PCR or qPCR. The key feature in RT-PCR is that amplification of DNA detected in real-time as PCR is in progress by the use of fluorescent reporter. The fluorescent reporter signal strength is directly proportional to the number of amplified DNA molecules \((10)\).

The current study uses qPCR-HRM assay to determine the SNP rs972283 of the \(KLF14\) gene \((G>A)\), in T2DM in Iraqi patients, by using specific designed primer and positive, negative control which ensure a high degree of specificity.

Distribution genotype and allele frequency of \(KLF14\) (rs972283 G>A) polymorphism in patients and controls.

\(KLF14\) is a master trans-regulator of multiple genes that are associated with metabolic phenotypes in adipose tissue. Table (1) illustrates genotypes and alleles frequencies, odds ratios, 95% confidence intervals and \(P\) values for the \(KLF14\) gene. In this study, statistical analyses of genotypic frequencies for the \(KLF14\) (rs972283) revealed significant difference between T2DM patients and controls in the examined population. GG genotype was significantly \((P = 0.0006)\) more frequent in the patient group.

The observed G/G, A/G, and A/A genotype frequencies were 50%, 42%, and 8%, respectively table (1). The A (wild-type) and G (variant) allele frequencies were 29% and 71%, respectively in patient group while the observed G/G, A/G, and A/A genotype frequencies were 22%, 48%, and 30, respectively and the A (wild-type), G (variant) allele frequencies were 54% and 46%, respectively in control group \((P <0.0003)\).
Table (1): Genotypes and alleles frequencies of KLF14 (rs972283 G>A) genes polymorphism in diabetic and control subjects.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients N=50</th>
<th>Control N=50</th>
<th>Odds ratio (95% CI of OR)</th>
<th>Chi-Square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>25</td>
<td>50%</td>
<td>11</td>
<td>22%</td>
<td>8.52(2.30-31.63)</td>
</tr>
<tr>
<td>AG</td>
<td>21</td>
<td>42%</td>
<td>24</td>
<td>48%</td>
<td>3.28 (0.94-11.44)</td>
</tr>
<tr>
<td>AA</td>
<td>4</td>
<td>8%</td>
<td>15</td>
<td>30%</td>
<td>1</td>
</tr>
</tbody>
</table>

Allele Frequency

<table>
<thead>
<tr>
<th>Allele</th>
<th>Patients N=50</th>
<th>Control N=50</th>
<th>Odds ratio (95% CI of OR)</th>
<th>Chi-Square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>71 (71%)</td>
<td>46 (46%)</td>
<td>0.35 (0.19-0.62)</td>
<td>12.87</td>
<td>P = 0.0003</td>
</tr>
<tr>
<td>A</td>
<td>29 (29%)</td>
<td>54 (54%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusion

The present case-control study focused on the contribution of KLF14 (rs972283 G>A) polymorphisms to the risk of having T2DM in Iraqi Population. The study also examined the relation between those polymorphisms and BMI and various biochemical parameters in the study sample. The results of the study can be summarized as KLF14 (rs972283 G>A) polymorphism revealed a significant difference between T2DM patients and controls; GG genotype was significantly more frequent in the patient group. The highly levels of Total Cholesterol, triglycerides, LDL and VLDL may associated with T2DM in people with risk variants in KLF14 gene.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

References


Theoretical Study of Cyclohexane -1,2-Diamine-Oxalate-Platinum Metal Complex and Comparing with Experimental Data: DFT Calculations

Shakir D. Al-Saeedi¹, Hamid Ibrahim Abbood², Wisam ShareefIrzooqi³

¹Asst.Prof., University of Thi-Qar, College of science, Iraq, ²Prof, Al-Zahraa University for Women, Karbala, Iraq, ³Asst. Lect. Thi-Qar Education Directorate, Thi-Qar

Abstract

Theoretical studies for calculating the molecular structure and electronic properties of Cyclohexane -1,2-diamine-oxalate- platinum metal complex by using density functional theory (DFT) for get electronic properties and time dependent density functional theory (TD-DFT) for get excited state with B3LYP-SDD basis sets at the Gaussian 09 of programs. The electronic states of the system have been calculated depend on Koopmans’ theorem.

The results showed that the excitation energies of oxaliplatinum lie in the UV region of electromagnetic radiation with very high biological reactivity, where The absorption spectra of complex recorded with wavelength (344.69nm), oscillator strength (0.0002). From calculations of the HOMO - LUMO energies, energy gap, hardness and softness, all this results showed oxaliplatinum are more soft and can easily interact with enzymes because the enzymes are big soft molecules. Thus, the optimized geometry shows a good agreement with the experimental results.

Keyword: Oxaliplatinum, B3LYP/DFT-SDD calculations, quantum chemical parameter and Enzymes.

Introduction

The basic components of cells picked by nature are metals [1]. In the enzyme catalytic domain, they are commonly located and are active in various biological processes, from electron exchange to catalysis and structural functions. They are commonly used in cellular activities [2].

Platinum coordination complexes have formed a new class of active anti-cancer agents in animals, and man, the most commonly studied compound, it’s now in experimental clinical use against a wide range of human cancers[3].

In the treatment of disease disorders, metal-based compounds have been commonly used. Platinum products, such as cis-platinum Pt(NH₃)₂Cl₂, carboplatinum C₆H₁₂N₂O₄Pt and oxali-platinum C₈H₁₄N₂O₄Pt are the main of the metal-based compounds in the treatment of cancer [4]. Many metal complexes through, redesigning the current chemical structure by ligand substitution or constructing the entire new compound with improved protection, the synthesis has been synthesized. However, a handful of these drugs are currently under clinical trial due to increased focus on the clinical importance of metal-based complexes and several compounds are waiting to enter the trial.

Oxaliplatinum was discovered by Professor Yoshinori Kidani at the University of Nagoya City. Where, platinum compounds of the present generation were tested, in recent years, they have been focused on those with the 1,2-diaminocyclohexane carrier ligand,
such as oxali-platinum.

The experiments molecular biology and in vitro cytotoxic screening by the National Cancer Institute find that diaminocyclohexane platinum, such as oxaliplatinum, belongs to a separate cytotoxic family, different from cisplatin and carboplatin [5].

Oxaliplatinum is a platinum compound that, mainly by inducing intrastrand cross-links in DNA [6]. The crystal structure of the drug oxaliplatinum anticancer compound consist of planar platinum(II) center binding with ligands 1,2 cyclohexane diamine, it also has oxalate group [7].

The formula for oxaliplatinum is [Pt (R, R-DACH) (oxalate)]. Fig.1 shows DACH are diaminocyclohexane and the formula of oxalate is CO$_2$. They bind together to form oxalplatinum structure.

**Fig.1: Geometrical structure cyclohexane-1,2-diamine-oxalate-platinum (oxaliplatinum)**

**Computational Details**

In this study, the measurements were carried out, theoretically by using Gaussian 09 programs and Gauss View 5.0.8 program at the hybrid functional B3LYP together with SDD basis set possible quality for the system of interest for heavy metals use relativistic ECP, it is powerfully recommended for the heavy metals[9]. DFT depends on the energy of the ground state and all other electrical properties of the ground state that are uniquely determined by the electron density [9-10]. Additionally, the exact ground state of the system corresponds to the electronic density for minimal total energy. TD-DFT which can be used to investigate the excited-state properties and dynamics of a system in the attendance of time-dependent. TD-DFT method has been verified to be effective in deciding the spectral properties of certain transition metal complexes [11].

**Results and discussion**

The Cyclohexane-1,2-diamine-oxalate-platinum (oxaliplatinum), the structure is designed at Gaussian view 5.0.8. The resulting theoretical optimized parameters for the studied structure included bond length in angstrom A° and bond angle in degree, seen in Table 1. The relaxation showed a suitable method is used to DFT-B3LYP/ SDD basis sets in our data theoretical framework are in good agreement with the experimental data [12-13].

When we compared our calculations with experimental data. One can see, the optimized bond lengths Pt-N, Pt-O, C-C, and C=O are approximately equal with experimental values in ref. [12]. While, bond
lengths are slightly larger than the experimental values in ref. [13]. Furthermore, much of the bond angles optimized are slightly larger than the experimental values.

Table 1: Bond lengths in Å and angles in degree of the oxaliplatinum.

<table>
<thead>
<tr>
<th>Bond length (Å)</th>
<th>Our data</th>
<th>Expt.α</th>
<th>Expt.β</th>
<th>Bond Angle (deg.)</th>
<th>Our data</th>
<th>Expt.α</th>
<th>Expt.β</th>
</tr>
</thead>
<tbody>
<tr>
<td>R(C-C)</td>
<td>1.57</td>
<td>1.53</td>
<td>1.19-1.50</td>
<td>A(C-C-C)</td>
<td>110.7-111.6</td>
<td>111-111.4</td>
<td>106-113</td>
</tr>
<tr>
<td>R(C-N)</td>
<td>1.51</td>
<td>1.49</td>
<td>1.49</td>
<td>A(N-Pt-N)</td>
<td>83.5</td>
<td>82.4</td>
<td>83.8</td>
</tr>
<tr>
<td>R(Pt-N)</td>
<td>2.10</td>
<td>2.10</td>
<td>2.4-2.6</td>
<td>A(C-N-Pt)</td>
<td>109.1</td>
<td>108.6-109.3</td>
<td>106-107</td>
</tr>
<tr>
<td>R(Pt-O)</td>
<td>2.01</td>
<td>2.01</td>
<td>2.1-2.4</td>
<td>A(N-Pt-O)</td>
<td>96.42-179.6</td>
<td>96.8-179.1</td>
<td>96-175.6</td>
</tr>
<tr>
<td>R(C-O)</td>
<td>1.36</td>
<td>1.34</td>
<td>1.21-1.32</td>
<td>A(Pt-O-C)</td>
<td>113.062</td>
<td>112.6</td>
<td>112-141</td>
</tr>
<tr>
<td>R(C=O)</td>
<td>1.23</td>
<td>1.22</td>
<td>1.29</td>
<td>A(O-C=O)</td>
<td>123.38</td>
<td>122.5</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A(C=O)</td>
<td>121.9</td>
<td>115.4</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A(C-C-N)</td>
<td>108.5-113.6</td>
<td>108.1-113.9</td>
<td>107-105</td>
</tr>
</tbody>
</table>

α from ref. [12].
β from ref. [13].

Table 2 displays the Calculated outcomes of the total energy $E_T$ (-843.0702914), $E_{HOMO}$, $E_{LUMO}$ and Egap ($E_{LUMO} - E_{HOMO}$) of the oxaliplatinum. In contrast to that, we showed a strong agreement with experimental data ref. [14], where listed in Table 2. Consequently, the present work is a suitable method a large separation was obtained between the valence and conduction bands. In our data, one can see LUMO energy (-1.1150658eV) greater than the HOMO energies (-5.852871), these results are similar to the result ref. [14]. The energy gap is a very important parameter as a symbol of the complexes biological reactivity, as can be seen in the table 2. Our Estimates, the Egap is 4.7378052eV in which the oxaliplatinum has insulating behavior. This outcome is in strong alignment with that of ref. [14], which corresponds to the high energy needed for oxaliplatinum to donate or accept an electron.

Biological activity of metal complexes can determine through study quantum chemical parameter HSAB [15-16]. Table 2 gather the calculated values hardness $H$ and softness $S$ for the oxaliplatinum complexes, the Quantum Chemical Parameter Pattern depends on the coordination and the molecular geometry of the complexes. It is important to address with HSAB the coordination tendencies of complexes against the enzymes. Soft complexes have small energy gap and can easily interact with enzyme because the enzymes are big and soft molecules.

Hard complexes have large energy gap and more complex to interact with enzymes, thus, one can note the results under study through table 3 are in good agreement with those in ref [14]. Also, from table 2 declare the results of electronic properties for our data and experimental ref. 14.
The results showed the virial ratio (-V/T = 2.1093) lies in the same range for the molecular systems, this an indication to a suitable basis set used for the relaxation of the studied metal complex.

The ionization energy IE and electron affinity EA measured values revealed the results of our data using B3LYP-SDD basis sets are in good agreement with ref. [14]. Ionization potential and electron affinity demonstrate the vibrational behavior and they can be expressed in terms of energies high molecular orbitals that are filled and low molecular orbitals that are unoccupied.

Ionization potential and electron affinity represents very important factors in symmetry, energy transfer and the stability of systems [17-18]. IE equal to (5.852871 eV) and EA equal to (1.1150658 eV) stands for closed shell system and these results are corresponding to the large value of energy gap of the compound and it has insulating behavior.

### Table 2: The HOMO, LUMO energies, IE, EA energy gap, virial ratio and quantum chemical parameter of the oxaliplatin.

<table>
<thead>
<tr>
<th>Method</th>
<th>Our data</th>
<th>Reference [14]</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHOMO (eV)</td>
<td>-5.852871</td>
<td>-5.201032</td>
</tr>
<tr>
<td>ELUMO (eV)</td>
<td>-1.1150658</td>
<td>-0.4652528</td>
</tr>
<tr>
<td>Egap (eV)</td>
<td>4.7378052</td>
<td>4.7357792</td>
</tr>
<tr>
<td>ET (a.u)</td>
<td>-843.0702914</td>
<td>-842.906902</td>
</tr>
<tr>
<td>Hardness (e V)</td>
<td>2.3689026</td>
<td>2.3678896</td>
</tr>
<tr>
<td>Softness (e V)</td>
<td>0.211068197</td>
<td>0.211158493</td>
</tr>
<tr>
<td>-V/T</td>
<td>2.1093</td>
<td>……</td>
</tr>
<tr>
<td>IE (eV)</td>
<td>5.852871</td>
<td>5.201032</td>
</tr>
<tr>
<td>EA (eV)</td>
<td>1.1150658</td>
<td>0.4652528</td>
</tr>
</tbody>
</table>

Fig.2 reflects the distribution of the HOMO and LUMO molecular orbital maps for oxaliplatinum. Where, A, B reflect present job, where red indicated negative regions with higher electronic density and green color indicated positive area. [15]. While C, D represent ref [14], where red indicated negative regions and blue color indicate positive regions. In present work one can see, the distribution map is similar with experimental data ref [14]. Where, the HOMO orbitals as an electron-donor region and LUMO orbitals as an electron-acceptor region. The LUMO distribution of oxaliplatinum presents a p-antibonding orbital spread over the oxalate group, which constitutes a special electronic feature of oxaliplatinum. Thus, the compound can be acting as oxidant and reduced relatively.
Fig. 2: Distribution of the molecular orbitals (MOs) of the oxaliplatinum maps: Present work HOMO(a) and LUMO(b). maps: Ref. [14] HOMO(c) and LUMO(d).

Fig. 3: The ESP distribution of the oxaliplatinum (ESP Counter left and 3D ESP right).

Fig. 3 illustrates the electrostatic potential ESP surfaces of Cyclohexane-1,2-diamine-oxalate-platinum Metal Complex by used to DFT-B3LYP/SDD basis sets. Oxaliplatinum ESP surfaces revealed that the potential was dragged into areas of high electronegativity. Thus, from figure 5 one can see

ESP demonstrates that, according to their mainly the high electronegativity, the charges are strongly dragged towards the oxalate group.

Excitation energy of the cyclohexane-1,2-diamine-oxalate-platinum metal complex was calculated by using the B3LYP-TD-DFT-SDD. The excitation energy of the main band appears at 3.597 eV, the oscillator strength, wave length, electronic transitions (HOMO→LUMO) and the transition characters were calculated and listed in Table 3.

Also, UV-Vis spectrum of oxaliplatinum appears at (344.69 nm), one can note, the excitation energies lie in the UV region of electromagnetic radiation with very high biological reactivity.
Table 3: The excitation energy, oscillator strength, wave length, of the cyclohexane-1,2-diamine-oxalate-platinum metal complex was calculated by using the B3LYP-TD-DFT-SDD.

<table>
<thead>
<tr>
<th>Excitation Energy (eV)</th>
<th>Oscillator Strength</th>
<th>Wave Length (nm)</th>
<th>Transitions HOMO→LUMO</th>
<th>Transition Character TC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.597</td>
<td>0.0002</td>
<td>344.69</td>
<td>H-3-&gt;L+1</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HOMO-&gt;L+1</td>
<td>78%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H-5-&gt;L+1</td>
<td>6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HOMO-&gt;L+3</td>
<td>2%</td>
</tr>
</tbody>
</table>

Conclusion

After discusses the results of the structural and electronic properties of Cyclohexane -1,2-diamine-oxalate-platinum Metal Complex using B3LYP-SSD/DFT, conclude the following:

· DFT/B3LYP-SDD basis sets used to study quantum chemical parameter and the electronic structure of the cyclohexane-1,2-diamine-oxalate-platinum (oxaliplatinum) metal complex.

· The result values of bonds and the angles are a good agreement with experimental data.

· Oxaliplatinum has insulating behavior due to $E_g$ is wide between the valence and conduction bands. Thus, the great contribution to the construction of the molecular orbitals is due to the outer electrons with few contributions from the ligands in the platinum metal. Oxalate group consist of oxygen atoms have able to interact with the receptor reactive sites due to high their negative charge densities in which they are the most electronegative atomic sites in complexes.

· A good relaxation was obtained without any imaginary frequency for the studied structure.

· Oxaliplatinum has a low electronic softness value and requires high excitation energy to transfer electrons or to accept an electron.

· Absorption spectra of oxaliplatin with wavelength 344.69nm, in the UV region.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required

References


Trend of Sex Related Crimes in Varanasi District

Shashank Shekhar Jha¹, Manoj Kumar Pathak²
¹ Junior Resident, Department of Forensic Medicine, IMS, BHU, Varanasi (UP), ² Professor and Head, Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, Patna (Bihar)

Abstract

Introduction: Sexual harassment and sexual assault both rely on dehumanizing the intended victims. Sexual violence is a significant cause of physical and psychological harm and suffering for women and children. The Indian Penal Code (IPC) is the crucial criminal code of India.

Material and Method: Consent of the victim and accused was taken prior to examination. In case of minor, the consent from his/her parents or legal guardian was taken. SAFE (Sexual Assault Forensic Evidence) kit was used for sample collection from sexual assault victims and accused.

Observation and Result: This study includes cases 160 victims of sexual assault.

Conclusion: The present study, being a pioneer one, is relevant in the context of crime against women of all age groups in the Eastern U.P., especially of Varanasi district in socio-demographic and medico-legal paradigms. The government must set up a special unit that recruits officers specifically to deal with sexual offences, and create easy access to doctors, forensic experts, rape survivors and psychologists.

Keywords: Sexual offence, SAFE Kit, Medico-legal examination, NCRB, Criminal force.

Introduction

A Sexual offence has been defined by the Law Reform Commission of Canada (1978) as sexual contact with another person (including touching of the sexual organs of another) or touching of another with one’s sexual organs without that person’s consent. Any person may understand sexual violence as any sexual act using coercion regardless of their relationship to the victim, in any setting, including but not limited to home and work. In sexual violence, coercion constitutes an important component, which covers a whole spectrum of degrees of force. Apart from physical force, it may involve psychological intimidation, blackmail or other threats (Bancroft, J., 1974).[1]

Sexual harassment and sexual assault both rely on dehumanizing the intended victims. Sexual assault justifications tend to rely on negatively stereotyping the behaviour of the intended victim [2]. As per Crime Statistics 2018 by NCRB, Uttar Pradesh has been ranked as the worst state for women’s security and Madhya Pradesh as the state where rape is most rampant. According to the statistics, while UP registered 59,445 cases of crime against women, Madhya Pradesh registered 5,450 rapes, the maximum in 2018[3]. The Criminal Law (Amendment) Bill, 2018[4] was passed to replace the Criminal Law (Amendment) Ordinance, 2018 which was promulgated after the Kathua and Unnao rape incidents. The bill covers the same amendments as were in the ordinance. CLAA 2018 provides amendments in The Indian Penal Code, 1860, The Criminal Procedure Code, 1973, The Indian Evidence Act, 1872 and The Protection of Children from Sexual Offences Act. The

Corresponding author:
Dr. Shashank Shekhar Jha
Junior Resident,
Department of Forensic Medicine,
IMS, BHU, Varanasi- 221005 (UP)
Contact No. - +91-9891831983,
Email id: shashank.nmch@gmail.com
CLAA, 2018 amended IPC in two ways; firstly, made amendments to existing sections, and secondly, inserted new provisions of law.

Sexual violence is a significant cause of physical and psychological harm and suffering for women and children. The World Health Organisation (WHO) defines Sexual Violence as “any sexual act, attempt to obtain a sexual act, unwanted sexual comments/ advances and acts to traffic, or otherwise directed against a person’s sexuality, using coercion, threats of harm, or physical force, by any person regardless of relationship to the victim in any setting, including but not limited to home and work. (WHO, 2003) Sexual assault, a form of sexual violence, is a term often used synonymously with rape.[5]

The Indian Penal Code (IPC) is the crucial criminal code of India. It is broad code intended to cover all substantive aspects of criminal law. The code was drafted in 1860 on the recommendations of first law commission of India established in 1834 under the Government of India Act 1833 under the Chairmanship of Thomas Babington Macaulay[6].

Being a holy religious city for many religions, regular visit by people around the globe is paid and the approximate annual footfall of tourists, both international and domestic is around 6-6.5 lakh. According to 2011 Census of India, population of Varanasi itself was 3,676,841. [7]

Material and Methods

Present study was carried out in the Department of Forensic Medicine at Institute of Medical Sciences, Banaras Hindu University, Varanasi for 10 post-mortem cases and District Women Government Hospital, Kabirchaura, Varanasi for 150 ante-mortem cases from September 2018 to March 2020. Also, for hands-on training, visit to state-of-the-art One Stop Centre at Seth G. S. Medical College and King Edward Memorial Hospital, Parel, Mumbai for 15 days was done and some data was collected from there too. Total 160 cases of victims of alleged sexual assault were examined.

Consent of the victim and accused was taken prior to examination. In case of minor, the consent from his/her parents or legal guardian was taken. If the accused was in police custody and refused to consent for medical examination, then the examination was carried out without the consent of accused as per provisions of Section 53(1) of CrPC. Similarly if the accused was a female then she was examined as per provisions of Section 53(2) of CrPC.

SAFE (Sexual Assault Forensic Evidence) kit was used for sample collection from sexual assault victims and accused.

Inclusion Criteria:

- All alleged victims of sexual assault brought for medico-legal examination with crime registered.
- All the victims of alleged sexual assault brought for examination directly to hospital without registering the crime at concerned police station.
- Dead bodies with alleged history of sexual assault wherein informed consent from the guardian of the deceased is obtained.

Exclusion Criteria:

- Victims of alleged sexual assault who refused to give consent for medico-legal examination.

Observation and Results

This study includes cases 160 victims of sexual assault. In this region such study has not been conducted, so the present study was carried out with the view of understanding the magnitude and pattern of alleged sexual assault cases to create a sense of awareness and to suggest preventive measures.

The data was entered and analysed by using MS-Excel SPSS software package. Frequency of all variables was derived to check completeness of data. Magnitude was expressed in percentages.
Table no. 1: DISTRIBUTION OF SEXUAL ASSAULT VICTIMS AS PER GENDER AND AGE GROUP

<table>
<thead>
<tr>
<th>AGE</th>
<th>FEMALE</th>
<th>MALE</th>
<th>TRANSGENDER</th>
<th>TOTAL (n=160)</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BELOW 18 YEARS</td>
<td>111 (69.375%)</td>
<td>7 (4.375%)</td>
<td>0</td>
<td>118</td>
<td>73.75</td>
</tr>
<tr>
<td>ABOVE 18 YEARS</td>
<td>40 (25%)</td>
<td>0*</td>
<td>2 (1.25%)</td>
<td>42</td>
<td>26.25</td>
</tr>
<tr>
<td>TOTAL</td>
<td>151</td>
<td>7</td>
<td>2</td>
<td>160</td>
<td>100</td>
</tr>
</tbody>
</table>

Above table shows that in total 160 victims, in 111 (69.375%) cases victims are female and male in 7 (4.375%) cases while no case of transgender below 18 years and in 40 (25%) cases victims are female while 2 (1.25%) are transgender above 18 years.

*As per Criminal Law Amendment Act 2013, only man can commit rape on woman. Hence concept of rape is above 18 years of male is not legally recognized in India u/s 375 of IPC.

Table No. 2: RELATIONSHIP OF ACCUSED WITH THE SEXUAL ASSAULT VICTIM

<table>
<thead>
<tr>
<th>RELATIONSHIP WITH VICTIM</th>
<th>NO. OF CASES (n=160)</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FATHER</td>
<td>5</td>
<td>3.125%</td>
</tr>
<tr>
<td>UNCLE</td>
<td>13</td>
<td>8.125%</td>
</tr>
<tr>
<td>BROTHER-IN-LAW</td>
<td>4</td>
<td>2.5%</td>
</tr>
<tr>
<td>FRIEND</td>
<td>55</td>
<td>34.375%</td>
</tr>
<tr>
<td>NEIGHBOUR</td>
<td>38</td>
<td>23.75%</td>
</tr>
<tr>
<td>TEACHER</td>
<td>3</td>
<td>1.875%</td>
</tr>
<tr>
<td>SCHOOL STAFF</td>
<td>1</td>
<td>0.625%</td>
</tr>
<tr>
<td>EMPLOYER</td>
<td>5</td>
<td>3.125%</td>
</tr>
<tr>
<td>STRANGER</td>
<td>24</td>
<td>15%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>160</td>
<td>100%</td>
</tr>
</tbody>
</table>
Above table shows that out of total 160 cases, in 85% of the cases the assailant is known to the sexual assault victim. In 4 (2.50%) cases accused is brother-in-law in 5 (3.125%) cases accused is employer, in 5 (3.125%) cases accused is father in 55 (34.375%) cases accused is friend, in 38 (23.75%) cases accused is neighbour in 1 (0.625%) case accused is school staff, in 24 (15%) cases accused is stranger, in 3 (1.87%) cases accused is teacher, in 13 (8.125%) cases accused is uncle.

Table No. 3: PLACE OF INCIDENT OF SEXUAL ASSAULT

<table>
<thead>
<tr>
<th>PLACE OF INCIDENT</th>
<th>NO OF CASES (n=160)</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOUSE OF ACCUSED</td>
<td>69</td>
<td>43.125%</td>
</tr>
<tr>
<td>SURVIVOR’S HOUSE</td>
<td>18</td>
<td>11.25%</td>
</tr>
<tr>
<td>FIELD</td>
<td>9</td>
<td>5.625%</td>
</tr>
<tr>
<td>HOTEL</td>
<td>16</td>
<td>10%</td>
</tr>
<tr>
<td>OFFICE</td>
<td>1</td>
<td>0.625%</td>
</tr>
<tr>
<td>RAILWAY STATION</td>
<td>4</td>
<td>2.5%</td>
</tr>
<tr>
<td>SCHOOL</td>
<td>2</td>
<td>1.25%</td>
</tr>
<tr>
<td>SECLUDED PLACE</td>
<td>40</td>
<td>25%</td>
</tr>
<tr>
<td>TOILET</td>
<td>1</td>
<td>0.625%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>160</td>
<td>100%</td>
</tr>
</tbody>
</table>

Above table shows that 69 (43.125%) victims are assaulted in the house of accused, 18 (11.25%) victims are assaulted in survivor’s house, 9 (5.625%) victims are assaulted in field, 16 (10%) victims are assaulted in the hotel, 4 (2.5%) are assaulted at the railway station, 1 (0.625%) is assaulted in the office, 2 (1.25%) are assaulted in school, 40 (25%) victims are assaulted in some secluded area while 1 (0.625%) victim in toilet.
Table No. 4: DISTRIBUTION OF SEXUAL ASSAULT VICTIMS AS PER THREATS GIVEN AND USE OF CRIMINAL FORCE/ THREATS BY ASSAILANT

<table>
<thead>
<tr>
<th>CRIMINAL FORCE/ THREATS</th>
<th>No. OF CASES (n=160)</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHYSICAL ASSAULT</td>
<td>27</td>
<td>16.875%</td>
</tr>
<tr>
<td>TO HARM FAMILY/ KIN</td>
<td>4</td>
<td>2.5%</td>
</tr>
<tr>
<td>TO ACQUIRE PROPERTY</td>
<td>2</td>
<td>1.25%</td>
</tr>
<tr>
<td>BLACKMAILING BY THREAT TO CIRCULATE PHOTOGRAPH/ VIDEO</td>
<td>11</td>
<td>6.875%</td>
</tr>
<tr>
<td>USING ABUSIVE/ CHEAP WORDS</td>
<td>5</td>
<td>3.125%</td>
</tr>
<tr>
<td>NONE</td>
<td>111</td>
<td>69.375%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>160</td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

As above table shows in total 160 cases, victim was physically assaulted in 27 (16.875%) cases, verbal threats to harm family/ kin in 4 (2.5%) cases, threat to acquire property was given in 2 (1.25%) case, blackmailing by threat to circulate photo/ video in 11 (6.875%) cases, using abusive/cheap words in 5 (3.125%) cases while no threat was used in 111 (69.375%) cases.

**Discussion**

Rape is the fourth most common crime against women in India according to NCRB records.[3] Rape in India received extensive media coverage after a fatal gang rape of a student in Delhi in December, 2012, and a village council-ordered gang rape of a young woman in West Bengal in January, 2014. Most recently a young veterinary doctor was raped, smothered and then burnt alive by a group of four people in November 2019. These incidents changed effect in policing ensured swift justice for most of the rape survivors and the complainants in cases of sexual harassment and molestation. India has been characterized as one of the “countries with the lowest per capita rates of rape”. [8]
In the present study cases below 18 years of age group, we found female preponderance 113 (70.625%) cases as compared to males 7 (29.375%) while in 40 (25%) cases victims are female while 2 (1.25%) are transgender above 18 years.

This is in agreement with the study by S. Bandyopadhay et al\textsuperscript{[9]} who observed almost 95% of cases of females as compared to 5% of males of sexual assault. Similarly our findings are consistent with other studies also Tamuli RP et al\textsuperscript{[10]} (98.43%), K. Bhowmik et al\textsuperscript{[11]} (97.08%) and Sarkar SC et al\textsuperscript{[12]} (88.9%).

![Relationship of Accused with Sexual Assault Victim](image)

**Figure No. 2: Relationship of Accused with the Sexual Assault Victim**

As per present study out of 160 victims, most common relation with survivor was friend 50 (31.25%) victims, followed by neighbour 43 (26.88%) victims and in 24 victims (15%) assailant was stranger, in 3 (1.87%) the assailant was teacher, in 5 (3.13%) victims the assailant was father, in 5 (3.13%) cases the assailant was employer. Out of 5 fathers 2 assailants were widowed. Out of 12 uncles, 2 were paternal and 1 was maternal uncle. Only 15% cases of sexual assault was committed by stranger and in 85% cases assailant was known to victim.

This finding is consistent with Rahul Jain et al\textsuperscript{[13]} who observed that in 87.5% of cases the rapist knew the victim (46.75% neighbour, 25% relatives, and 15.75% were friends) and Ian Mclean et al\textsuperscript{[2]} found that 69% of the assailants were known to the victim prior to assault.
In our study, the most reported site of offence was the house of accused i.e. 69 (43.125%) cases, Secluded area 43 (26.875%) and Survivors house 18 (11.26%) and in hotel 16 (10%) cases. Victims with known assailants are considerably more likely to be assaulted in a house/apartment where he resides than those assaulted by strangers. Victims assaulted by strangers are most likely to be assaulted outdoors.

Similarly study by Bhoi S et al\textsuperscript{14} found that maximum incidence took place at accused house in 146 cases (38.52%) followed by victims house in 127 cases (33.50%). Also our study is consistent with Rahul Jain et al\textsuperscript{13}, who found that most of the cases 46.75% and, in the study of Bijoy S et al\textsuperscript{15} 36.53% cases, where these victims were sexually assaulted in the house of the accused. Similarly as per study by Kumar Pal et al\textsuperscript{16} most incidences occurred at the house of alleged accused (31.40%).
We observed in this study that among total 160 cases, victim was physically assaulted in 27 (16.875%) cases, verbal threats to harm family/ kin in 4 (2.5%) cases, threat to acquire property was given in 2 (1.25%) case, blackmailing by threat to circulate photo/ video in 11 (6.875%) cases, using abusive/ cheap words in 5 (3.125%) cases while no threat was used in 111 (69.375%) cases.

This is consistent with Rahul Jain et al [13] (2008) and a similarly a study conducted at Massachusetts Executive Office of Public Safety and Security Research and Policy Analysis Division in 2008 [17], which concludes that for both strangers and known assaults, verbal threats were the most commonly reported type of force, reported in 58% of all assault cases.

**Conclusion**

The present study, being a pioneer one, is relevant in the context of crime against women of all age groups in the Eastern U.P., especially of Varanasi district in socio-demographic and medico-legal paradigms.

Of all the crimes, sex related crimes are the most barbarous and humiliating. Women and children remain the most vulnerable group of this crime. Justice Arijit Pasayat had observed that while a murderer destroys the physical frame of the victim, a rapist degrades and defiles the soul of helpless female.

The government must set up a special unit that recruits officers specifically to deal with sexual offences, and create easy access to doctors, forensic experts, rape survivors and psychologists. This will help victims feel confident in coming forward to seek justice. All registered offences must be dealt with by this unit within a month using fast-track courts. Predators must know that justice is swift and favourable to victims. India’s approach to curbing sexual aggression must steer clear of diminishing women, and root out reckless patriarchal attitudes instead.

The latest NCRB data for the year 2018 states, majority of cases under crimes against women out of total IPC crimes against women were registered under ‘Cruelty by Husband or His Relatives’ (31.9%) followed by ‘Assault on Women with intent to Outrage her Modesty’ (27.6%), ‘Kidnapping & Abduction of Women’ (22.5%) and ‘Rape’ (10.3%).

To conclude, the words of Robert F Kennedy seems appropriate:


> “Laws can embody standards, governments can enforce laws but the final task is not a task for government. It is a task for each and every one of us. Every time we turn our heads the other way when we see the law flouted when we tolerate what we know to be wrong, when we close our eyes and ears to the corrupt because we are too busy, or too frightened, when we fail to speak up and speak out we strike a blow against freedom and decency and justice.”

**References**


Comparative Study of Cold Physical Plasma Effect on Modulation of Basic-Fibroblast Growth Factor and Tumor Necrosis Factor Alpha in Full Thickness Skin Wound Healing Process in Normal and Diabetic Dogs

Shatha M. Al Qaseer¹, Serwa I.Salih¹, Ruqaya M. Ali², Mohammed K. Khalaf³

¹Scholar Researcher, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, ²Scholar Researcher, Ministry of Agriculture, Central Veterinary Laboratories, Baghdad, Iraq, ³Scholar Researcher, Ministry of Higher Education Science and Technology, Baghdad, Iraq

Abstract

Non-thermal atmospheric pressure plasma (N-APP) a physical method that recently had been extensively studied by researchers as a possible therapy in biomedical researches such as wound healing. In clinical dermatology, cold plasmas are mainly used for the treatment of chronic wounds and pathogen-based skin diseases, in which stimulation of tissue repair and decontamination. In this research, home-made Helium Non-Equilibrium atmospheric pressure plasma jet (He -NAPPJ), that had been generated using a DBD configuration for exceptional standardization protocol of this plasma source that was used in treatment of full-thickness skin tissue wound of normal and diabetic dogs. This clinical research in veterinary medicine is the first one in Iraq that study role of cold physical plasma in wound healing in diabetic wounds used the dogs as a model. The results were evaluated by quantitative real-time polymerase chain reaction qRT-PCR, which showed enhancement in wound healing process by cold plasma jet, by modulation of gene expression of basic-Fibroblast Growth Factor and Tumor Necrosis Factor alpha(TNF-α) in normal and diabetic dogs.

Key words: Growth factor, Skin Wound healing, non-thermal plasma jet.

Introduction

Plasma medicine is an emerging field incorporating physics, chemistry, life science, and medicine. To estimate the role of non-thermal plasma in wound healing process in this study, basic fibroblast growth factor (b-FGF) was used as a parameter, it plays a vital role in the repair of injured tissue in many studies,b-FGF has ability to promote cell division and proliferation related to injury repair and tissue reconstruction, moreover, during acute wound there is a rise in the production of b-FGF normally, which is responsible for angiogenesis, granulation tissue formation, re-epithelialization and tissue remodeling. Furthermore, Injury-induced b-FGF promotes the aggregation of monocytes, neutrophils, macrophages and fibroblasts via chemo taxis in the injured tissues (1). Tumor necrosis factor alpha (TNF-α) is a multifunctional pro-inflammatory cytokine, which is secreted mainly by monocytes and macrophages, the main supply of TNF-α are macrophages and T-cells, yet many other cells such as B-cells, neutrophils, and endothelial cells have been described to produce TNF-α. The functional relevance is broad, like the mediation of cell survival and pro-inflammatory response and, TNF-α instigates signaling pathways of the cell death (2).

Diabetes mellitus (DM) is one of the common endocrinopathy of dog characterized by hyperglycemia, glycosuria and weight loss. The treatments for diabetic wounds include surgical and non-surgical procedures. As a non-surgical treatment, the application of non-thermal plasma jet, is one of the promising methods to promote wound healing or to provide healthy wound beds for surgical treatments, a non-equilibrium
atmospheric pressure plasma jet (N-APPJ) is considered as environment friendly substances, have been studied all over the world, as reliable effects, safe, non-toxic and no adverse reactions have been reported in the treatment of wounds, but remains as dose dependent radiation. However, the interrelationship between diabetes, non-thermal plasma treatment and the wound healing process acts together with the immune system and the immunomodulatory function (3).

The aim object of this study is to demonstrate the effect of non-thermal plasma jet in treatment of diabetic wounds, through demonstration of tissue levels of gene expression of growth factors and cytokine using quantitative Real Time Polymerase chain reaction (qRT-PCR) technique, to measure the b-FGF and TNF-α gene expression level in alloxan- induced diabetic dogs compared to the normal ones. Also, studying the modulation effect of NAPPJ for both b-FGF and TNF-α.

Materials and Methods

Experimental design:

The experiments performed at the Department of surgery & obstetrics in the college of veterinary medicine, University of Baghdad, and all procedures conducted within the guidelines for humane care of laboratory animals and approved by the University of Baghdad ethics Committee (no. 1364/ P.G).

Twelve adult male dogs were used. All surgical and non-surgical procedures were performed under aseptic conditions and general anesthesia. Each dog was subjected to (4) full-thickness open cutaneous wounds of (3×3cm) of the animals back. Dogs were put into two groups: 1- The non-diabetic animal groups(N): six) The dogs were divided in two sub-groups: Non diabetic control (NC);Non diabetic treated (NT) by(N-APPJ); 2- The diabetic animal groups (D): (six) dogs were subjected to experimental induction diabetes, and after two weeks from induction they underwent the same previous procedure (grouping, wounding and treatment), and the sub- groups were: Diabetic control (DC); Diabetic treated (DT) with (N-APPJ). All the wound healing process was evaluated by Tissue level detection of (b-FGF), and (TNF-α), in consequence periods (0, 3, 7, and 14 days) post-surgery.

Induction of Experimental Diabetes:

Six dogs were placed on a fasting regimen for 24 hours before the induction of diabetes via intravenous injection of Alloxan which was dissolved in normal saline 0.9 %, at a dose of 100 mg/kg. After 32 hours, the dogs showed fasting hyperglycemia.

Non-thermal Plasma Jet System (Experimental Setup):

![Figure (2.1): Schematic representation of the DBD plasma jet system.](image-url)
In this research, the characterization of home-made Helium Non-Equilibrium atmospheric pressure plasma jet (He-NAPPP), that generated using a dielectric barrier discharge (DBD) configuration device (figure 2.1), driven by a (8 kVp−p) voltage, the frequency (12 kHz). As a working gas, Helium (He) was used in flow rates (6) slm, with 32 °C He gas temperature, plume length 40 mm. The distance is 15 mm from the distal end of Pyrex tube (DBD) and the wound surface, the power density 44 mWatt/cm², and plasma radiation energy dose 76J/cm².

**Molecular diagnosis:**

The biopsies from skin tissue of each animal were collected from normal skin and wounds of all sub-groups, and kept at deep freezing (-80 °C), then evaluated using RNA extraction kit for extract m-RNA then Real Time Polymerase chain reaction (PCR) technique for house-keeping, (b-FGF) and (TNF-α) primers as mentioned below:

**Primers:** Three primers were used in this study including the specific gene of cutaneous tissue in dogs which including Anterior Reverse glyceraldehydes-3-phosphate dehydrogenase (AR-GAPDH) gene primer that was used as Housekeeping gene (HKG) (4), anterior reverse b-Fibroblast Growth Factor (AR-FGF) (5) and anterior Reverse (AR-TNF-α) genes primers (4) that were used as target genes. The primers were used in quantification of genes expression by using Real–Time quantification Polymerase Chain Reaction (q-RTPCR) techniques based BRYT Green DNA binding dye (Promega–USA).

**The Kits used in molecular techniques** as SaMag Total RNA Extraction Kits, the GoScript™ Reverse Transcription System kit (Como–Italy), The GoScript™ Reverse Transcription System kit (Promega–USA), and GoTaq® qPCR Master Mix kit (Promega–USA).

**B. m-RNA Extraction:** m-RNA was extracted by automated nucleic acid extraction system according to with SaMag Total RNA Extraction Kit by Sacace Biotechnologies Srl Como – Italy company instructions. The extracted m-RNA samples were kept at freezing temperature (-20) C°.

**C. Examination of m-RNA Concentration and purity:** The concentration of m-RNA was measured by using NanoDrop ND-1000 spectrophotometer (thermo-fisher, USA), and according to the manufacture’s manual (NanoDrop Technologies, 2006).

**D. cDNA synthesis for easy transition to gene-specific target amplified:**

By using GoScript™ Reverse Transcription System KIT, and according to the manufacture`s manual. The following procedure is designed to convert up to 5µg of mRNA Mix and briefly centrifuge each component before use: Experimental RNA (3µl/reaction); Primer Oligo (dT)15 (1µl/reaction); Nuclease-Free Water(1µl/reaction) the Final volume was (5µl) For each reaction. The PCR component for syntheses of cDNA: Nuclease-Free Water (to a final volume of 15µl) (7.3µl); GoScript™ 5X Reaction Buffer (4.0µl); MgCl2 (1.2µl); PCR Nucleotide Mix(1µl); Recombinant RNasin® Ribonuclease Inhibitor(5.0µl); GoScript™ Reverse Transcriptase(1µl) and the final volium was (15.0µl) amount for each reaction. 15µl aliquots of the reverse transcription reaction mix to each reaction tube were added to ice. a final reaction volume of 20µl per tube

**Quantitative Real-Time PCR (q RT-PCR) master mix preparation:**

Assembling the Reaction Mix: Prepared the reaction mix by combining the GoTaq® qPCR Master Mix with cDNA, PCR primers and Nuclease-Free Water, the component and their volume used in reaction mixed of qPCR, the target genes primers TNF-α, or b-FGF were used, also the calibrator gene HKG. Each q-RT-PCR mixture reactions were prepared with (10µl) of GoTaq® qPCR Master Mix, (1µl) Forward Primer (20X), (1µl) Reverse Primer (20X), (3µl) Nuclease-Free Water, (5 µl) cDNA, and the final volume was (20µl). PCR program was used with Rotor-Gene cycler: the first step was (1) cycle of GoTaq® Hot Start Polymerase activation at (95°C) for (2 minutes), then denaturation
was used (40) cycles at (95°C) for 15 seconds, finally, Annealing and extension programmed at (60°C) for (1 minute).

**Statistical Analysis:** The data were analyzed using the following software, Microsoft excel, Minitab v17, and IBM SPSS V26. The results reported in this study were expressed as mean + _ SE. Two-way ANOVA were used to test between groups and days after treatments. Mean were compared using the least significant difference test (L.S.D).

### The Results

**Tissue level detection qRT-PCR:**

The dogs in diabetic group were kept in hyperglycemic state. The results showed slight non-significant increase in expression of (b-FGF) in wound tissue by qPCR after 14 days from alloxan-induced diabetes. While at the same days the results showed significant increase in TNF-α level in wound tissue in diabetic dogs.

**Fibroblast growth factor (b-FGF)& (TNF-α) detection:**

Table (3-1): The final result is presented as the fold change of target gene expression a. (b_FGF), b. (TNF-α) in treatment groups by using $2^{\Delta\Delta Ct}$ method(6).

<table>
<thead>
<tr>
<th></th>
<th>0 days</th>
<th>3 days</th>
<th>7 days</th>
<th>14 days</th>
<th>Mean groups</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-Diabetic treatment</strong></td>
<td>1.26±0.011</td>
<td>8.42±0.133</td>
<td>7.20±0.173</td>
<td>2.74±0.427</td>
<td>4.91±0.91</td>
<td>0.001***</td>
</tr>
<tr>
<td><strong>Diabetic treatment</strong></td>
<td>1.06±0.034</td>
<td>5.48±0.369</td>
<td>5.06±0.029</td>
<td>1.06±0.067</td>
<td>3.16±0.642</td>
<td></td>
</tr>
<tr>
<td><strong>Mean days</strong></td>
<td>1.16±0.048</td>
<td>6.95±0.680</td>
<td>6.13±0.485</td>
<td>1.90±0.423</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.001***</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>0 days</th>
<th>3 days</th>
<th>7 days</th>
<th>14 days</th>
<th>Mean groups</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-Diabetic treatment</strong></td>
<td>1.04 ± 0.017</td>
<td>3.02 ± 0.075</td>
<td>1.18 ± 0.104</td>
<td>1.06 ± 0.017</td>
<td>1.57±0.254</td>
<td>0.001***</td>
</tr>
<tr>
<td><strong>Diabetic treatment</strong></td>
<td>1.06±0.040</td>
<td>2.00±0.162</td>
<td>0.80±0.133</td>
<td>0.61±0.098</td>
<td>1.12±0.169</td>
<td></td>
</tr>
<tr>
<td><strong>Mean days</strong></td>
<td>1.05±0.020</td>
<td>2.51±0.242</td>
<td>0.99±0.114</td>
<td>0.84±0.110</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.001***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD Group=0.324 Days=0.458 Group*Days=0.648

Data presented as mean ±SE, Two way a nova were used to test between groups and days, *, **, *** significant (P<0.05), highly significant (P<0.01), very high significant (P< 0.001) respectively.
Discussion

The results showed slight non-significant increase in expression of (b-FGF) in wound tissue by qPCR in hyperglycemic animals, while a significant increase in TNF-α level in wound tissue sample in diabetic dogs. This finding was explained by many researchers, who stated that the uncontrolled diabetes is characterized by hyperglycemia that has been shown to drive oxidative and nitrative stress. Hyperglycemia also stimulates expression of multiple survival factors, including (FGF-2), and vascular endothelial growth factor (VEGF), which is generally protective against endothelial cell death, which was similarly elevated in high glucose conditions (7). The elevation of in the TNF-α level has been measured in diabetic laboratory animals (rats), in study (8) used streptozotocin-induced diabetic rats, with significant increase in TNF-α messenger RNA expression were twice as high in diabetic rats than in no diabetic control rats.

In the present study it has been found that there is a relationship between the results of both (b-FGF and TNF-α). In other study the researchers examined the possible role of basic fibroblast growth factor (b-FGF or PGE2) in regulating the effects of TNF-α, they find that FGF enhanced the amount of prostaglandin (PGE2) produced in response to TNF-α between (3-11) fold, and increases TNF-α receptor expression in fibroblast cells. Glucose and (TNF-α) concentrations are increased in diabetes, these factors correlated both with diabetic endothelial cell apoptosis, vasculopathy and induced cell viability inhibition that led to the proliferative inhibition. The b-FGF associated elevation in TNF-α-induced cell death. b-FGF release by endothelial cell in high glucose goes to cell cycle progression, which makes cells more liable to TNF-α-induced cell death (9).

In the present study the level of b-FGF gene expression appeared high in both treatment sub-groups (NT &DT) when compared to control sub-groups (NC& DC) significantly at (P< 0.001) between the periods of time 0, 3, 7, and 14 days post wounding, also there was significant difference between groups (N&D) at (P< 0.001). In more details, the results showed high up-regulation of b-FGF gene expression (7.16) at 3 days post wounding compared with the zero day; (5.94) at 7 days, then normal regulation (1.48) at 14 days, post wounding in comparison with the zero day, in NT group attributed to NC group. Also in DT group attributed to DC group there were up-regulation of gene expression at (4.42) at 3 days and (4) at 7 days, while normal regulation in gene expression found at 14 days post wounding compared with the zero day. In a study (10) found that the endothelial cells treated with plasma for 30 seconds demonstrated twice as much proliferation as untreated cells, in that way they suggested that low dose non-thermal plasma enhances endothelial cell proliferation due to reactive oxygen species mediated b-FGF release. The same results found in (11) they suggested increase in b-FGF mRNA expression in murine skin wound tissue treated with CAP that was compared to control group. Also, the secretion of angiogenesis-related molecules is affected after the CAP treatment in endothelial cells, fibroblasts and keratinocytes.

This clinical study had proved the effect of AP-He-PJ in boost the gene expression of TNF-α in both treatment sub-groups (NT &DT) at 3 days, when compared to control sub-groups (NC& DC) significantly at (P<0.001), also there was significant difference between groups (N&D) at (P< 0.001). There is significant difference in (TNF-α) gene expression of treatment sub-group NT in comparison to control NC between all periods of time, the same results found in DT in comparison to control DC between the periods of time. In the same way the results in NT sub-group showed up-regulation of TNF-α gene expression (1.98) at 3 days, while there was normal regulation in gene expression at 7 and 14 days post wounding compared with the zero day. However, in DT sub-group, there were up-regulation of gene expression at 3 days (6.42±0.360), while there were slight down-regulation in gene expression at 7 and 14 days post wounding, when compared with the zero day. Many molecular researchers studied the TNF-α, they differ in explaining the role of this cytokine that involved in the process of wound healing. At wound tissues,
TNF-α is quickly released and initiates inflammation, synthesis of TNF-α was detected just after wound was developed, then increased during the first several hours, reached a peak level at day 1, and then reduced to the basal level, and they indicated that TNF-α is involved in the early healing process (12). While other studies reported the harmful effects of TNF-α were significantly amplified in the presence of high glucose. The excessive TNF-α is closely related to diabetic complications by inducing endothelial cell apoptosis under high-glucose condition, which exacerbated inhibitory effects of TNF-α by increasing its expression (9). A study (13) revealed the selective apoptotic effect of CAP on cytotoxic and T-helper cells, B-lymphocytes, and natural killer (NKT) cells may be involved in regulating the healing process, also, in vitro study by (11) demonstrated the beneficial effect of short time (60 s) application of NTP on parameters of molecules and cell function involved in wound healing in osteoblast-like cells. Their results showed the significant increase in the mRNA expression of pro-inflammatory cytokines IL-1β, IL-6, chemotactic factor IL-8, also, TNF-α and COX2 after 1 day compared to untreated cells. These mediators play an important role in wound healing managing the primary inflammation process, and the extra cellular matrix organization, which are both crucial for the entire wound regulation.

Conclusion

In conclusion, these findings not only support the fact that non-thermal plasma has a potent multifunctional agents during tissue regeneration, but also highlight the potential multimodulatory therapy advantages of non-thermal plasma. The enhancement in wound healing process by cold physical plasma jet, by modulation of gene expression of basic-Fibroblast Growth Factor and Tumor Necrosis Factor-alpha in normal and diabetic dogs.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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10. Kalghatgi S, Friedman G, Fridman A, Clyne AM. Endothelial cell proliferation is enhanced by low dose non-thermal plasma through fibroblast growth


Effect of Ammivisnagawater Extract Compared to Proximol (Halfa Bar Extract) in Treatment of induced Renal Lithiasis in a Mature Female Rabbit

Shatha Mahdi Younis1, Abdulrazaq N. Khudair2, Asia Selman. Abdullah3

1MSC Student, 2Professor, Department of Physiology / College of Veterinary Medicine/ University of Basrah, 3Assistant Professor, Department of Pharmacology and Toxicology/ College of Pharmacy / University of Basrah

Abstract

Nowadays people worldwide uses several medicinal plants especially for curing urinary lithiasis. Ammivisnaga is extensively distributed medicinal plant used for management of numerous diseases including urinary lithiasis. Cymbopogon proximus or called Halfa-bar suggested for use in medicine for the ejection of ureteric and renal calculi. Ammivisnaga and Halfa-bar found in Asia and several parts of the world. This study aimed at investigating and comparing both Ammivisnaga and Halfa-bar extracts for their antiurolithic activity using a mature female rabbit. Forty-two rabbits randomly divided into four groups. Group A negative control, group B positive control (chemically induced urolithiasis), group C like group B but treated with Ammivisnaga extract 5 ml orally twice daily for 2 weeks and group D like group B but treated with Proximol (Halfa Bar extract) 5 ml twice daily for 2 weeks also. GC-MS analysis of alcoholic extract of AmmiVisnaga revealed the detection of main alkaloids Khellin, Visnagin, adulicin and enidimine, with other compounds in lower quantity. The results show that group of induced urinary lithiasis for 14 days and 21 days showed clear body weight loss, higher consumption of drinking water and increased morning urine excretion. In addition to significant increase of blood urea, uric acid, serum creatinine and calcium levels. After 14 days of treatment with AmmiVisnaga extract and proximol we noticed reversed body weight loss and overcome low blood urea, serum uric acid, creatinine and calcium. Furthermore, urinary lithiasis groups showed clear crystallization compared to the control urine samples, but after 14 days treatment with AmmiVisnaga and proximol extracts, there was nearly clearance of the urine and disappearance of the crystals from the urine. In conclusion, AmmiVisnaga and Proximol are safe and good diuretic herbs causes improvement in the body weight, high clearance in urine crystals and amelioration in kidney function compared to untreated group.

Key words: Ammivisnaga, Proximol, Cymbopogonproximus, urinary lithiasis.

Introduction

Kidney lithiasis considered as a common disease of high number of people exhibited for many years ago until now. It causes severe pain and several disturbances in the biochemical and hematological characteristics, that urinary lithiasis may be formed due to the consumption of canned food that contain several chemical materials used as food preservative as well as drinking non-pure water for long period. Although treatment of renal stone nowadays is undertaken by new methods, such as using waves to destroy renal calculi, or by endoscopy or even surgical removal of stone. The mentioned methods have disadvantages like the need to professional people to do it, also, the high cost and possibility of relapse and reformation of the stones in the kidney. In addition to the

Corresponding author
Dr Asia Selman Abdullah (PhD), Assistant professor, Department of Pharmacology and Toxicology, College of Pharmacy, University of Basra, Iraq E-mail: <asiaselman2016@gmail.com> Phone: 00967-7719532558 Orcid: 0000-0002-7384-0313

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numerous side effect such as kidney tissue destruction resulting in elevation of blood pressure, hemorrhage and ureter obstruction in addition to multiple inflammations which may lead to severe sepsis. The source of stone formation can be calcium oxalate or uric acid stone$^{1, 2}$.

Therapeutic drugs such as diuretics and antibiotics were not sufficiently effective in treatment of renal stones and associated with different side effects$^{3, 4}$. Nowadays people all over the world uses several medicinal plants especially for curing urinary lithiasis, after analysis medicinal plants are found to contain several materials which can affect urolithiasis by different pathways such as relieving spasm, increasing diuresis or decreasing pain$^{5, 6}$.

Ammivisnaga L. is a short annual or biennial herb indigenous to the Mediterranean region of North Africa, Asia, and Europe$^7$. The plant also distributed throughout North America (North Carolina, Pennsylvania, Oregon, Alabama, California, Florida, and Texas), the Atlantic islands, Argentina, Mexico, and Chile. In Asia, the plant is found in Iraq, Iran, and other western and southern countries. The plant, especially its fruit, has a wide range of applications either in traditional or modern medicine$^8$.

Many common drugs developed from this plant, khella. These drugs includescromolyn, amiodarone andnifedipine. Khellin, which isolated from khella, used for the treatment of asthma and angina pectoris. Furthermore, it is used with the phototherapy for vitiligo, alopecia areata and psoriasis.

Khellincaninhibit citrate metabolism. It was reported that urinary citrate is important to reduce the recurrences of calcium oxalate urinary stones$^7$. The most popular type of urinary stone is calcium oxalate stones, constitute about 80% of total stone types and citrate is a known inhibitor of calcium oxalate crystallization$^9$.

_Cymbopogonproximus_ or called Halfa-bar is an aromatic grass extensively growing in Upper Egypt, the genus _Cymbopogon_, from Gramineae family. It was suggested for use in medicine as diuretic, abdominal or renal antispasmodic, act by relaxation of the fibers of smooth muscle without eliminating propulsivemove of the tissue; therefore, it is used for the ejection of ureteric and renal calculi$^{10}$.

This study aimed at investigating and comparing both Ammivisnaga and Halfa-bar extracts for their antiurolithic activity using a mature female rabbit. Ammivisnaga and Halfa-bar found in Asia and several parts of the world.

### Material and Methods

Current study done at the department of Physiology, Pharmacology and biochemistry of College of veterinary medicine, University of Basrah; in the period from October 2019 to March 2020. Twenty-four healthy adult female rabbits weighing 1-1.5 kg, obtained from local animal market at Basrah city, and housed in the animal house of the college of Veterinary medicine / University of Basrah. The rabbits housed separately in cages under controlled room temperature around 25°C and 12 hours dark-light cycle. The animals have free access to food and water (ad libitum) throughout the study. However, the food and water were restricted 24 hours before urine collection. Cages of the animal were cleaned regularly, and animal kept for seven days for adaptation prior to the start of the experiment.

**Preparation of AmmiVisnaga plant extract:**

Seeds of Ammivisnaga plant bought from the Basrah market. The seeds air-dried in 40°C oven, after that dried seeds were crushed. About 180g of seeds placed in 500 ml of distilled hot water and soaked for 3 days in pure water with occasional shaking at room temperature, the filtration was done using clean muslin cloth and through Whatman qualitative grade1 filter paper. The procedure repeated twice, and all the filtrate combined and called as crude extract. This extract used for administration to animals.

This crude extract of Ammivisnaga screened for the presence of different phytochemical materials by drying to harvest dried powder. After that, the dried powder soaked in 70% ethanol as a solvent and a rotary vacuum evaporator under reduced pressure used to get alcoholic
extract and to eliminate the solvent. This extract screened
for the presence of different phytochemical materials
such as alkaloids, saponin, coumarins, khillin, visnagin
and flavonoid by using GC mass.

**Experimental induction of urolithiasis**

Calcium oxalate urolithiasis was induced in the
kidney of female rabbits in both untreated and treated
groups using 2 ml of (0.75%) ethylene glycol orally
for each animal daily for 21 days and 2 ml of 1 %
ammonium chloride orally for the first 5 days only and 2
ml of disodium oxalate orally for 21 days. Control group
was drenched 2 ml of normal saline for 21 days.

Ethylene glycol administration is a common method
for the induction of experimental urolithiasis, it has been
used alone or in combination with other drugs such as
ammonium chloride for the study of kidney calcium
oxalate crystals deposition. To achieve uniformly high
rate of kidney crystal deposition, ammonium chloride
has been drenched in the first 5 days of the experiment
as it causes acidification of the urine and hence enhances
calcium oxalate crystallization.

**Animal's treatment**

The rabbits were divided randomly into four groups
(6 rabbits each), the first group (Group A) drenched
only 2 ml of normal saline daily orally throughout the
experiment period (21 days) and considered as negative
control group. The second group (group B) was drenched
ethylene glycol (0.75%) twice daily and disodium
oxalate (350 mg in 100 ml distilled water) 2ml daily
orally for 21 days. In the last week, the dose increased
to 3 ml twice daily and considered as a positive control.
The third group (group C) was drenched like group B but treated with Ammivisnaga extract 5 ml orally twice
daily for 2 weeks. The fourth group (group D) drenched
like group B but treated with Proximol (Halfa Bar
extract) 5 ml twice daily for 2 weeks also.

**Sample collection for analysis:**

Urine samples collected at the morning from all
groups and analyzed by urine strips method to know the
changes in biochemical constituents.

Blood samples were collected from all groups by
cardiac puncture to estimate renal function test including
blood urea, serum creatinine, serum uric acid and serum
calcium.

**Statistical Analysis**

Result gained in this experiment were estimated
as a mean ± standard error of the mean (SEM). The
significant variance between groups were measured by
ANOVA using the SPSS followed by post-hoc test.
The comparison between groups was estimated by least
significant difference (LSD) test (P≤ 0.05).

**Findings**

The samples collected and investigated after the
chemical induction of urinary lithiasis and after two
weeks treatment of rabbits with AmmiVisnaga and
proximol extracts.

The group of induced urinary lithiasis for 14 days
and 21 days showed clear body weight loss, higher
consumption of drinking water and increased morning
urine excretion. In addition to significant increase of
blood urea, uric acid, serum creatinine and calcium
levels (table 1).

After 14 days of treatment with AmmiVisnaga
extract and proximol we noticed reversed body weight
loss and overcome low blood urea, serum uric acid,
creatine and calcium as shown in (table 1).

After three weeks of induction, urinary
lithiasis groups showed bad effects in urine examination
significantly different from control group, these effects
includes urinary crystals, increased bilirubin, increased
urine total protein loss, decreased urinary acidity,
increased leucocyte contents, increased nitrite, increased
urobilinogen, increased blood content as well as
significant increase in urine glucose and ketone bodies.
The only parameter, which is not significantly affected,
is the urine specific gravity, as shown in (table 2).
Table 1: Effect of AmmiVisnaga and Proximolextractson kidney function of chemically induced lithogenic female rabbits (mean ± SD).

<table>
<thead>
<tr>
<th>Parameters (mg/dl)</th>
<th>Control</th>
<th>14 days lithogenic treatment</th>
<th>21 days lithogenic treatment</th>
<th>14 days AmmiVisnaga</th>
<th>14 days proximol</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood urea</td>
<td>45.3 ± 1.2 A</td>
<td>49.2 ± 1.3 A</td>
<td>246.0 ± 3.4 A</td>
<td>68.6 ± 2.5 C</td>
<td>127.4 ± 4.2 D</td>
<td>7.5</td>
</tr>
<tr>
<td>S. Creatinine</td>
<td>0.59 ± 0.16 A</td>
<td>0.69 ± 0.18 A</td>
<td>4.55 ± 1.12 C</td>
<td>1.18 ± 0.67 B</td>
<td>2.12 ± 0.71 D</td>
<td>0.15</td>
</tr>
<tr>
<td>S. Uric acid</td>
<td>0.28 ± 0.12 A</td>
<td>0.30 ± 0.13 A</td>
<td>0.60 ± 0.15</td>
<td>0.24 ± 0.10 A</td>
<td>0.38 ± 0.11 A</td>
<td>0.10</td>
</tr>
<tr>
<td>S. Calcium</td>
<td>10.03 ± 1.2 A</td>
<td>11.80 ± 1.4 A</td>
<td>14.18 ± 1.6 B</td>
<td>10.30 ± 1.8 AC</td>
<td>11.91 ± 1.85 A</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Means bearing different capital letters differ significantly at 5 % level (P< 0.05)

Table 2: Effect of AmmiVisnaga and Proximolextractson the urine examination of chemically induced lithogenic mature female rabbits (Mean ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>14 days lithogenic treatment</th>
<th>21 days lithogenic treatment</th>
<th>14 days AmmiVisnaga</th>
<th>14 days proximol</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin mg/dl</td>
<td>0.03 ± 0.01 A</td>
<td>15 ± 2 A</td>
<td>17 ± 2 C</td>
<td>0.1 ± 0.01 D</td>
<td>0.2 ± 0.02 E</td>
<td>2</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>0.0 ± 0.0 A</td>
<td>10 ± 3 B</td>
<td>11 ± 2 B</td>
<td>2.1 ± 0.5 C</td>
<td>1± 0.0 A</td>
<td>3</td>
</tr>
<tr>
<td>Nitrite</td>
<td>0.0 ± 0.0 A</td>
<td>3.4 ± 1.4 A</td>
<td>4.1 ± 1.3 B</td>
<td>0.0 ±0.0 C</td>
<td>0.0 ±0.0 C</td>
<td>3</td>
</tr>
<tr>
<td>Urobilinogen mg/dl</td>
<td>0.8 ± 0.1 A</td>
<td>0.9 ± 0.2 A</td>
<td>16 ± 1.0 C</td>
<td>3 ± 1.1 C</td>
<td>0.5 ± 0.1 C</td>
<td>3</td>
</tr>
<tr>
<td>Protein mg/dl</td>
<td>0.423 ± 0.10 A</td>
<td>1.5 ± 0.5 A</td>
<td>3 ± 1.2 C</td>
<td>0.0 ± 0.0 A</td>
<td>0.2± 0.02 D</td>
<td>1</td>
</tr>
<tr>
<td>pH</td>
<td>5.01 ± 1.1 A</td>
<td>5.5 ± 1.2 A</td>
<td>9.5 ± 1.4 B</td>
<td>5 ± 1.6 A</td>
<td>6 ± 1.5 A</td>
<td>2</td>
</tr>
<tr>
<td>Blood mg/dl</td>
<td>0.01 ± 0.0 A</td>
<td>80 ± 5 B</td>
<td>200 ± 6 C</td>
<td>0.0 ± 0.0 A</td>
<td>0.0 ±0.0 A</td>
<td>10</td>
</tr>
<tr>
<td>Glucose mg/dl</td>
<td>0.3 ± 0.01 A</td>
<td>5 ± 0.2 B</td>
<td>7± 0.4 C</td>
<td>0.2 ± 0.02 A</td>
<td>0.2 ± 0.02 A</td>
<td>4</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.01 ± 0.01 A</td>
<td>1.03 ± 0.02 A</td>
<td>1.1 ± 0.02 A</td>
<td>1 ± 0.03 A</td>
<td>1 ± 0.03 A</td>
<td>0.01</td>
</tr>
<tr>
<td>Ketone mg/dl</td>
<td>0.0 ±0.0 A</td>
<td>0.5 ± 0.05 B</td>
<td>0.5 ± 0.05 B</td>
<td>1.0 ± 0.0 A</td>
<td>0.0 ± 0.0 A</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Means bearing different capital letters differ significantly at 5 % level (P<0.05)
GC-MS analysis of AmmiVisnaga extract:

GC-MS analysis of alcoholic extract of AmmiVisnaga revealed the detection of the main four components Khellin, Visnagin, adulicin and enidimine, appeared as high peaks in the figure, while the lower peaks represented other less contents of other compounds, as illustrated in figure 1.

![Figure 1: Histogram of AmmiVisnaga alcoholic extract showing the main chemical compounds.](image)

Urine Crystallization:

After 14 days and 21 days from induction, urinary lithiasis groups showed clear crystallization compared to the control urine samples, but after 14 days treatment with AmmiVisnaga and proximol extracts, there was nearly clearance of the urine and disappearance of the crystals from the urine as shown in figures 7, 8, 9, 10.

![Figure 2: Image of urine samples collected in the morning from rabbits (A) control group (B) lithogenic group after 14 days of induction showing clear crystals and (C) lithogenic group after 21 days of induction showing severe crystallization. Investigated using the high power field (HPF), under light microscope at 400 X magnification.](image)
Figure 3: Image of urine samples collected in the morning from rabbits after treatment by AmmiVisnaga extract (A) for 14 days and (B) for 21 days showing disappearance of crystallization Investigated using the high power field (HPF), under light microscope at 400 X magnification.

Figure 3: Image of urine samples collected in the morning from rabbits after treatment by proximol extract (A) for 14 days and (B) for 21 days showing partial disappearance of crystallization Investigated using the high power field (HPF), under light microscope at 400 X magnification.
Discussion

Results of this study showed that urine crystals formation induced by drenching ethylene glycol and ammonium chloride caused damage to the renal tissue, as in previous studies\textsuperscript{11, 12}. This damage might induced by the reactive oxygen species (ROS) which is regarded as the dangerous material produced by the crystals deposited in the renal tissue forming renal calculi\textsuperscript{9}.

In this study AmmiVisnaga extract induced significant rise in urine production (diuresis effect) compared to the control group, this may be due to the diuretic materials found in AmmiVisnaga extract which may possess several treatmental actions present in several phytochemicals. So diuretic effect of AmmiVisnaga increases urine amount is voided leading to decrease urine saturation of salts crystals and help in discharging out of the previously formed crystals\textsuperscript{16,17}. Previous studies on AmmiVisnaga revealed removing of DPPH free radicals and caused inhibition of fat peroxidation assisting its antioxidant properties\textsuperscript{13-15}.

In this experiment high calcium oxalate crystals were induced in the kidney by ethylene glycol (0.75% in the drenching water) for 21 days and ammonium chloride (1%) was given only for the first 5 days, as drenching ammonium chloride for more than 5 days will causes severe weight loss and may result in rabbits death\textsuperscript{18}. Drenching of ethylene glycol and ammonium chloride causes more crystallization in the urine as well as forms larger crystals because of hyperoxaluria. Higher water consumption and more urine excretion may happened because of kidney damage\textsuperscript{19}. Furthermore, there was significant rise in the serum creatinine, blood urea and total protein loss in the lithogenic group when compared to the control group, these overcome by AmmiVisnaga and Proximol extracts.

These results in cope with other past results recorded that crystallization of salts in the urine caused by increase in oxalate consumption and less calcium excretion in the group of induced urolithiasis was overcome by drenching AmmiVisnaga extract\textsuperscript{20, 21}.

AmmiVisnaga accelerate the recovery compared to untreated group which was obviously seen by improvement in the body weight, high clearance in urine crystals and amelioration in kidney function compared to untreated group.

This study provided us with a good knowledge on the treatment bases and its action in the urinary calculi or stones. Furthermore, the plant can be considered as safe. Previous study found no toxic effect of AmmiVisnaga extract on rats and the plant has a good history of medical uses consumed by human and regarded safe and nontoxic\textsuperscript{22}.

Result of Table 1 showed a significant effects of lithogenic treatment on blood urea, serum creatinine, uric acid and serum calcium and their overcoming and ameliorative effects by both AmmiVisnaga and proximol. Those result are consistent with previous studies on sheep\textsuperscript{23} and on rats\textsuperscript{24}.

The significant increase in serum calcium level in this study due to lithogenic treatment may be due to calcium reuptake under the effect of aldosterone\textsuperscript{25}. This explanation supported by reduction of serum calcium level by AmmiVisnaga and proximol significantly nearly to the control. Therefore, the dose of AmmiVisnaga extract and Proximol need to be adjusted or refined in the case of combined treatment.

The results of this study indicates that there is a bad effect on the Kidney function caused by the lithogenic effect indicated by significant increase in the blood urea, serum creatinine and uric acid as well as serum calcium, these effects are ameliorated by AmmiVisnaga extract and Proximol. These results are consistent with the results of previous study\textsuperscript{25}, and with other study that explained high salt consumption resulted in the formation of kidney damage, which may lead to Kidney failure\textsuperscript{26}.

When rabbits with renal impairments treated with AmmiVisnaga or proximol extracts, the high serum level of uric acid, creatinine and blood urea were regressed to the non-significant levels in comparison with the control.
Other parameter such as ketone bodies, PH, protein, Nitrite and bilirubin were increased due to lithogenic formation. Treatment with AmmiVisnaga or Proximol extracts ameliorate these parameters and restore their concentration to nearly normal levels due to their diuretic, antioxidant, anti-inflammatory and antispasmodic effects.

Conclusion

In conclusion, AmmiVisnaga and Proximol are safe and good diuretic herbs causes improvement in the body weight, high clearance in urine crystals and amelioration in kidney function compared to untreated group.

Acknowledgments: This study is a part of M.Sc. thesis submitted to the Department of Physiology, College of Veterinary Medicine, and University of Basrah. The authors acknowledge the college for encouragement and support.

Conflicts of Interest: The authors state that they have no conflicts of interest.

Ethical Statement: All tests performed in agreement with the National Institute of Health Guidelines for the Treatment and Use of Laboratory Animals (86/609/EEC) and permitted by Basrah University, College of Veterinary Medicine Ethical Committee.

References


Studying the Influence of Maternal Factors on Iraqi Pediatrics Patients Presented with Neonatal Hyperbilirubinemia

Shaymaa Hasan Abbas1, Lubab Tarek Nafea1, Rasha Saadi Abbas1

1Assistant Lecturer, Department of Clinical Pharmacy, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq

Abstract

Background: Hyperbilirubinemia regarded as common problem in neonates. Risk factors for elevated indirect bilirubin include maternal age, race, prematurity, and breast-feeding.

Material and Methods: This study include mothers of term newborn infants with neonatal hyperbilirubinemia requiring phototherapy and/or exchange transfusion that are combatable or have maternal-fetal ABO incompatibility to evaluate maternal contributing factors which related to development of hyperbilirubinemia in newborns.

This study found that higher serum bilirubin level (31.32 ± 2.30mg/dl) found in 28% neonate for mother were ≥30 years old. There was no significant difference found in serum bilirubin level according to parity (primi or multi). There was association between the neonatal serum bilirubin level with maternal ABO blood groups which showed highest mean of neonatal serum bilirubin reported in neonate for mothers carry blood group A and B (17.92 ± 10.32), (17.28±6.85) respectively. While a result of the association of neonatal serum bilirubin level to maternal Rh reuses factor found that there was no significant differences among them (P>0.05).

Conclusions: hyperbilirubinemia was more frequent in neonate for mothers≥30 years old. Presence of highest mean of neonatal serum bilirubin reported in neonate for mothers carry blood group A or Band Rh - women.

Keywords: Hyperbilirubinemia, Maternal factors, Neonate’s blood group.

Introduction

Hyperbilirubinemia regarded as common and non-threatening problem in neonates. Jaundice is observed during the 1st week of life in approximately 60% of term infants and 80% of preterm infants(1,2). The raises of indirect, unconjugated bilirubin are potentially neurotoxic. Thus health care providers monitor bilirubin levels and provide treatment for the newborns jaundice to prevent the growth of kernicterus (also called bilirubin encephalopathy)(3). The general presentation may be asymptomatic in physiological jaundice or ill neonate (vomiting, lethargy, poor feeding, behavioral changes, tachypnea, pale stools and dark urine)(4). Risk factors for elevated indirect bilirubin include maternal age, race (Chinese, Japanese, and Native American), maternal diabetes, prematurity, drugs (vitamin K3), altitude, polycythemia, male sex, trisomy 21, breast-feeding, and a family history(1).

In relating with “maternal-fetal ABO blood group incompatibility”, in which the mother has blood group O and the newborn has blood group A or B, in which the hemolytic disease grows in approximately 10% of such newborns and may be associated with clinically significant neonatal hyperbilirubinemia(5). The hemolytic disease of the newborn due to ABO incompatibility frequently noticed during the first pregnancy, and about 50% of infants are affected unlike rhesus hemolytic disease of the newborn which noticed in subsequent babies rather than the first baby(6). Recent study showed that there is
no need of exchange transfusion for ABO incompatible neonates and incompatible neonates needed the duration of phototherapy for ≥24 hours (7).

Material and Methods

This study is a cross-sectional study and descriptive analytic study has been done from March 2020 to December 2020 include mothers of term newborn infants (gestational age >36 weeks) with neonatal hyperbilirubinemia (serum bilirubin level more than 5 mg/dl) requiring phototherapy and/or exchange transfusion who are combatable or have maternal-fetal ABO incompatibility to evaluate causes, maternal contributing factors which related to development of hyperbilirubinemia in newborns with jaundice. Jaundice was already diagnosed clinically and laboratory inside the hospital. This study has been accomplished over a half-year, on 75 newborns and their mothers who admitted to [Neonatal and Immaturity Department] in child central teaching hospital CCTH hospital and from consultation private clinic in Baghdad, Iraq. The ethic committee of approval was done in this study. A special questionnaire was used to collect and record information from all newborns and their mothers.

The maternal data like socio-demographic and clinical parameters, and neonatal plasma bilirubin level on admission were all recorded. Finally, several laboratory tests made such as (indirect and direct bilirubin, blood type, Rh factor).

Patients Criteria:

-Inclusion Criteria:-

1- Neonates babies (age less than 1 month) with hyperbilirubinemia that require phototherapy or exchange transfusion.

2- Mothers of neonates who involved in the study.

-Exclusion Criteria:-

1- Neonate babies with any other severe diseases or with other known causes of jaundice and hemolysis.

2- Mothers with hemolytic disease or with other serious diseases.

Statistical Analysis

Statistical analysis was carried out using the Minitab 16.1 (2010). Data expressed as numbers (%) and (mean±SD). Chi-square (X^2) was to detect significant value differences among study variables.

Results

Table (1) showed the maternal socio-demographic characteristics of 75 women were delivered children with hyperbilirubinemia. Regarding maternal age the study found that 54(72%) of women were (<30 years) and 21 (28%) were (>30 years) with highly significant differences between them (P<0.01). On the other hand 13(17.3%) of women were primi parity and 62(82.7%) were multi parity and highly significant differences between them (P<0.01). Considering the smoking history, most of women had no history for smoking 63(84%) and only 12(16%) had a positive history for smoking with highly significant differences between them (P<0.01).

Of the study population, higher percentage 40(53.3%) was found in women with blood group O, while 15(20%), 11(14.7%), 9(12%) was women with blood group A, AB, B respectively and highly significant differences found between them (P<0.01).

Interestingly, most of women included in this study 48(64%) were delivered by cesarean section (C/S) while 27(36%) were delivered by vaginal delivery and significant differences found between the two types of delivery (P<0.05).
Table (1): Maternal socio-demographic characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age(years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (¶)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 54 72</td>
<td></td>
<td>0.001**</td>
</tr>
<tr>
<td>&gt;30 21 28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td>P-value</td>
</tr>
<tr>
<td>N (¶)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primi 13 17.3</td>
<td></td>
<td>0.001**</td>
</tr>
<tr>
<td>Multi 62 82.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking Hx</td>
<td></td>
<td>P-value</td>
</tr>
<tr>
<td>N (¶)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes 12 16</td>
<td></td>
<td>0.001**</td>
</tr>
<tr>
<td>No 63 84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood group (mother)</td>
<td></td>
<td>P-value</td>
</tr>
<tr>
<td>N (¶)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 15 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B 9 12</td>
<td></td>
<td>0.001**</td>
</tr>
<tr>
<td>AB 11 14.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O 40 53.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery type</td>
<td></td>
<td>P-value</td>
</tr>
<tr>
<td>N (¶)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal 27 36</td>
<td></td>
<td>0.015*</td>
</tr>
<tr>
<td>C/S 48 64</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data presented as number of patients (n) and percentage (%).

NS: No significant differences (P>0.05), (*) Significant difference (P<0.05), (**) Highly Significant difference (P<0.01).

C/S: Caesarian Section

Table (2) found that 54 (72%) women who were below 30 years old the mean serum bilirubin was (24.34 ± 3.08 mg/dl) while (31.32 ± 2.30mg/dl) serum bilirubin level found in 21(28%) of woman who were equal or higher than 30 years old and highly significant level between them (P<0.01).
Table (2): Comparison of serum bilirubin for neonate according to maternal age in the study group

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Serum bilirubin level (mg/dl)</th>
<th>N</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30 years</td>
<td>24.34 ± 3.08</td>
<td>54</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>≥ 30 years</td>
<td>31.32 ± 2.30</td>
<td>21</td>
<td>28</td>
<td>0.001**</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD, number of patients (n) and percentage (%).

NS: No significant differences (P>0.05), (*) Significant difference (P<0.05), (**) Highly Significant difference (P<0.01).

Table (3) found that in women with primi parity the mean serum indirect bilirubin level for neonate was (14.73 ± 6.75 mg/dl) while in women with multi parity the mean was (13.68 ± 4.56 mg/dl) but no significant differences found between them (P>0.05).

Table (3): Comparison of serum bilirubin according to parity in the study group.

<table>
<thead>
<tr>
<th>Parity</th>
<th>Serum Indirect bilirubin level (mg/dl)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primi</td>
<td>14.73 ± 6.75</td>
<td>0.495NS</td>
</tr>
<tr>
<td>Multi</td>
<td>13.68 ± 4.56</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as number of patients (n) and percentage (%).

NS: No significant differences (P>0.05), (*) Significant difference (P<0.05), (**) Highly Significant difference (P<0.01).

Table (4) reported highest mean of neonatal serum bilirubin in neonate for mothers carry blood group A and B (17.92 ± 10.32), (17.28 ± 6.85) respectively, while lowest mean of the serum bilirubin reported in neonate for mothers carry blood AB (10.24 ± 4.05) with highly significant differences between them (P<0.01).

Table (4): Association of the neonatal serum bilirubin level with maternal ABO blood groups

<table>
<thead>
<tr>
<th>Maternal blood groups</th>
<th>Serum Indirect bilirubin level (mg/dl)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17.92 ± 10.32 a</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>17.28 ± 6.85 ab</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>10.24 ± 4.05 c</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>13.86 ± 3.75 bc</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.008**</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as mean±SD, ©One way ANOVA were used to test Maternal blood groups,

** Highly significant (P<0.01), Means that do not share a letter are significantly different according to Fisher test.

On the other hand, the serum bilirubin level in Rh + women was (14.28 ± 5.04) while for Rh – women was (14.64 ± 6.85) with no significant differences among them (P>0.05), table (5).
Table (5): Association of neonatal serum bilirubin level with maternal Rh factor

<table>
<thead>
<tr>
<th>Rh factor</th>
<th>Serum Indirect bilirubin level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh+</td>
<td>14.28 ± 5.04</td>
</tr>
<tr>
<td>Rh-</td>
<td>14.64 ± 6.85</td>
</tr>
<tr>
<td>P-value</td>
<td>0.804 N.S</td>
</tr>
</tbody>
</table>

Discussion

This study was assumed to find out the contributions of each of the associated risk factors. One of the most serious clinical problems inside the hospitals with the mysterious etiology was hyperbilirubinemia between neonates; including not only a danger to infants but also taking much valuable time of laboratory and medical supervise\(^8\).

Table 1 reveals the maternal socio-demographic variables. The results showed that most of mother’s age was ≤ 30 years old while the remaining mothers was ≥ 30 with highly significant differences between them; these results compatible with Sumangala Devi D et al\(^9\).

Instead (17.3\%) of women were primi parity and (82.7\%) were multi parity and highly significant differences between them (\(P<0.01\)), the results of the study compatible with Oyapero O et al\(^9\) which showed that most women were multiparity or parity of the mothers included in this study was more than P1\(^10\). While this study is in contrast with Taneja S et al\(^11\) study which showed that most mother’s presented with primi parity rather than multi parity. Also Oyapero O et al\(^9\) study which showed that majority of the mothers of the study individuals had a 1–2 children\(^10\).

Allowing for the smoking history of mothers, data suggested significant results, the results did not reach for review of the mothers’ smoking history and did not permit us to count the accurate number of cigarettes smoking mothers. In a preliminary analysis of the data has found negative association between neonatal jaundice and other factors such as maternal smoking\(^12\).

Regarding ABO compatibilities higher percentage of mothers 53.3\% were with blood group O, while 20\%, 14.7\% and 12\% was in mothers with blood group A, AB, B respectively and highly significant differences found between them (\(P<0.01\)). These results consistent with Akanmu AS et al\(^13\) which found that blood group O of females were 54.3\% of the donor population, equally if it can be assumed that sex factor has no influence on ABO gene inheritance and blood group distribution\(^13\).

Considering mode of delivery for mothers in the study, most of mothers (64\%) were delivered by caesarian section (C/S) while 27(36\%) were delivered by vaginal delivery and significant differences found between the two types of delivery (\(P<0.05\)). Concentrating study on the use of oxytocin on mother’s babies showed that the induced labor was associated with more neonatal jaundice\(^14\). In contrast with other study that shows the delivery outcomes for cases and controls, mode of delivery did not show any statistically significant difference between vaginal delivery and caesarean delivery. About 74\% of babies delivered vaginally and 25\% caesarean delivered babies developed neonatal jaundice\(^9\).

This results of the study institute that 72\% of mothers were below 30 years old with the mean serum bilirubin was \((24.34 ± 3.08 \text{ mg/dl})\) while \((31.32 ± 2.30 \text{ mg/dl})\) serum bilirubin level found in 28\% of mothers who were equal or higher than 30 years old and highly significant level between them (\(P<0.01\)). These results well matched with Norman M et al\(^1\) and Olusanya BO et al\(^1\) which showed that mothers age was found to be statistically significant in statistical analysis and it also suggested that a higher serum bilirubin levels in
neonates who born for older mothers (more than ≥30 years old) can be linked to increased risk for neonates hyperbilirubinemia\(^{(15)}\).

The present study showed that women with primi parity the mean serum indirect bilirubin level for neonate was \((14.73 \pm 6.75 \text{ mg/dl})\) while in women with multi parity the mean was \((13.68 \pm 4.56 \text{ mg/dl})\) but no significant differences found between them \((P>0.05)\). Moreover the present study finding suggested that birth in the first pregnancy could be important risk factors in current study population , it could agree with Agarwal V etal, Ekwochi U etal, study which suggested that obstetric factors for mothers associated with an increased risk for developing hyperbilirubinemia in neonates involved primi parity\(^{(16,17)}\).

Although a number of parameters, including maternal age and parity have been studied to predict the presence of a newborn with ABO incompatibility and the development of a subsequent hyperbilirubinemia, it stills difficult to expect the real causes behind that\(^{(18)}\). We therefore aimed in this study to determine prospectively the relation between maternal factors and presence of hyperbilirubeinemia in healthy term newborns.

The previous studiesshowed the association between the neonatal serum bilirubin level and maternal ABO blood groupsand OA was more commonly associated than OB blood group\(^{(19,20)}\).

Moreover the present results showed highest mean bilirubin in neonate whom their mothers carry blood group A and B \((17.92 \pm 10.32)\), \((17.28 \pm 6.85)\) respectively, while lowest mean of the serum bilirubin in neonate for mothers carry blood AB \((10.24 \pm 4.05)\) with highly significant differences between them \((P<0.01)\). These results compatible with Sarici SU etal which found that ABO incompatibility played a major role in development of significant hyperbilirubinemia similar to previous studies. 70/150 (46.66%) babies had ABO incompatibility as compared to 6 babies (4%) who had Rh incompatibility and highly significant differences between them\(^{(21)}\).

Then again, a result of the correlation between neonatal serum bilirubin level and maternal Rh reuses factor found to be with no statistical differences among them \((P>0.05)\)\(^{(22-24)}\). These results compatible with Harry C etal which found that ABO blood group incompatibility, was seen in 70 babies as opposed to 6 babies with Rh incompatibility. ABO \((17.98)\) incompatibility resulted in higher serum bilirubin levels with the difference being statistically significant \((P=0.026)\)\(^{(25)}\).

**Conclusion**

Multiple risk factors were associated with development of significant hyperbilirubinemia. The higher percentage of mothers were with blood group O consequently the ABO blood group incompatibility between neonates and their mothers have higher chances of developing significant hyperbilirubinemia in neonates. Moreover the result of the occurrence of neonatal hyperbilirubinemia and maternal Rh reuses factor found to be with no statistical differences among them. Thus, it was concluded that an early follow up is still one of important steps to recognize the development of significant jaundice early, and if the above mentioned risk factors was diagnosed a more careful follow up and management of those babies can be prepared to prevent development of significant hyperbilirubinemia.

**Conflicts of Interest:** Nil

**Source of Funding:** self

**Ethical Clearance:** The patients signing a written consent before doing the questionnaire

**Acknowledgment:** The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq), Baghdad - Iraq for its support in the present work.

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2. Patra LB. Prediction of neonatal hyperbilirubinemia


The Antibacterial and Antioxidant Activity of *Moringa Oleifera* Seed Oil Extract Against Some Foodborne Pathogens

Shaymaa Rajab Farhan¹, Ahmed H. AL-Azawi², Warqaa Y. Salih³, Alaa A. Abdulhassan⁴

¹Researcher, Ministry of Science and Technology, Iraq, ²Assistant Professor, ³Assistant Lecture, ⁴Engineer, Biotechnology Dept., Genetic Engineering and Biotechnology Institute for Post Graduate Studies, University of Baghdad, Baghdad, Iraq

Abstract

*Moringa oleifera* is one of the most important and useful plants because it is both a medical and nutritional plant. The aim of this study is to determine the antioxidant and antibacterial activity of *Moringa oleifera* seed oil extract. Petroleum ether solvent in Soxhlet apparatus was used to prepare the seed oil. Many tests were conducted included, Gas Chromatography-Mass Spectrum (GC-MS), evaluation of antioxidant activity utilizing 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay, total phenolic content. The oil extract of *Moringa oleifera* was investigated against some foodborne pathogens include *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumonia*. The results of GC-MS revealed to the identification of twenty two components, the prevalent compounds were Oleic acid (14.61 %), Butyric acid (11.55 %), 8-Octadecenoic acid, methyl ester (11.16 %) and 1-Pentanol (10.75 %). The radical scavenging activity of the seed oil was 75.81% in concentration 100 µl/ml, and the (EC₅₀) was 25 µl/ml. Furthermore, the total phenolic content of the seed oil extract increased gradually with the increases of concentration. The results of the antibacterial activity of *Moringa oleifera* oil extract showed that the oil extract have the antibacterial effect against all tested bacteria in all concentrations except *E. coli* and *K. pneumonia* which were resistant to the seed’s oil extract in concentration 125 µl/ml. The highest antibacterial activity was observed on *Staph. aureus*, *B. cereus*, *E. coli* and *K. pneumonia* with inhibition zone 18.50, 14.16, 11.83 and 11.33 mm in concentration 500 µl/ml respectively. Furthermore, the MIC of the oil extract was 64 µl/ml for *Staph. aureus*, 128 µl/ml for *Bacillus cereus* and 256 µl/ml for each *E. coli* and *K. pneumonia*.

Keywords: Antibacterial activity, DPPH, MIC, Moringa oleifera, Seed oil extract, Total phenol.

Introduction

Natural antioxidants have an important role in the inhibition of many diseases and improving health. It was also reported that phenolic compounds rich food may have a protective effect against certain diseases such as heart disease, inflammatory diseases, cancer and diabetes¹, ², ³. *Moringa oleifera* is a wonderful tree in the plant kingdom. It does not only possess rich nutritive values (e.g vitamin, mineral, protein, energy, carbohydrate and electrolyte) but contains a lot of medicinal value with important chemical constituents also ⁴. A number of medicinal properties have been ascribed to various parts of this tree. Most parts of this plant: root, bark, gum, leaf, fruit (pods) flowers, seed and seed oil have been used in folk medicine in Africa and South Asia ⁵. The effectiveness of *M. oleifera* as an antioxidant became evident after the identification of some natural antioxidants which include vitamin C, flavonoids, tocopherols and other phenolic compounds. It was reported that the Moringa plant provides a rich and rare combination of zeatin, quercetin, kaempferol and many other phytochemicals ⁶.
Moringa oleifera seed oil is pleasant tasting, highly edible, and resembles olive oil in its fatty acid composition. The characteristics of M. oleifera seed oil can be highly desirable especially with the current trend of replacing polyunsaturated vegetable oils with those containing high amounts of monounsaturated acids. Furthermore, M. oleifera has been found to be a potential new source of oil especially with the advent of the need for oleo-chemicals and oils/fats derived fuels (Biodiesel) all over the world. Thus, the objectives of the present study include detection of the active ingredients in Moringa oleifera seed oil cultivated in Iraq and assessment of the antibacterial and antioxidant activity of oil on some foodborne pathogens.

Materials and Methods

Chemical reagents

The chemical reagents DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin Ciocalteu reagent were purchased from Sigma Aldrich chemicals (Sigma-Aldrich, Germany), Resazurin dye, Nutrient agar (Himedia, India), Muller-Hinton agar and broth (Oxoid, England).

Collection of Moringa oleifera

Moringa oleifera seed was obtained from the plantation of Al-Diwaniyah city, Iraq. The seed was identified by the specialist, Department of Biology, College of Science, University of Baghdad. The seed was washed with water and dried at room temperature, and shelled by using mortar and pestle. The husk and kernel were ground to a fine powder and stored at 4°C for further analysis.

Preparation of seeds oil extract

Soxhlet apparatus was used to extract oil from seeds, 100 g of Moringa oleifera whole crushed seeds (husk and kernel) were put in a thimble and 700 ml of petroleum ether was added within 40-60°C for 6 hours. The solution was filtered through a filter paper Whitman No.1 and evaporated under vacuum at 40°C by a rotary evaporator to get rid of petroleum ether; the oil was stored in glass vials at 4°C until analyzed.

Gas chromatography mass spectrophotometer analysis (GC-MS)

Analysis of the moringa oleifera seed oil extract was carried out on GC-MS equipment. The experimental conditions of the equipment are: HP-5MS ultra inert capillary non-polar column, dimensions: 30 mm × 0.25 mm; ID: 0.25 mm, film thickness: 0.25 μm. The flow rate of mobile gas: 1.0 ml/min. The oven temperature for the gas chromatographic part was 50°C raised to 300°C at 7°C/min for 10 min. The nature and structure of compounds were identified by the mass spectrometer. The spectrum of unidentified components was compared with the spectrum of identified components stored in the national institute standard and technology (NIST) library.

Evaluation of the Antioxidant activity (DPPH assay)

The radical scavenging activity of samples was determined according to Kedare and Singh. 5ml of a freshly prepared 0.004% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was mixed with 100 µl of different concentrations (20, 40, 60, 80 and 100 µl/ml) of the seeds oil extract, then the mixture was left to stand for 30 min. The absorbance was measured at 517 nm. All tests were performed in triplicate. The percentage of DPPH reduction was calculated as:

\[
\text{% Reduction} = \left( \frac{\text{Abs DPPH} - \text{Abs Dil.}}{\text{Abs DPPH}} \right) \times 100
\]

Where:

\[
\text{Abs DPPH} = \text{average absorption of the DPPH solution}
\]

\[
\text{Abs Dil.} = \text{average absorption of the three absorption values of each dilution.}
\]

With the obtained values, a graphic was made using Microsoft Excel. The EC₅₀ of each extract (concentration of extract or compound at which reduced 50% of DPPH) was taken from the graphic.
Determination of total phenolic contents

Total phenolic content of *Moringa oleifera* seed oil extract was determined spectrophotometrically using the Folin-Ciocalteu method described by Jayaprakasha

Two ml of Folin-Ciocalteu reagent (diluted 10 times) was mixed with 1.6 ml of 7.5% sodium carbonate solution and 0.4 ml of *Moringa oleifera* extracts. The volume was completed to 5 ml by adding distilled water. The tubes were covered with parafilm for 30 min. at room temperature, and then the absorbance was read at 760 nm spectrophotometrically.

Bacterial isolates

*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* isolated from food, obtained from the Department of Food Sciences, College of Agricultural Engineering Sciences, University of Baghdad, and emphasize diagnoses by using VITEK-2 System.

Antibiotic susceptibility test

Kirby-Bauer method was followed as described by WHO, to carry out the antibiotics susceptibility test for 10 different antibiotics. The bacterial suspension was prepared by picking 1-2 isolated colonies of bacteria from the original culture and introduced into a test tube containing 4 ml of normal saline to produce a bacterial suspension of moderate turbidity compared with the standard turbidity solution. This approximately equals to 1.5x10^8 CFU/ml. By a sterile cotton swab, a portion of bacterial suspension was transferred and carefully and evenly spread on Mueller- Hinton agar medium, and then it was left for 10 min. Thereafter the antimicrobial discs were placed on the agar with a sterile forceps pressed firmly to ensure contact with the agar. Later the plates were inverted and incubated at 37°C for 18-24 hours. Inhibition zones developed around the discs were measured by a millimeter (mm) using a metric ruler according to Clinical Laboratories Standards Institute.

Agar well diffusion method

Agar well diffusion method was employed for the determination of this study. Muller- Hinton agar plates were swabbed (sterile cotton swabs) with broth culture of respective bacteria. Wells 6 mm diameter was made in each of these plates using a sterile cork borer. 100 µl from each concentration (125, 250 and 500 µl/ ml) of the seed oil extract was put in each hole by using micropipette and allowed to diffuse at room temperature for 30 min. The plates were incubated at 37 °C for 18-24 hours. All tests were performed in triplicate. The diameter of any resulting zone of inhibition was measured in millimeters.

Determination of the minimum inhibitory concentration (MIC) of the *Moringa oleifera* oil extract

Broth microdilution method was used to determine the MIC of *Moringa oleifera* oil extract using the 96-well microtiter plate. The extracts were prepared in a double concentration, the desired final concentration as it will be diluted with an equal amount of bacteria in broth. 200 µl of the prepared oil extract was introduced into the first wells in columns 1-4 (in row A). Rows B-H in columns 1-4 had 100µl of broth alone. Twofold serial dilutions using micropipette was done systematically down the columns 1-4 (from rows B-H). 100 µl was removed from the starting concentrations (columns 1-4 in row A) and transferred to the next row with the 100µl broth, properly mixed, and the procedure was repeated up to the last row (H) where the last 100µl was discarded. This brings the final volume in all the test wells with the extracts to 100 µl except the 5th, 7th, 9th and 11th columns which had 200 µl of the broth that served as sterility negative control (Broth only). The columns 6th, 8th, 10th and 12th served as positive control (Bacteria + Broth). 100µl of the 1x10^6 CFU/ ml bacterial inoculum was transferred into all the wells except sterility negative control columns to give us the desired final inoculum load of 5x 10^5 CFU/ml. Microtiter plates were incubated at 37°C for 18-20 hrs. After incubation, 20 µl of resazurin dye was added to all the wells and incubated for some minutes to observe any color changes. The minimum inhibitory concentrations were determined visually in broth microdilution as the lowest concentrations of the extracts at which color

...
changed from blue to pink in the resazurin broth assay.

**Statistical Analysis**

The Statistical Analysis System was used to detect the effect of different factors in study parameters. Least significant difference-LSD test was used to significant compare between means in this study.

**Results and Discussion**

Gas chromatography mass spectrophotometer analysis

The GC-MS analyses of the *Moringa oleifera* seed oil extract lead to the identification of twenty two components. The prevalent compounds were Oleic acid (14.61 %), Butyric acid (11.55 %), 8-Octadecenoic acid, methyl ester (11.16 %) and 1-Pentanol (10.75 %); all compounds were identified and listed in Table 1. Efeovbokhan in his investigation to study the effect of different solvents on composition of *Moringa oleifera* seed’s oil referred to the presence of different percentage of palmitolic acid in the seed oil extracted by different solvents. Furthermore, it was reported that oleic acid present in crude extract of *Moringa oleifera* seed oil.

<table>
<thead>
<tr>
<th>No.</th>
<th>Possible compound name</th>
<th>Ret. time (min)</th>
<th>Peak area (%)</th>
<th>Molecular formula</th>
<th>Molecular weight (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hexanoic acid</td>
<td>5.497</td>
<td>6.5</td>
<td>C₇H₁₂O₂</td>
<td>116.160</td>
</tr>
<tr>
<td>2</td>
<td>Pentanoic acid</td>
<td>6.844</td>
<td>2.54</td>
<td>C₆H₁₀O₂</td>
<td>102.133</td>
</tr>
<tr>
<td>3</td>
<td>Butyric acid</td>
<td>7.235</td>
<td>11.55</td>
<td>C₄H₉COOH</td>
<td>88.106</td>
</tr>
<tr>
<td>4</td>
<td>Heptanoic acid</td>
<td>7.728</td>
<td>10.75</td>
<td>C₅H₁₄O₂</td>
<td>106.176</td>
</tr>
<tr>
<td>5</td>
<td>Decane</td>
<td>8.565</td>
<td>8.57</td>
<td>C₁₃H₂₆</td>
<td>142.286</td>
</tr>
<tr>
<td>6</td>
<td>Ether, 6-methylheptyl vinyl</td>
<td>9.473</td>
<td>2.59</td>
<td>C₁₀H₂₂O</td>
<td>156.26</td>
</tr>
<tr>
<td>7</td>
<td>Malonic acid</td>
<td>10.257</td>
<td>1.15</td>
<td>C₄H₆O₂</td>
<td>104.061</td>
</tr>
<tr>
<td>8</td>
<td>Hexyl octyl ether</td>
<td>10.506</td>
<td>0.27</td>
<td>C₁₄H₃₀O</td>
<td>214.39</td>
</tr>
<tr>
<td>9</td>
<td>Heptanoic acid</td>
<td>10.720</td>
<td>0.81</td>
<td>C₇H₁₄O₂</td>
<td>130.187</td>
</tr>
<tr>
<td>10</td>
<td>3-Acetoxytridecane</td>
<td>11.390</td>
<td>0.30</td>
<td>C₁₃H₂₆O₂</td>
<td>242.4</td>
</tr>
<tr>
<td>11</td>
<td>6,8-Doixetadecane</td>
<td>11.657</td>
<td>0.27</td>
<td>C₁₂H₂₄O₂</td>
<td>202.33</td>
</tr>
<tr>
<td>12</td>
<td>Cyclohexanecarboxylic acid</td>
<td>12.702</td>
<td>1.84</td>
<td>C₁₂H₁₂O₂</td>
<td>128.171</td>
</tr>
<tr>
<td>13</td>
<td>Isoamyl levulinate</td>
<td>14.577</td>
<td>1.75</td>
<td>C₁₃H₂₃O₃</td>
<td>186.25</td>
</tr>
<tr>
<td>14</td>
<td>Oxalic acid</td>
<td>15.195</td>
<td>0.69</td>
<td>C₂H₂O₄</td>
<td>90.034</td>
</tr>
<tr>
<td>15</td>
<td>Isophytol</td>
<td>15.747</td>
<td>1.80</td>
<td>C₂₀H₄₆O₂</td>
<td>296.539</td>
</tr>
<tr>
<td>16</td>
<td>Pyrrolidine</td>
<td>16.744</td>
<td>2.23</td>
<td>C₄H₇N</td>
<td>71.123</td>
</tr>
<tr>
<td>17</td>
<td>Heptadecyl heptfluorobutyrate</td>
<td>18.091</td>
<td>0.44</td>
<td>C₁₉H₃₃F₂O₂</td>
<td>452.49</td>
</tr>
<tr>
<td>18</td>
<td>Palmitoleic acid</td>
<td>20.163</td>
<td>0.42</td>
<td>C₁₃H₂₅O₂</td>
<td>254.414</td>
</tr>
<tr>
<td>19</td>
<td>Isopropyl myristate</td>
<td>20.513</td>
<td>2.26</td>
<td>C₁₃H₂₄O₂</td>
<td>270.44</td>
</tr>
<tr>
<td>20</td>
<td>8-Octadecenoic acid, methyl ester</td>
<td>23.065</td>
<td>11.16</td>
<td>C₁₉H₃₈O₂</td>
<td>296.48</td>
</tr>
<tr>
<td>21</td>
<td>Heptadecanoic acid, 16-methyl- methyl ester</td>
<td>23.344</td>
<td>1.36</td>
<td>C₁₉H₃₈O₂</td>
<td>298.50</td>
</tr>
<tr>
<td>22</td>
<td>Oleic acid</td>
<td>24.602</td>
<td>14.61</td>
<td>C₁₈H₃₄O₂</td>
<td>282.47</td>
</tr>
</tbody>
</table>

DPPH assay

The antioxidant activity of oil extract was 45.21% in concentration 20 µl/ml and the activity was 75.81% in concentration 100 µl/ml as shown in Table 2. The effective concentration 50 (EC₅₀) is an important parameter to evaluate the antioxidant activity of materials and it could be used to compare the antioxidant capacity of various materials. The radical scavenging capacity (EC₅₀) of the seed oil extract was 25 µl/ml.
The antioxidant activity of seed’s oil may be attributed to the presence of 8-octadecenoic acid, methyl ester, oleic acid and Isopropyl myristate.

Table 2: Radical scavenging activity of *moringa oleifera* seed oil extract

<table>
<thead>
<tr>
<th>Concentration (µl/ml)</th>
<th>Oil Seed extracts (µl/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>45.21 ± 0.21</td>
</tr>
<tr>
<td>40</td>
<td>57.94 ± 0.18</td>
</tr>
<tr>
<td>60</td>
<td>66.98 ± 0.07</td>
</tr>
<tr>
<td>80</td>
<td>69.23 ± 0.03</td>
</tr>
<tr>
<td>100</td>
<td>75.81 ± 0.13</td>
</tr>
<tr>
<td>LSD value</td>
<td>0.450 *</td>
</tr>
</tbody>
</table>

Total phenolic content of seed oil extract

The results of total phenolic content of seed oil extract increased gradually with increases of concentration, with significant differences (P≤0.05) as shown in Table 3. The result in the current study is disagreement with Ogbunugafor which referred in his investigation to study the antioxidant properties of *Moringa oleifera* seed oil, the total phenolic content was 40.17 ± 0.01 mg/g.

Table 3: Total phenolic content of *moringa oleifera* seed oil extract

<table>
<thead>
<tr>
<th>Concentration (µl/ml)</th>
<th>Seed oil (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.5</td>
<td>14.38 ± 1.05</td>
</tr>
<tr>
<td>125</td>
<td>18.96 ± 0.22</td>
</tr>
<tr>
<td>250</td>
<td>23.93 ± 0.06</td>
</tr>
<tr>
<td>LSD value</td>
<td>2.154 *</td>
</tr>
</tbody>
</table>

* (P≤0.05)

**Antibiotic susceptibility test**

The antibiotic susceptibility of the bacterial isolates was performed on ten antibiotics represented in Table 4. The antibiogram of the studied isolates revealed that *B. cereus* was resistance to Cefotaxime, Chloramphenicol, Doxycycline, Erythromycin, Penicillin and Trimethoprim, *Staph aureus* was resistance to Cefotaxime and penicillin, *E. coli* was resistance to Erythromycin, Penicillin and Tetracycline and *K. pneumonia* was resistance to Erythromycin and Penicillin, while the isolates were sensitive and intermediate to another antibiotics used in this study.

Over the past decades, overreliance and use of antibiotics have led to the emergence and dissemination of multidrug resistant strains of several groups of microorganisms. Due to the increase in resistant clinical isolates, there is a paramount need to develop new and innovative antimicrobial agents. Therefore,
researchers are looking for new leads in the discovery of better alternatives against multidrug resistant microbial strains. Among the potential sources of new agents, plants have long been investigated owing to their popular use as remedies for diverse infectious diseases because they contain many bioactive compounds that could be interest in therapeutics. 

Table 4: Antibiotic Sensitive Test

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Microorganisms</th>
<th>B. cereus</th>
<th>Staph. aureus</th>
<th>E. coli</th>
<th>K. pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacine</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>R</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Penicillin</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>I</td>
</tr>
</tbody>
</table>

S=Sensitive, R=Resistant, I=Intermediate

Antibacterial activity of seed oil extract
The antibacterial activity of *Moringa oleifera* seed’s oil extract was reported in Table 5. The oil extract had antibacterial effect against all tested bacteria in all concentrations except *E. coli* and *K. pneumonia* which were resistant to the seed’s oil extract in concentration 125 µl/ml. The highest antibacterial activity was observed on *Staph. aureus* with inhibition zone 10.50 ± 0.28, 13.16 ± 0.16 and 18.50 ± 0.28 mm in concentrations (125, 250 and 500 µl/ml) respectively, and the lowest effect was observed on *E. coli* with inhibition zone of 7.33 ± 0.33 mm in 250µl/ml concentration with significant difference (P≤0.05). Abdulrasheed \(^{27}\) revealed that *Moringa oleifera* seed oil was inactive against all the tested organisms even at 100% concentration. Results obtained by Othman \(^{28}\) showed that *M.oleifera* seed oil had low effect on gram positive and gram negative test bacteria.

Table 5: Antibacterial activity of *moringa oleifera* seed oil extract

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Inhibition zone (mm)</th>
<th>LSD value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>125 (µl/ml)</td>
<td>250 (µl/ml)</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>10.50 ± 0.28</td>
<td>13.16 ± 0.16</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>6.83 ± 0.33</td>
<td>11.16 ± 0.16</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0.00 ± 0.00</td>
<td>7.33 ± 0.33</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>0.00 ± 0.00</td>
<td>7.66 ± 0.33</td>
</tr>
<tr>
<td>LSD value</td>
<td>0.719 *</td>
<td>0.859 *</td>
</tr>
</tbody>
</table>

* (P≤0.05)

The antibacterial activity of *Moringa oleifera* seed oil extract attributed to the presence of oleic acid (fatty acid) , 8-Octadecenoic acid, methyl ester (fatty acid ester) \(^{22}\), Butyric acid and Hexanoic acid, Pyrrolidine \(^{29}\), Heptadecyl heptafluorobutyrate, Isophytol \(^{30}\), and other compounds that exhibit antimicrobial activity.

**Minimum Inhibitory Concentration (MIC) of seed oil extract**

Different standard methods have been used to evaluate the antimicrobial activities of plant’s extracts. However, dilution methods have been favored over others for the determination of MIC and MBC. A method using the oxidation-reduction colorimetric indicator resazurin has been proposed for the determination of drug resistance and MIC of antimicrobial agents against pathogenic organisms \(^{31}\). The results of seed oil extract showed that the MIC was 64µl/ml for *Staph. aureus*, 128 µl/ml for *Bacillus cereus* and 256 µl/ml for each *E. coli* and *K. pneumonia* as shown in Figure 1.

Phenolic compounds of plants are of noticeable interest due to their antioxidant and antibacterial properties \(^{32,33}\). The means by which microorganisms are inhibited by phenolic compounds involves a sensitization of the phospholipids bilayer of the cell membrane, causing an increase in permeability and leakage of vital intracellular constituents, or impairment of bacterial enzyme systems. Phenolic compounds act by inhibiting the amino acid decarboxylase in target bacteria \(^{34}\).
Conclusion

*Moringa oleifera* seed oil extracts have a wide variety of fatty acid like Oleic acid, Butyric acid, 8-Octadecenoic acid, methyl ester and 1-Pentanol that show antioxidant activity and antimicrobial agent against both gram positive bacteria (*Staph. aureus* and *B. cereus*) and gram negative bacteria (*E. coli* and *K. pneumoniae*).

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Not required

References


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A Study to Assess the Effectiveness of Planned Teaching on Knowledge Regarding Prevention of Nutritional Anemia among Adolescents in Kasturba Vidya Mandir, Sevagram, Wardha

Sheetal Sakharkar¹, Prinumol Punnoose², Shakuntala Pande³, Priya Navarate⁴

¹Assistant Professor, Dept. Medical Surgical Nursing, Smt. Radhikabai Meghe Memorial College of Nursing,
²Nursing Officer, INHS Kalyani Navy Hospital Vishakhapatnam,
³Nursing Officer, S.V.N.G.M.C. Yavatmal,
⁴Nursing Officer, S.B.H.G.M.C. Dhule

Abstract

Introduction: - Growth of all living organism depends on nutrition. In human being growth period is said to be from conception to adolescence and after that nutrition is required for maintenance of body. Lot of work has been done regarding role of nutrition during pregnancy, infancy and childhood but hardly any attention is paid to the group called adolescence i.e. period between childhood and adulthood. The most prevalent nutritional condition is anaemia worldwide. Prevalence in India of anemia among young girls were 56 % and this amount to an average 64 million girls at any point in time.

Aim: To determine the effectiveness of planned teaching on knowledge regarding prevention of nutritional anemia among adolescence.

Materials and Methods: A pre-experimental pre-test, post-test research design was used to conduct this study. The setting was selected in the Kasturba Vidya Mandir Sewagram, Wardha (Maharashtra) after getting ethical permission. Purposive sampling was the sampling method used to collect data from 9th standard students on the basis of structured knowledge questionnaire. After collecting pre-test data, planned teaching was given for intervention regarding prevention of nutritional anemia to adolescents. Seven days were provided to the samples for utilizing planned teaching which was organized for 45-50 minutes. Post-test information was gathered after seven days from the day of intervention. The data were described by frequency, percentage and t-test was used to describe the difference between pre-test and post-test knowledge score. Chi-square test was also used to find out the association between knowledge of 9th standard students regarding prevention of nutritional anaemia and selected demographic variables.

Results: There was a significant difference between pre-test and post-test knowledge scores interpreted that planned teaching on knowledge regarding prevention of nutritional anemia among adolescents were effective. Mean value of pretest was 10.10 and posttest was 19.08 and standard deviation values of pretest was ±2.27 and posttest was ±2.21. The calculated t-value was 19.66 and p-value was 0.000.

Conclusion: - The study was effective because the post-test knowledge score improved than the pre-test knowledge score. So the planned teaching has proved to improve 9th standard students knowledge regarding prevention of nutritional anaemia.

Key Word: avoidance, teenage and vitamin-deficiency anemia

Introduction

The teen age demographic is the window of opportunity for children to correct their dietary status.
We will avoid potential effects of nutritional problems if we act appropriately during this time.

The most prevalent nutritional condition worldwide is anaemia. The life period between 10-19 years is specified according to the WHO teenage age group. The prevalence of anaemia among teenage girls in India was 56 percent, which at any point of time amounts to an average of 64 million girls. Incidence of anaemia in university health science students to be 43%. Anemia incidence is found to be more common in students of both sexes due to many causes such as insufficient ingestion of nutrients, socio-economic background etc.

Studies performed in various regions of India found that in Madhya Pradesh, 37 % in Gujarat, 41.1 % in Karnataka, 85.4 % in Maharashtra, 21.5 % in Shimla, 56.3 % in Uttar Pradesh, 77.33 % in Andhra Pradesh, 58.4 % in Tamil Nadu and Kerala, the prevalence of anaemia was 52.5 %(19.13 % among college students and 96.5 % in tribal area). Socio-economic condition, menstrual blood loss, nutritional status, hand hygiene and worm infestation were the key risk factors found by the above reports.

Girls’ dietary requirements are usually neglected during puberty, resulting in stunting and ill health. Anaemia is one of the key implications of the physiological changes and nutritional negligence happening during this time.

Hence, present study was conducted with an aim to determine the effectiveness of planned teaching on knowledge regarding prevention of nutritional anaemia among adolescents.

When the condition progresses, anaemia causes adverse effects. It influences not only the development of teenage girls, but also their concentration, memory and achievement at school and success of school and attendance. It also induces delay in the initiation of menarche, leading to infections, affecting the immune system. If the anaemic teenage girl becomes pregnant, fetal morbidity and mortality may increase, perinatal risk may increase, low birth weight (LBW) occurrence may increase, and infant mortality rate (IMR) and maternal mortality rate (MMR) may increase (MMR). As growing pregnant adolescents compete with the growing foetus for nutrients anaemia in pregnancy will be worse than in older women.

Anemia accounts for much of the nutritional issues in worldwide. Owing to low socioeconomic status and inadequate access to health care, the incidence of anaemia is considerably higher in developing countries. The teenage phase is characterized by marked physical activity and accelerated growth spur; thus, they require extra nutritional nutrients and are at greatest risk of developing nutritional anemia.

The teenage community is more vulnerable to food problems in developed countries and adolescent girls are more vulnerable to the disease. Studies have found that the biggest nutritional problems in developed countries is adolescent anemia. India had reported a high prevalence of anemia among teenage girls, which is obviously higher than in other developed countries.

The World Health Organisation has described the era between 10 and 19 years of age as adolescence.

This time is known to be the transition from childhood to adulthood. During this phase, due to marked physical activity and rapid growth spur adolescence, significant psychological, behavioral, and physical developments require additional nutritional requirements. According to recent statistics, there are approximately 1.2 billion adolescents worldwide, representing one-fifth of the world’s total population, and the numbers are increasing. Developing countries account for about 5 million adolescents of the total adolescent population, and in India about 21% of the total population are adolescents.

Hypothesis:-

H1: There will be significant difference between pre and post-test knowledge scores of adolescents (9th standard students) on prevention of nutritional anemia.

H0: There will not be significant difference between pre and post-test knowledge scores of adolescents (9th
standard students) on prevention of nutritional anemia.

Materials and Methods

- A pre-experimental pre-test, post-test research design was used to conduct this study. The setting was selected in the Kasturba Vidya Mandir Sewagram, Wardha (Maharashtra) after getting ethical permission. The 50 9th standard students were informed and explained the objective of the study. The written informed consent dully signed individually by them was obtained.

Inclusion criteria: Those students who gave consent for participation in the study, and who were studying in 9th standard of Kasturba vidya mandir, and included male and female adolescents, and those who were available during data collection period.

Exclusion criteria: Who had participated in similar type of research.

Assumption:- Adolescents may have some knowledge regarding prevention of nutritional anemia.

Demographic variables were collected in terms of gender and dietary pattern. A structured questionnaire, which is attached in [Annexure V], has 40 multiple choice questions were used. The sections were - (i) introduction (ii) causes of nutritional anemia and (iii) and management of nutritional anemia and (iv) prevention of nutritional anemia. Each correct answer carries one mark and the total score is 40. The prepared tool was validated by 10 experts who included two from departments of statistics, one each from department of English, department of general medicine and, department of respiratory medicine and five from department of medical surgical nursing. Parallel form method was adopted for reliability test and it was found as $r=0.82$. And hence tool was reliable and valid. The data collection process was planned to gather demographic information and the knowledge regarding prevention of nutritional anemia. The planned teaching organized on (i) introduction (ii) causes of nutritional anemia and (iii) and management of nutritional anemia and (iv) prevention of nutritional anemia There were two sessions conducted for education in two groups, each session had 25 students in each group in 45 minute. Each sample required mean time of 30 minutes to complete the pretest structured questionnaire. Then the planned teaching was intervened to the sample. The post test structured questionnaire was administered after 7 days. Based on the 40 questions each study participant was asked individually for his / her answers with the same questionnaire. As collected, the responses were arranged in tabular form to conduct statistical analyzes which are mentioned in the following sections.

Statistical Analysis

The collected data were coded, tabulated, and analyzed by using descriptive statistics (mean percentage, standard deviation) and inferential statistics. Significance difference between pre and posttest readings was tested by using a t-test; association of knowledge with demographic variables was done by one way ANOVA test and independent t-test. For statistical analysis SPSS version 15.0 was used.

Results

Association of knowledge with their demographic variables are depicted in Table No.1

<table>
<thead>
<tr>
<th>Table No.1 Association of knowledge with their demographic variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic Variable</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Dietary Pattern</td>
</tr>
<tr>
<td>Vegetarian</td>
</tr>
<tr>
<td>Non-Vegetarian</td>
</tr>
</tbody>
</table>
Table No.1 showed that
Majority of the 28(56%) sample were males whereas only 22(44%) of sample were belonged to female gender.

The majority of the 29(58%) sample belong to non-vegetarian and 21(42%) belong to vegetarian.

The demographic variables were not associated with (p>0.05) knowledge score of adolescence i.e., gender and dietary pattern.

Table No.2:-The significant difference between the pre-test and post-test knowledge of adolescents n=50

<table>
<thead>
<tr>
<th>Overall</th>
<th>Mean</th>
<th>SD</th>
<th>Percentage</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Test</td>
<td>10.10</td>
<td>2.27</td>
<td>33.66</td>
<td></td>
<td>19.66</td>
</tr>
<tr>
<td>Post Test</td>
<td>19.08</td>
<td>2.21</td>
<td>63.60</td>
<td></td>
<td>0.000</td>
</tr>
</tbody>
</table>

The effectivenss of planned teaching was analyzed as follows:

The mean value of the pre-test is 10.10 and the post-test is 19.08 and the standard deviation values of pre-test are ±2.27 and post-test is ±2.21. The calculated t-value is 19.66 and the p-value is 0.000 [table 2]. Hence it was statistically interpreted that the planned teaching on knowledge regarding prevention of nutritional anemia among adolescence was helpful. Thus the H 1 is accepted and H 0 is rejected in this research.

Discussion

Present study was conducted to evaluate the effectiveness of planned teaching on knowledge regarding prevention of nutritional anemia among adolescence. It intends to promote adolescence knowledge regarding prevention of nutritional anemia among adolescence. The mean value of the pre-test is 10.10 and the post-test is 19.08 and the standard deviation values of pre-test are ±2.27 and post-test is ±2.21. The calculated t-value is 19.66 and the p-value is 0.000. Hence it is statistically interpreted that the planned teaching on knowledge regarding prevention of nutritional anemia among adolescence was helpful. Similarly for a period of 6 months, pre-experimental research was carried out on 60 teenage high school girls aged 10-19 years of age studying at Handignur High Schools, Belgaum, Karnataka conducted on Effectiveness of Planned Teaching Programme on Prevention of Anaemia among School Going Adolescent Girls. Analysis of the data showed that 100% of adolescent girls in pre-test had average knowledge, whereas in post-test majority 73.33% of the adolescent girls had good knowledge and 26.67% had average knowledge, which indicates that the Planned Teaching Programme has impact in prevention of anaemia. The post test score was high and statistically significant that is the planned teaching on on Prevention of Anaemia among School Going Adolescent Girls was effective.

The present study illustrated quasi experimental research design among 50 adolescence in which majority of the sample belong to non-vegetarian i.e., 58% and 42% belong to vegetarian.

The calculated t-value is 0.04 and the p-value is 0.96 and In another similar study on Prevalence of Anemia and Its Associated Risk Factors Among Adolescent Girls of Central Kerala in which majority of the sample belong to non-vegetarian i.e., 51 and 3 belong to vegetarian. The calculated t-value is 0.853 and the p-value is 0.356.

Limitation:

The study was limited to sample size i.e., 50, which might be inadequate to generalize the study findings.
More time duration would give more relevant results with variations of any research, but the investigator planned to complete the research work within one week to get more feasibility of getting sample. Therefore, sufficient number of sample and time duration was required to establish the effect of planned teaching, in general.

**Conclusion**

Study concluded that knowledge regarding prevention of nutritional anaemia improved after administering planned teaching among adolescents.

**Acknowledgement:** Authors express their sincere thanks to all faculties of Kasturba Nursing College Sevagram, Wardha, Maharashtra India for smooth completion of my research work.

**Ethical Clearance:** Taken from Institutional Ethics Committee

**Source of Funding:** No funding.

**Conflict of Interest** - Nil

**References**


Evolution of Electronic Root Canal Length Measurement Device -A Literature Review

Shivangi Shekhar¹, P.Laxmish Mallya²
¹Post Graduate, ²Associate Professor, Department of Conservative Dentistry and Endodontics, Manipal College of Dental Sciences, Mangalore, Affiliated to Manipal Academy of Higher Education (MAHE), Manipal

Abstract

Summary: An electronic apex locator (EAL) is a device used to measure the working length (WL) of the root canal space (RCS) and proved to be a great help to the endodontist reducing radiation exposure of both patients and clinicians and providing accurate reading for the WL. Evolution of different generations of EAL helped to eradicate the shortcomings of the previous generations providing precise measurement of the WL. Electronic Apex locators are used to detect any perforations, furcation involvement, also used to detect horizontal fractures, also to detect internal or external resorption. With so many uses, it is still contraindicated in patients with pace makers and inaccurate reading in canals with common irrigants like Ethylenediamine tetraacetic acid and sodium hypochlorite. It requires dry or partially dry canals to give readings with accuracy. It also gives inaccurate reading in the presence of semi-conductive materials and hence the canals should be free of them. Though it provides almost precise measurement of the WL it cannot be replaced with radiographs but can be used as an adjunct with it. Pre- and post-operative radiographs are still necessary for the success of the endodontic treatment as it provides an idea for any periapical lesion and degree of curvature of roots. Acceptance of an electronic apex locator to determine the correct WL has still not gained approval worldwide. Lack of exposure to the technology by the operator, inadequate knowledge of the root canal anatomy and cost of the instrument are also the factors for it not gaining worldwide acceptance.

Keywords: Apex locators, Apical constriction, Endodontics, Generation, Working length

Introduction

The accomplishment of an endodontic treatment or its success depends on complete elimination of the contents from the root canal system (RCS) before shaping that include biofilm, planktonic bacteria, bacterial toxins, organic infected tissue, inorganic debris and smear layer which refers to cleaning of the root canal.¹

Shaping refers to de-roofing of the pulp chamber, removal of obstacles, pre-flaring to reach middle and apical third also smoothing the walls of the RCS. Bio mechanical preparation plays a vital role for the accomplishment of the endodontic treatment. This can be achieved with accurate endometrics. The success of endodontic treatment with periapical lesions and necrotic pulps depends on the apical end of the filling material used in root canal.²

Traditionally, radiographs were used to measure and regulate the extent of the RCS and point of termination at the minor constrictor.³ The development of the electronic apex locator came as a boon for the endodontist to evaluate the accurate working space and to reduce radiation exposure of the clinician and the patient and minimizing on the number of radiographs.⁴

An apex locator is an device applied to measure the length and position of the minor constrictor. The apical portion of the root has specific resistance to electrical current, and it is measured using a pair of electrodes which is attached to the endodontic file on one end and hooked to the lip at the other end to form an circuit.⁵
**Importance of working length:**

Working length (WL) is defined as the distance from a coronal reference point to the point at which canal preparation and obturation should terminate,” according to Glossary of Endodontic terms. \(^3,6\)

The determination of a precise WL is a crucial step in the root canal therapy. The biomechanical preparation of the RCS cannot be done correctly if the WL is not determined with accuracy. Thus, the predictable endodontic treatment success stresses on achieving a precise WL determination.\(^7\)

Over & underestimation of the WL can result in failure. Overestimated WL can lead to preparation of the RCS beyond the apex, which can bring about damage in the peri-apical area. Underestimated WL results in inadequate debridement in the canal which eventually leads to failure of the treatment causing dissatisfaction to both the dentist and the patient.\(^1\) Since determination of the WL plays a vital role in root canal treatment, some procedures may be suggested:\(^1\)

A: Application of tactile sensation using paper cones is one of the methods that is used due to their ease and relative efficacy. This technique can be inaccurate in patients with apical curvature and teeth with an open apex

B: Radiograph is a most used method for determining WL where the apex is kept 0.5-1 mm shorter than the radiographic apex. The radiographic technique has its recompenses, such as direct opinion of the RCS and the canal curvature or any incidence of any peri-apical lesions, but radiographs give a 2D view of a 3D object. In an addition, a drawback of radiation is that it can be hazardous to the dental staff, clinician and the patients.

C: Introduction of the electronic apex locators (EAL) came with advantages like reduction in number of radiographs and elimination of radiographic obstacles.

The development and the principal design of the initial apex locator’s dates back to 1942, in which Suzuki investigated the circuit on dogs and came to a conclusion there is some electrical resistance observed between the periodontal ligament (PDL) and the oral mucosa which had a persistent charge. These proceedings were taken into consideration and presented into daily clinical practice by Sunada (1962) which measured the electrical resistance between periodontal ligament and oral mucosa.\(^5\) Therefore calculation of WL should be done with accuracy, using methods that have already proven to give precise and correct results and by procedures that are easy and practical to perform and should be confirmative.\(^8\)

An understanding of the anatomy of the apex and the apical region is required to gain the idea of the WL. The structure of the minor & major constrictor changes with increase in time of life due to hard tissue deposition.\(^1\)

**Mode of action of Apex Locators:**\(^4,6\)

Electronic Apex Locators works by completing the electric circuit using the human body. There are two sides of an apex locator, one part of the circuit is connected to the oral mucosa via a clip that attaches to the lip whereas the additional part is fixed on to an endodontic instrument. The circuit gets completed when the endodontic instrument is sited in the RCS and is moved apically till it reaches the apex and touches the periodontal tissue. The system specifies that the apex has been reached when the electrical resistance of the electronic apex locator and the resistance between oral mucosa and the endodontic instrument are same.

When the tip of the endodontic instrument contacts the tissue, an electrical circuit is completed and the resistance decreases evidently and the current swiftly begins to flow. This current flow signalled by a beep or a buzzer or digital readout or pointer on the screen display. The electrical characteristic of the tooth structure is measured and a precise location of the endodontic instrument in the RCS is determined.

Classification of electronic apex locators according to Generations of EALs (Evolution of EALs) \(^3\)

1. The first-generation apex locators determine the WL of the RCS by means of the resistance technique. This generation of apex locators measured the obstruction
to the flow of direct current (resistance) and henceforth called Resistance based apex locators.

These devices gave false interpretations in obstructed and wet canals and also in carious and defective restoration, in cases with perforations and also in patients who had cardiac pacemakers. For example: The Root Canal Meter, Dentometer

2. The 2nd generation apex locators determine the WL using the impedance method. This generation of apex locators measures the obstruction to the flow of alternating current hence known as impedance-based devices. These units perform the task by utilizing the current of a single frequency.

Second generation apex locators are devices based on impedance measurement that functions on the principle that there is electrical impedance across the root canal wall as there is transparent dentin present. The tooth increases in electrical impedance across the root canal walls, which is greater apical area compared to the coronal area. At the cemento-dentinal junction the impedance level drops intensely. Examples: Endocater, Sono explorer Apex finder endoanalyzer, Digi pex

One major disadvantage of these devices was that it gave false reading of electro-conductive materials. The RCS should be completely unrestricted by any electro-conductive materials to gain correct interpretation.

3. Third Generation Electronic Apex locators are known frequency dependent apex locators. These work on the principle of comparative impedance and frequency. These electronic apex locators are based on the aspect that different locations in the RCS can give difference in impedance ranging from extremely high (8KHz) and extremely low 400(Hz) frequencies. This difference in the frequencies is seen minimum in the coronal portion of the root canal. Difference in frequencies increases as the endodontic instrument penetrate deeper into the canal. Frequencies are seen highest at cemento-dentinal junction. For examples: Apit –Endex (Osada, Japan)/ Apit or Endex

The disadvantage of this device was that it needs to be “reset” or “calibrated” for every canal. For example: The Root ZX

Other third generation apex locators include:

1. Apex Finder AFA Model 7005 (EIE Analytic Endodontics, USA).

4. The 4th Generation electronic Apex Locators are the ratio type apex locators which regulate the impedance at 5 different frequencies and they have a pulp tester which is in-built. These devices take the capacitance and resistance measurement and evaluate them with a database to estimate the distance from the apex of the root canal to the tip of the file.

Multi-frequency measurement system is a system employed to estimate the length from the tip of the endodontic instrument being used to the apical foramen by measuring changes in impedance occurring between the two electrodes. This technique apparently leads to less specimen mistake and more reliable readings.

The electronic apex locators of this particular generation are by far the most reliable in their group due to their high accurateness and dependability.

A noteworthy disadvantage of this generation devices is that, for accurate readings they require partially or completely dry canals.

e.g., Bingo 1020/, The Ray-Pex 4 and 5

5. The Fifth Generation Electronic Apex Locators is a recent measurement technique that was developed based on the judgement of the facts engaged from the electrical distinctive of the canal and additional mathematical processing. And so, 5th generation apex locators are dual frequency ratio type and they are being used currently.

These devices were introduced in 2003. It calculates resistance & capacitance of the circuit discretely. It comes with a diagnostic table which comprises the statistics of the file. It has greatest exactness in all root canal condition like bleeding, dry, wet canals.
(saline, NaOCl, EDTA). The apex locators retaining this technique experience significant problems while working in dry canals.

During any of the clinical procedure it was seen that the accuracy of electronic root canal length measurement varies in conditions in periapical lesions or any damage to the pulp.

The fifth-generation electronic apex locators come with a graphic illustration, digital read out and a signal which is audible. The pulp testing machine which is in built can be used to access tooth vitality.

These devices were introduced to handle the problems related with previous generations apex locators.

6. The Sixth Generation Electronic Apex Locators is an adaptive apex locator. The efficiency of this generation electronic apex locators in long term use are yet to be known. A main advantage of this device is eradicating the need of wetting and drying of the canal.

Adaptive 6th generation apex locators immediately adapt to wet or dry canal and is also possible to be used in canals with blood or exudate.

Additional uses of Electronic Apex Locators: 
- It has the ability to detect root perforations.
- It helps to determine the location of pulpal floor or root perforations
- It can detect horizontal fractures
- It helps to confirm an assumed periodontal or pulpal perforation
- It helps to recognize any connection between the periodontal membrane & the root canal such as internal or external resorption root fractures or cracks.
- They detect vitality of the tooth

Combination of electronic handpiece and EALs (Ex. Root ZX II) are also becoming popular as they achieve outstanding outcomes with same and exact precision as the stand-alone units. The Root ZX has been combined with a handpiece to measure root canal length with a rotary instrument being used. The handpiece uses rotary instruments made from nickel-titanium that rotate at the speed of 240-280 rpm.

Contraindications associated with Apex locators:
1. Non- Isolated Tooth with rubber-dam.
2. Immature Tooth with Open Apex.
4. Inflamed and Infected Periapical Area
5. Periapical-Periodontal lesions.
6. Severe Intra-Canal Resorption
7. Auxiliary canals

Problems associated with apex locators: 
- Root ZX can give inaccurate readings if the canal is filled with blood or exudate.
- Presence of any calcification or dentin debris can affect the accurate determination of the WL.
- The accuracy of the reading of the apex locator interferes with cardiac pacemakers.
- They do work appropriately when common irrigants in the canal such as sodium hypochlorite and EDTA are used.
- High cost of the instrument.
- Technique sensitive

Hence, the of apex locators cannot be used alone but should be just as an adjunct with pre- and post-operative radiograph as it through radiographs that a clinician can judge any periapical lesion and also the anatomy and morphology of the tooth. Radiographs are used for medico-legal records.
Conclusion

No particular method is suitable in determining the WL.\(^3\) The apex or the apical terminus is an anatomic termination point for the preparation of the root canal and obturation of the root canal and this should be determined with the assistance of both radiographs and an EAL. This will give results with precisions greater than 90%.\(^4\) Hence, appropriate familiarity of the root canal anatomy, judicious use of radiographs and the accurate use of an electronic apex locator will help clinicians to attain an expectable working length of the root canal system for further endodontic treatment.\(^5\) Though at any stage apex locators will not be to replace radiographs, but can definitely serve as an effective adjunct.

Ethical Clearance: Nil

References

Surrogacy as an Alternative for Depressive Infertile Couples

Shyamantak Misra¹, Prafulla Chandra Mishra²
¹Research Scholar, ²Dean, School of Law, KIIT, Bhubaneswar, Odisha, India

Abstract

This piece of work aims to provide a clear picture of the available alternatives to the intending infertile couples and how far surrogacy is acceptable as an alternative to the prolonged infertility treatments that ultimately lead to depression symptoms like anxiety and other expressed emotional patterns. It’s a bestowed power on women to cherish the experience of motherhood but unfortunately not all the women are lucky enough to conceive and enjoy the joyous feeling of being a mother and experiencing motherhood. It’s usually a dubious decision to make for the intending infertile couples whether to continues with the infertility treatments to have a child of their own or opt for an alternative like Surrogacy or Adoption. This article states the need of taking up an alternative before it’s too late and emphasizes on the reasons for taking up surrogacy as an alternative above the other available ones and also mentions the reasons why couples do not opt for surrogacy so that one takes care of the same while choosing the same. The procedure to be followed by such couples while selecting this alternative has also been stated so that they find themselves in a better position than the once suffering to get through.

Keywords: Surrogacy, Infertile, Depression, Artificial reproductive techniques, Anxiety, Stress.

“Little souls find their way to you whether they are from your womb or someone else’s”. - Sheryl Crow

Introduction

The capacity to procreate a life has been bestowed by the nature to women and this experience of motherhood is cherished by every women barring a few, who suffer from certain physiological conditions or infertility problems and unfortunately cannot give birth to their own offspring. However the desire to have a child of their own leads them to search for alternatives and out of several available options, Surrogacy and Adoption are most preferred ones. Few infertile couples without opting for the available alternatives continue with their fertility treatment for years together which then on being unfruitful leads to certain symptoms of depression like anxiety and other expressed emotional patterns.

Barrenness and Its Impact:

Every couple has an inherent desire to have a genetically related or bloodrelated child of their own. In most of the religions around the world this need for a child has been emphasized on, may it be for taking forward the family traditions or performing the funeral pyre, for preserving the community they belong to and so on. A deviant behaviour in marriage that leads to adverse effects on the relationship of such married couples while pertaining to the lack of a child being involuntary may be considered as childlessness. Many research papers highlight the social or cultural, stigma and isolation suffered by the infertile married couples. Such childlessness suffered due to infertility, physiological or other psychological medical problems involves anxiety and stress. The infertility treatment that such couple or women go through involves treatment that might be very painful, expensive, and might create a strain on the relationship of such couple which is difficult to handle with patience. Even after all this there’s no guarantee

Corresponding Author:
Mrs. Shyamantak Misra
Research Scholar, School of law, KIIT, Bhubaneswar-751004, Odisha, India, Email-Id: shyamantakmisra@gmail.com, Mob: 7978707612

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that you could be able to procreate a child of your own.

Ways to Overcome Childlessness:

In the ancient times people believed that children are a gift of God and if one is unable to conceive that means it’s a curse of God due to some sins that the couple might have committed. Therefore, the only way to overcome this was by performing religious rituals to appease God.\(^1\) Even second marriages to have a child was approved or to beget proved to be unsatisfactory in the long run. As we improved in legal arena, the method of adopting a child legally became a way to overcome childlessness, even then one’s wish of a genetically related child remained unfulfilled. With the development in science and technology in the field of human reproduction, various techniques were evolved like Artificial Insemination (AI), In Vitro Fertilization (IVF), Surrogacy etc.\(^2\)

According to “INDIAN SOCIETY OF ASSISTED REPRODUCTION” - “Infertility currently affects about 10-14% of the Indian population which is nearly 27.5 million couples, especially in urban areas where one out of six couples are impacted”.\(^3\)

Therefore, its high time to think of the available alternatives instead of brooding over the infertility treatment for years together that impact the physical and mental health as well. In order to reduce the rate of such couples suffering from infertility there are various available options but the point is the major reason behind not opting for any of them being the genetic link in maximum of cases. Thus, only surrogacy can fulfill the dream of having a child with genetic link too. Whereas in case of adoption the child may not have anything in similar with the adopting parents.

The reproductive environment has been revolutionized and advancement in Assisted reproductive techniques like embryo transfer methods, donor insemination etc. are the major reason behind surrogacy being the most desirable option. This process of surrogacy has been the ray of hope for thousands of infertile couples, who longed to have a kid of their own.

The word surrogate means substitute\(^4\) . Surrogate mother acts as a substitute for the genetically biological mother who is unable to give birth for various infertility problems. Thus, a surrogate mother is a person who is recruited to rent her womb and bear the child till birth and ultimately hand over the child to her employer couple.

As per the ART (Artificial Reproductive Technique) Guidelines Government of India :“Surrogacy is an arrangement in which a woman agrees to a pregnancy, achieved through assisted reproductive technology, in which neither of the gametes belong to her or her husband, with the intention of carrying it to term and handing over the child to the person or persons for whom she is acting as surrogate and a surrogate mother is a woman who agrees to have an embryo generated from the sperm of a man who is not her husband, and the oocyte for another woman implanted in her to carry the pregnancy to full term and deliver the child to its biological parents(s)”.\(^5\)

Earlier surrogacy arrangements were limited to family, friends, and close relatives as an altruistic deed (Altruistic Surrogacy). But in the past few years surrogacy has widely spread its wings across the country with financial arrangements leading to commercialization. Thus, leading to a contract of the biological functioning of a woman’s body. Surrogacy advertisements are common nowadays where the whole process of commercial surrogacy involves recruitment of Surrogate mothers and making contracts between the infertile couple and such surrogate and making huge profits out of such arrangements. This commercialization of Surrogacy in India has raised the fear of black marketing, however finally giving a child to a childless couple seems to take over all the other sins and materialistic values that one needs to sacrifice in the process.

**REASONS FOR TAKING UP SURROGACY AS AN ALTERNATIVE:**

- Surrogacy agreements benefits both the parties in need. In the surrogacy arrangement, the barren lady
gets a baby and poor lady gets monetary consideration for the same.

- Get to complete your family with genetic connection with your child using gestational surrogacy and maintain a biological relationship.

- Get a legally sound relationship and confirm your legal rights by undergoing a legally binding contract, well negotiated and signed and obtain court order prior to the birth of the child.

- Get to be a part and parcel of the whole process from conceiving to birth along with the surrogate and help her in having a healthy pregnancy, whereas in Adoption you have no idea of all these.

- Get a better assurance of success rate since surrogacy has a good track record for successfully delivering babies to the intending parents then the other fertility treatments.

- Get to raise the child from birth and can inculcate moral values from the beginning.

**REASONS FOR NOT OPTING FOR SURROGACY AS AN ALTERNATIVE:**

- The intending parents might get to go through a complex medical procedure in case of gestational surrogacy.

- The intending couple might find it overwhelming at times complying with the legal procedures involved. However, appointing trusted legal professionals for the same can be of great help.

- The Surrogacy in practice exploits women physically and emotionally.

- The surrogate mothers are mostly from poor financial background and hence unaware of their legal rights and unable to appoint lawyers for themselves. They however sign the contract in want of money and later find it difficult to escape from the contract.

- The intending couple may experience lack of patience in need of a greater sense of control over your surrogate, so you will have to trust your surrogate for the pregnancy.

To what extent the factors like Anxiety, depression, emotional distress etc. corelates to functional infertility isn’t clear yet. Many investigations showed contradictory results while demonstrating a link between infertility and psychopathology, some authors did not find significant differences, whereas few others reported greater degrees of maturity fear and interpersonal distrust, anxiety and dissatisfaction, depression, and a tendency toward somatization in women with ‘functional’ infertility.

Several descriptive studies relating to infertile couples suggests a greater susceptibility to depression and anxiety as a factor that reduces the ability to become pregnant or conceive.

**Conclusion**

Thus, the Gestational surrogacy market in India provides the barren couples with the opportunity to create offspring’s that are biologically related. It also gives the financially weak surrogate women an economic opportunity to carry a baby and get financial benefits. However, there are other less harmful ways to get benefits and create opportunities than the ones created by India’s gestational surrogacy market. Providing proper higher education to women and then proper training can make them capable for jobs that aren’t putting them at risk physically and emotionally. Further surrogacy India commodifies women unlike the Americas non-commercial surrogacy arrangements that create biological offspring’s too with a friend or family member. Since the benefits are achievable through less detrimental means the harms that occurs due to India’s gestational surrogacy market highly out weightsthe benefits.

**Ethical Clearance:** Not required, as the research article is based on surrogacy and the literature is discussed from the decided judicial decisions. The research is doctrinally undertaken.

**Source of Funding:** Self
Conflict of Interest: Nil

References

Surrogate Motherhood: Challenges and Legal Implications

Shyamantak Misra¹, Prafulla Chandra Mishra²

¹Research Scholar, ²Dean, School of Law, KIIT, Bhubaneswar, Odisha, India

Abstract

Surrogacy wasn’t a commonly used term until the 21st century, it came up with the rising level of infertility in couples and the stringent procedure of compliance for adoption. Surrogacy has always been the choice of couples for whom genetic link is of utmost importance. At a point of time the rate of commercial surrogacy was at its peak which leads to several issues and challenges that needs to be treated on an urgent basis and obviously an enactment to guide alongside which isn’t present till date in India. However, after the passing of the Surrogacy Regulation Bill 2019 in the Lok Sabha, a hope for betterment has arose. This piece of study showcases the challenges faced since time immemorial relating to Surrogacy and how it was being tackled with, without any legislation being present in India. It also stresses on the key points of the upcoming bill of 2019 and how it would prove to be a turning point for the commercial world that developed due to the Surrogacy in India.

Keywords: Surrogacy, Altruistic Surrogacy, Commercial Surrogacy, Assisted Reproductive Technologies.

Introduction

The tremendous breakthrough in science have opened the new contours of nature whose existence have remained as secret since then. The contribution of the bio-medical sciences to the mankind is unimagined. These unprecedented advances in science come with their own attached risks and concerns whether it may be legal, moral, technical or ethical. Advances in the bio-medical sciences and the reproductive techniques have catered to the desire of the men and women who have no or little reproductive capabilities to have their children through techniques known as Assisted Reproductive Technologies (ART) methodized to achieve pregnancy by artificial means which is also termed as fertility treatment. Surrogacy is also one of those techniques which substitute the natural conception and birth and has witnessed a phenomenal growth. Surrogacy is not a new concept as far as Artificial Reproductive Technology is concerned.

Surrogacy has always been a controversial topic in India mainly due to the absence of a cohesive legislation although in ancient India it was practiced, accepted and respected as well. Its quite weird that surrogacy existed during those times but Indian Hindu Mythology widely mentions instances of surrogacy like Karthikey (the God of war), 8 the son of lord Shiva but not born out of goddesses Parvati, the Surrogate mother being Ganga. In Mahabharat Dhritarastras wife Gandhari conceived and after a long span of 2 years gave birth to a mass which was then put in a nutrient medium by Rishi Vyasa, according to whom there were 101 cells in the mass delivered by Gandhari and surprisingly those cells finally developed into hundred male and one female child.

As per the Assisted Reproductive Technologies (Regulation) Bill, 2010, which for the first time tried to get a specific legislation pertaining to the critical issues involved in Surrogacy defines Surrogacy as “an arrangement in which a woman agrees to a pregnancy, achieved through assisted reproductive technology,
in which neither of the gametes belong to her or her husband, with the intention to carry it and hand over the child to the person or persons for whom she is acting as a surrogate.”

Bad health condition of a woman which makes her pregnancy dangerous and weak uterus to hold the fetus also calls for surrogacy arrangement.

**Indian Perspective:**

Coming to the legal protection and regulation governing the law of surrogacy, the contract and other allied issues, however is considered legitimate in India and in the absence of any legislation to that effect, the only guidelines currently related to this field are those of the Indian Council of Medical Research (ICMR) in 2005 issued guidelines for accreditation, supervision and regulation of ART clinics in India. However, this could not check the abuses of exploitation of surrogate mothers in the background of no strict penal provisions and implementation. Looking into the pressing need and demand for protection against all such misuses the government appointed an expert committee has drafted a legislation known as Assisted Reproductive Technology (ART Regulation) Bill, 2010 for legalizing surrogacy.

There is an urgent need for a strong regulation is called for to meet the demand of the legislature. The 228th Report on “Need for Legislation to regulate Assisted Reproductive Technology Clinics as well as Rights and Obligations of Parties to a Surrogacy” reflects the background and the need for developing legislation to regulate this widely unregulated industry in India. The Commission said, “It seems that wombs in India are on rent, which translates into babies for foreigners and dollars for Indian surrogate mothers.” The approach “The parents construct the child biologically, while the child constructs the parents socially” has been widely reflected in the works of many scholars while dealing with surrogacy and related aspects. This certainly reflects, the complexities involving the parentage and legitimacy of the child and rights that would come with this report.

As stated above issues majorly arose due to the commercialization of surrogacy and after a thorough research recently the Minister of Family and Health Welfare, Dr. Harsh Vardhan introduced the Surrogacy Regulation Bill 2019.

On July 5, 2019: Cabinet approved the surrogacy regulation Bill 2019

On July 15, 2019: It was introduced in the lower house.

The Special features of this bill are:

- It aims at banning commercial surrogacy and legalizing only the altruistic surrogacy (involves no monetary compensation only the medical expenses).
- It has fixed the criteria’s relating to the persons going for the same. It’s for Intending couples who suffer from proven infertility, for any condition or diseases fixed under the regulations or altruistic one.
- Certificate of essentiality and certificate of eligibility are both required.
- Appropriate authorities are to be appointed by the Central and State government within 90 days of the Bill becoming an Act.
- Surrogacy clinics cannot undertake surrogacy related procedures unless registered under the appropriate authority.
- National and State surrogacy Boards would be constituted.
- Penalties have been fixed for the offences under this act.

**Surrogacy- Issues and Concerns:**

Surrogacy raises serious issues concerning legal rights of the parties concerned and parenthood i.e. paternity and maternity rights, guardianship and custody of the child along with the matters relating to maintenance and financial support. Whether the human body is an object that is possessed by an individual
and is that individual the owner of her body so as to be entitled to put it through abuse i.e. undergoing artificial reproductive techniques for procreation is a pertinent question.

The legitimacy and legal status of the babies born out of ART technique and the question as to who are the legal father and mother of the child is a matter which needs address. The status of the ova donor and the sperm donor is not clear. The succession rights and the citizenship of the baby raise huge concern as well. The concern regarding the surrogate mother to recover to normal life after the handing over of the child to the commissioning parents raises an issue as to the responsibility of the surrogacy clinics whether they can be made liable for their fault or not if afterwards the surrogate still persists with health problems.

Current International Scenario:

Risking the life is another very critical issue in a country where no doubt the health care sector is very advanced but the womb lent might not be taken proper care of and the contractual terms might not be sound enough to insure you about your health. So that is why it is a tourist place for surrogacy and instances are many which put spotlight on the murky commercial surrogacy market. The practice of surrogacy is popular beyond the borders or territorial jurisdiction of the government of the commissioning parents. Australian parents prefer surrogacy the most amongst the other nationals of the world and India, Thailand and some other Asian countries have been the most viable cost-effectivetourist spots for them and thus are usually preferred to USA where the best facilities and proper regulatory environment is available.

In this backdrop, another very pertinent question that rises here –Is not it a forced removal of indigenous children from the community, going overseas and asking for a womb to brought up your intended offspring for reasons not so befitting and if question being raised which woman, would willingly, freely, rent out their womb with availability of other choices for earning enough money and to live decently?

Analyzing the global trend in various countries it is found that in the U.K, USA surrogacy is highly regulated and comparatively costlier to other countries whereas, in Germany and Canada, surrogacy is outlawed or prohibited. In a series of controversies surrounding surrogacy and question of citizenship of the baby born out of such procedure, the German authorities refused to give passports to children born of surrogate procedures. In an article under the Sydney herald “Babies left in limbo as India struggles with demand for surrogacy” (2008) the story of a German couple, Jan Balaz and Susan Lohle, in the wait of their twin sons, Nikolas and Leonard, have been trapped in citizenship limbo is highlighted owing to the issues of claiming citizenship of German and who were denied passport because , German nationality is determined by the birth mother. These issues left the slow-moving Indian judicial system to wrestle with their citizenship status. When born to an Indian surrogate mother, the Constitution of India entitles the Indian citizenship, but it remains critical as to what happens if the biological mother is a foreign citizen and the child applies for citizenship of that country and is denied? This question when raised by a Bench led by Justice Ranjan Gogoi in the above case, the concept of dual citizenship for surrogate children which is not allowed in India, whether could be considered in any circumstances? This dual citizenship can give limited entitlements to such children.

Judicial Analysis:

The Supreme Court, in the 2008 case of Baby Manji Yamada v. Union of India, discussed surrogacy and noted that “commercial surrogacy is reaching industrial proportions because of the ready availability of poor surrogates. It mentioned the Commissions for Protection of Child Rights Act, 2005 but stopped short of demanding that the government take immediate action to regulate the whole surrogacy industry, and not just address the issue of the rights of the child once it is born”. In Jan Balaz v. Anand Municipality, the Court held that Surrogate babies born in India are Indians.
In K Kalaisevi v Chennai Port Trust\(^1\), the court stated that “Surrogacy is a well-known method of reproduction whereby a woman agrees to become pregnant for the purpose of gestating and giving birth to a child she will not raise but hand over to a contracted party. She may be the child's genetic mother (the more traditional form for surrogacy) or she may be, as a gestational carrier, carry the pregnancy to delivery after having been implanted with an embryo. In some cases, surrogacy is the only available option for parents who wish to have a child that is biologically related to them.” This was also stated in Sadhna Agarwal v. state of Chhattisgarh\(^2\) and 5 other cases which are “State of Punjab v. Sodhi Sukhdev Singh, AIR 1961 SC 493, p. 502 27 Liverpool and London SP&I; Association v. M.V. Sea Success & Asso. Ltd. (2004) 9 SCC 512 (para 65) 28 Senior Electric Inspector v. Laxminarayan Chopra, AIR 1962 SC 159, p. 163 W.P.(S)No.4927/2016 Manji Yamada v. Union of India.”

**Suggestion and Conclusion**

From all the above discussed matters it can be rightly said that surrogacy being and awarding experience for infertile intending couples cannot be banned completely due to the large number of issues and challenges arising because of it but can be curbed and minimized if dealt in the right way. However, the Surrogacy Regulation Bill 2019 might rise to our expectations by doing the needful since it has the proviso of banning the commercial surrogacy, reducing the complicacies involved as well as giving a remarkable boost to the adoption Industry which might further reduced the number of uncared or orphaned children. All this depends on the implementation procedure of the same. Thus, it is suggested to tighten and provide a stricter method of implementation of the provisions of the Surrogacy Regulation Bill 2019 if it turns out to be the only legislation in India on Surrogacy.

**Ethical Clearance:** Not required, as the research article is based on surrogacy and the literature is discussed from the decided judicial decisions. The research is doctrinally undertaken.

**Source of Funding:** Self

**Conflict of Interest:** Nil

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Red Dragon Fruit (*Hylocereus Polyrhizus*) to Reduce Cholesterol Level in People With Excessive Nutritional Status

Siti Fadlilah¹, Adi Sucipto¹, Mohamad Judha¹, Tia Amestiasih¹, Cornelia Dede Yoshima Nekada¹, Eko Mindarsih¹, Cipta Pramana²

¹The Lecturer at The Nursing Programme Study, Universitas Respati Yogyakarta, Indonesia, ²The Lecturer at The Medical Faculty Tarumanagara University, Jakarta Indonesia

Abstract

**Background:** Nutritional status is closely related to high cholesterol levels. High cholesterol levels as a trigger for other metabolic diseases. Fruits high in fiber and vitamin C can be used to keep blood cholesterol levels regularly.

**Aim:** To determine the effect of red dragon fruit on blood cholesterol levels in people with excessive nutritional status.

**Methods:** Research used an experimental approach with a pretest and post-test control group design. The sample consisted of 2 groups, namely the control group and the intervention group, with 50 respondents in each group. The sample was taken using purposive sampling. The intervention group got red dragon fruit juice for seven days. Blood cholesterol levels are measured by laboratory tested using intravenous blood. The statistical test used the Paired T-Test and Independent T-Test.

**Results:** The difference mean posttest-pretest control group and intervention groups were 13.56 mmHg and -13.06 mmHg. The analysis of pretest-posttest blood cholesterol levels among the control and intervention groups were p=0.514 and p=0.035. The difference between the control group and the intervention group was 0.022.

**Conclusion:** Red dragon fruit is effective in reducing blood cholesterol levels in people with excessive nutritional status.

**Keywords:** Cholesterol, *Hylocereus polyrhizus*, obesity, overweight, phytotherapy.

Introduction

The incidence of obesity in the world has doubled since 1980. Worldwide in 2014, it showed that > 1.9 billion adults aged > 18 years were overweight, of which > 600 million people were obese. The highest prevalence of overweight is in the United States, and the lowest is in the South-East Asia region.¹ Indonesia's 2018 basic health research shows that from 2007 to 2018, the proportion of overweight in adults aged > 18 years has increased, from 8.6% to 13.6%. The ratio of obesity at age > 18 years from 2007 to 2018 has also increased, namely 10.5% to 21.8%.² Nutritional status is more capable of leading to dyslipidemia, an increased risk of coronary heart disease (CHD), diabetes mellitus, and other serious diseases. More nutritional status is also closely related to dyslipidemia, one of which is high cholesterol levels.³,⁴,⁵

Cholesterol is one of the most important substances in the human body. There are two types of cholesterol in the blood, namely bad cholesterol and good cholesterol.⁶ Bad cholesterol in the body is Low-Density Lipoprotein (LDL) cholesterol, triglycerides, and lipoproteins. High-Density Lipoproteins (HDL) is a type of good cholesterol that is harmless in the body. Normal total cholesterol levels in the blood are less than 200 mg/dL or 160-200 mg/dL.⁷ High blood cholesterol levels
in the long term are bad for the body. Long-term LDL buildup causes atherosclerosis, which blocks blood flow and is an indicator of heart disease risk. World Health Organization data shows that 17 million people (31%) each year die from Cardiovascular Diseases (CVDs) worldwide. The prevalence of heart disease in Indonesia in 2018 reached 1.5%. Management to prevent the accumulation of cholesterol levels in the can use pharmacologic and non-pharmacological treatments.

It decreased cholesterol levels with a nonpharmacologic diet combined with herbal medicines or components of active substances in fruits or vegetables. One of the fruits that can lower cholesterol levels is dragon fruit. Dragon fruit is rich in antioxidants (anthocyanins, polyphenols, vitamin C, vitamin E) and is high in fiber. Dragon fruit can reduce total cholesterol, triglycerides, and LDL cholesterol levels in rats with hypercholesterolemia, dyslipidemia, and diabetes mellitus.

Dragon fruit is a plant that is easy to grow and cultivate in Indonesia. Dragon fruit is also cheap and easy to consume. Researchers are interested in examining the benefits of dragon fruit on cholesterol levels. Based on the results of a preliminary study, people with excessive nutritional status are found. After measuring cholesterol levels in these 10 people, there were 6 people with cholesterol levels > 200mg/dL, 2 people with cholesterol levels > 190mg/dL (<200mg/dL) and 2 people with cholesterol levels of 178mg/dL. The results of other interviews were as many as eight people who did not make good pharmacological or non-pharmacological efforts. One person had consumed cholesterol-lowering drugs, and one person took herbal medicine for approximately six months.

Methods

1. Study Design and Participants

This study used a experimental approach with a pretest and post-test control group design. The study was conducted on excessive nutritional status in the Kebumen, Central Java, Indonesia, in June 2020.

The independent variable was dragon fruit juice. The dependent variable is the total cholesterol level. The sample consisted of 2, namely the control group and the intervention group. The control group did not get any treatment, while the intervention group received dragon fruit juice. The number of samples in each group was 50 people who were selected by purposive sampling method. The inclusion criteria were age 20-60 years, BMI ≥ 25.0, and willingness to become respondents. The exclusion criteria were smoking, consuming alcohol, consuming cholesterol medication, consuming vitamin C regularly, consuming high fiber fruit regularly, diabetes mellitus, and gastritis. The drop-out criterion does not follow the research process from the beginning or the first day to the last day.

During the research process, the respondents were arranged in their diets and activities. Respondents got a diet of carbohydrates and protein. Respondents are not allowed to consume foods high in fat. The activities that are allowed are light and moderate. Select samples of excessive nutritional status by measuring height and weight directly, then calculating BMI. The digital weight scales used have been tested for calibration at the Legal Metrology Unit of Yogyakarta City with the certificate number 212/MET/TE-72/II/2020. Microtoice has conducted a calibration test at the Legal Metrology Unit of Yogyakarta City with certificate number 212/MET/UP- 20/II/ 2020.

2. Instruments and Data Collection

The intervention group was given dragon fruit juice. Red dragon fruit juice is a fruit drink made by separating the skin’s red dragon fruit flesh. The red dragon fruit flesh was weighed using a digital food scale with a dose of 2.86 g / kg. Then it is blended for about 30 seconds with the addition of water as much as +/- 70 ml without adding sugar. The red dragon fruit that has been blended is put into a drinking glass and is directly consumed by the respondents. Dragon fruit juice is given once a day for 7 days and is given in the afternoon at 16.00-17.00 WIB on an empty stomach or a maximum of lunch around 12.30 WIB. Dragon fruit juice was made directly
in front of the respondents so that it was consumed fresh.

Total cholesterol level is the total amount of cholesterol in the blood measured using a digital cholesterol test strip or an auto check. Cholesterol tests were carried out in the peripheral blood vessels or one of the respondent’s fingertips, namely the index, middle, and ring fingers, previously disinfected. Cholesterol levels were measured pretest and post-test in the morning around 07.00-09.00 WIB or after respondents had fasted for approximately 9-12 hours. Cholesterol levels were measured using a three cc median cubital vein and checked in the laboratory. The pretest cholesterol levels were measured on day one before the intervention. Post-test cholesterol levels were measured after the respondent was given intervention for seven days or was carried out on the 8th day. Sampling blood was carried out according to standard operating procedures. The data is recapitulated in the observation sheet.

### 3. Data Analysis and Ethical Consideration

All respondents stated their willingness to become respondents by signing informed consent. The research was conducted after obtaining an ethical clearance letter with letter number 084.3/ FIKES/PL/III/2020 and research permit number 900.2/ FIKES/PL/V/2020. Univariate data were presented with minimum, maximum, mean, and frequencies-percentages for categorical variables. Before the bivariate test, the normality test was carried out using the Shapiro-Wilk test. The results showed that the data were normally distributed (p > 0.05) to analyze the pretest and post-test; each group used a Paired T-Test. The differences between groups were measured using the Independent T-Test.

### Results

#### 1. Sample Characteristic

<table>
<thead>
<tr>
<th>Variable</th>
<th>∑</th>
<th>Total Pretest Cholesterol Levels (mg/dl)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>24</td>
<td>113</td>
<td>233</td>
</tr>
<tr>
<td>Elderly</td>
<td>12</td>
<td>152</td>
<td>287</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>2</td>
<td>171</td>
<td>233</td>
</tr>
<tr>
<td>Women</td>
<td>34</td>
<td>113</td>
<td>287</td>
</tr>
<tr>
<td>Nutritional Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>11</td>
<td>113</td>
<td>239</td>
</tr>
<tr>
<td>Obesitas</td>
<td>25</td>
<td>114</td>
<td>287</td>
</tr>
</tbody>
</table>

∑=Number Min=Minimum Max=Maximum SD=Standard Deviation

*Tested using Pearson
Table 1 shows that most of the respondents are adult, female, and have an obese nutritional status, namely 24 people (66.67%), 34 people (94.44%), and 25 people (69.44%). The bivariate test results between age, sex, and nutritional status with the pretest value of cholesterol levels were $p=0.010$, $p=0.883$, and $p=0.695$. The results showed a significant relationship between age and pretest cholesterol levels, while gender and nutritional status had no significant relationship.

2. Differences in Characteristics of Respondents with Changes in Cholesterol Levels Pretest-Posttest

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\sum$</th>
<th>Cholesterol Levels at Pretest-Posttest (mg/dl)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>13</td>
<td>114-132</td>
<td>233-214</td>
</tr>
<tr>
<td>Elderly</td>
<td>5</td>
<td>152-206</td>
<td>226-276</td>
</tr>
<tr>
<td>Intervention Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>11</td>
<td>113-131</td>
<td>233-227</td>
</tr>
<tr>
<td>Elderly</td>
<td>7</td>
<td>178-182</td>
<td>287-236</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intervention Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>2</td>
<td>171-165</td>
<td>233-190</td>
</tr>
<tr>
<td>Women</td>
<td>16</td>
<td>113-131</td>
<td>287-235</td>
</tr>
<tr>
<td>Nutritional Status</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>2</td>
<td>171-171</td>
<td>178-183</td>
</tr>
<tr>
<td>Obesitas</td>
<td>16</td>
<td>114-132</td>
<td>233-276</td>
</tr>
<tr>
<td>Intervention Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>8</td>
<td>113-147</td>
<td>239-235</td>
</tr>
<tr>
<td>Obesitas</td>
<td>10</td>
<td>114-131</td>
<td>287-235</td>
</tr>
</tbody>
</table>

$\sum$=Number Min=Minimum Max=Maximum SD=Standard Deviation

**Tested using Paired Samples Test
Table 2 shows the results of differences in respondents’ characteristics with changes in cholesterol levels in the pretest and post-test in each group. There was no difference between adults and the elderly in the control and intervention groups with changes in pretest-posttest cholesterol levels with p=0.276 and p=0.133. Although statistically there is no difference, seen from the difference between the mean posttest-pretest in the control group, it shows that the elderly have increased cholesterol levels more than adults (62 mg/dl> 1.31 mg). Whereas in the intervention group, the reduction in cholesterol levels in the elderly was more than in adults (13.71 mg/dl> 12.63 mg/dl).

Judging from gender, in the control and intervention groups, there was no difference between men and women with changes in pretest-posttest cholesterol levels with p=0.307 and p=0.121. Although statistically, there is no difference, seen from the results of the mean posttest-pretest difference in the intervention group, it shows that there is a decrease in cholesterol levels more than women (24.5 mg/dl> 11.63 mg). Based on nutritional status, there was no difference between overweight and obesity in the control and intervention groups with changes in pretest-posttest cholesterol levels with p=0.302 and p=0.132. Although statistically, there is no difference, seen from the difference between the mean post-test-pretest in the control and intervention groups, there is a decrease in cholesterol levels more than overweight.

3. Effects of Red Dragon Fruit on Cholesterol Level

Table 3 The Effect of Red Dragon fruit (Hylocereus Polyrhizus) to reduce blood cholesterol level in people with excessive nutritional status

<table>
<thead>
<tr>
<th>Cholesterol level (mg/dl)</th>
<th>Control Group</th>
<th>Intervention Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretest</td>
<td>Post-test</td>
</tr>
<tr>
<td>Min</td>
<td>114</td>
<td>132</td>
</tr>
<tr>
<td>Max</td>
<td>233</td>
<td>276</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>173.5±32.311</td>
<td>181.5±39.193</td>
</tr>
<tr>
<td>Difference mean</td>
<td>13.56</td>
<td>-13.06</td>
</tr>
<tr>
<td>Pvalue***</td>
<td>0.514</td>
<td>0.035</td>
</tr>
<tr>
<td>Pvalue****</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** Paired T-Test

*** *Independent T-Test

Table 3 shows an increase in the average cholesterol level between the pretest and post-test in the control group, which was 13.56 mg/dl. In contrast, there was a decrease in cholesterol levels between the pretest and post-test in the intervention group, as much as 13.06 mg/dl. The bivariate test results in the control group obtained p=0.514, indicating no difference in cholesterol levels in the pretest and post-test. The intervention group showed red dragon fruit juice on total cholesterol levels in people with excessive nutritional status with p=0.035. The comparison between the control group and the intervention group obtained p=0.022, indicating a difference in the two groups, namely, the intervention group had a decrease in cholesterol levels.
Discussion

Cholesterol is one of the most important fat components in the body, found in cell membranes in all body tissues. Cholesterol is produced naturally by the body, namely in the liver, which builds cell walls and makes hormones. Apart from being produced naturally by the body, cholesterol is also obtained from the food consumed daily. Total cholesterol is the total amount of cholesterol in the body, namely High-Density Lipoprotein (HDL), Low-Density Lipoprotein, and other lipid components. The range of normal values for total cholesterol levels in the body has 3 categories, namely normal values <200mg/dl, high enough 200-239 mg/dl, and high cholesterol levels> 240 mg/dl. Measuring total cholesterol levels on the first day and the eighth day when the respondent has fasted for approximately 9-12 hours. Researchers gave red dragon fruit juice with a fruit pulp dose of 2.86 g/kg body weight for each person. This dosage is following the results of previous studies.12

Total cholesterol levels in the blood can be influenced by several factors, namely genetic factors, diet and lifestyle, body weight, age, physical activity, smoking, and certain diseases.16 This study did not consider all the risk factors that affect cholesterol levels in this study, only attention to age, gender, and the problem of nutritional status. Respondents are not allowed to consume high-fat foods during the study because it will affect the results. The content of foods that contain cholesterol is fried, meat, brain, offal (intestines, liver, kidney, lungs, heart), egg yolk, seafood. Cholesterol in the body is also produced by the liver.17

Table 1 shows that the mean pretest cholesterol level in the elderly is higher than that of adults with a 5.22 mg/dl difference. The bivariate test results show a significant relationship between age and cholesterol levels (p=0.010). Increasing age is a factor in improving cholesterol levels. Cholesterol levels tend to grow at the age of more than 20 years. With age, there will be an increase in LDL production and a decrease in the level of fractional clearance of LDL, responsible for the rise in blood cholesterol levels in the long term.18 Previous studies show that cholesterol levels get higher with age.19,20 This study’s results do not support the survey results. There is no relationship between age and cholesterol levels, where all ages risk increasing cholesterol levels.21

At the start of menopause, cholesterol levels begin to fall in women, and after that, cholesterol levels will increase as in men. After 50 years, cholesterol levels in men tend to continue to grow.22 This is consistent with the study results, although the number of men but the mean cholesterol level of men was higher by a difference of 17.47 mg/dl. Statistically, there was no relationship between gender and cholesterol levels in respondents (p=0.883), supporting previous studies with the results that there was no relationship between sex and cholesterol levels (p=0.847). The absence of a relationship in this study was due to an unbalanced ratio between men and women. The majority of the study samples were adults and had not yet menopause.

Table 1 shows that the higher the BMI value, the higher the mean total cholesterol level but does not reach the cholesterol level limit in the reasonably high category (> 200 mg/dl). However, statistically, there is no relationship between excess nutritional status and cholesterol levels (p=0.695). Excess weight can harm health. Being overweight can raise triglyceride levels and lower HDL.21 Biochemical disturbances can also occur due to excess nutrition or obesity, which causes an increase in cholesterol, triglyceride, blood sugar, and insulin levels.23 The study results supported previous studies: there was no relationship between excess nutritional status and total cholesterol levels.4,24

Table 2 shows no difference in respondents characteristics with changes in cholesterol levels post-test-pretest in all aspects. This indicates that cholesterol levels in the control and intervention groups are not influenced by age, sex, and nutritional status. Table 3 shows the pretest value of total cholesterol levels between the control and treatment groups, namely the minimum value and the mean value in the normal
category. In contrast, the maximum value is categorized as high enough and in the high class. So it can be interpreted that respondents with nutritional status in this study had an average total cholesterol level in the standard type (<200 mg/dl). The results also showed the same effects on total cholesterol levels post-test in the control and treatment groups. The minimum value and the mean value indicate typical values. In contrast, the maximum value is included in the high category in the control group and the intervention group’s high enough category. The minimum, maximum, and mean values in the intervention group were lower than in the control group.

Table 3 shows a difference between the pretest and post-test total cholesterol values in the intervention group (p=0.035) and is strengthened by a decrease in the mean value of 13.06 mg/dl. Meanwhile, the control group showed no significant difference between the pretest and post-test total cholesterol levels (p=514), and there was an increase of 13.56 mg/dl. This indicates that dragon fruit juice is effective in reducing cholesterol levels in people with excessive nutritional status. The research supports previous studies that red dragon fruit juice on total cholesterol levels in hypercholesterolemic men and women with diabetes mellitus.12,13

Red dragon fruit contains many substances that are beneficial to health. Dragon fruit content is rich in antioxidants (anthocyanins, polyphenols, vitamin C, and vitamin E) and high fiber content, which controls blood cholesterol levels.10 The content in 100 grams of red dragon fruit is 10.1 grams of fiber and 9.4 mg of vitamin C. The fiber content in dragon fruit can reduce fat absorption, especially cholesterol, to lower cholesterol levels. In this case, the long-term effect can provide benefits in preventing heart attacks and strokes. High fiber content can reduce cholesterol in the blood by inhibiting the re-absorption of bile acids in the intestine as compensation for the liver to synthesize more bile acids and require cholesterol. The liver will multiply receptors to capture cholesterol from the blood.12,25

Vitamin C plays a role in cholesterol metabolism, which increases the rate of removal of cholesterol removal in the form of bile acids, increases HDL levels, and plays a role in reducing bile acids’ re-absorption. The content of vitamin C in dragon fruit can prevent coronary heart disease by inhibiting LDL oxidation, and vitamin C have the effect of avoiding HDL damage due to lipid peroxidase, free radical formation, and increased bile acid excretion.26,27,28 The anthocyanin content of 8.8 mg/100gr is useful for reducing the risk of cardiovascular disease. The anthocyanin content in dragon fruit can also provide a cholesterol-lowering effect by inhibiting CETP and inhibiting the HMG-CoA reductase enzyme. Therefore, cholesterol concentrations in the liver and plasma are expected.11,29 Nurses can promote complementary therapies through consumption of red dragon fruit which is used to keep blood cholesterol levels normal. This can be used as a preventive measure in the occurrence of other diseases.

This study had several constraints. The difficulty in this research is maintaining the freshness of the juice to be given. Good juices are those that are made and consumed at a distance of no more than 2 hours. To overcome this, the researchers prepared the juice directly in front of the respondents.

**Conclusion**

The results showed that red dragon fruit effectively reduced total cholesterol levels in people of excessive nutritional status. This study’s results can further increase the intake of healthy nutrients from fruit to keep cholesterol levels regular. Normal cholesterol levels can prevent dangerous metabolic diseases.

**Conflict of Interest:** There is no conflict of interest

**Source of Funding:** Nil

**Ethical Clearance:** Taken from Ethics Commission for Health Research, Faculty of Health Sciences, Respati University Yogyakarta
References


Physical Exercise in Clinical Stage II Human Immunodeficiency Virus Infection Patients’ Increases Skeletal Muscle Mass Through the Increasing of Myogenic Regulatory Factors Expression

Sri Mardjiati Mei Wulan¹, Hening Laswati¹, Usman Hadi², Nasronudin Nasronudin²,³
¹Lecturer at The Department of Physical Medicine and Rehabilitation, Faculty of Medicine Airlangga University/ Dr. Soetomo General Hospital, Surabaya Indonesia, ²Lecturer at The Department of Internal Medicine, Faculty of Medicine Airlangga University/ Dr. Soetomo General Hospital, Surabaya Indonesia, ³Lecturer at Airlangga University Hospital, Faculty of Medicine Airlangga University Surabaya Indonesia

Abstract

Background: Human Immunodeficiency Virus infection is a chronic disease that threatens the health of millions of people in the world and causing disability. One of the factors that caused disability is HIV muscle wasting, causing a decrease in quality of life that interfere the daily activities and even increased mortality. Proper physical exercise is needed to prevent and treat muscle wasting.

Methods: This study using human subjects with clinical stage II HIV infection. Subjects were grouped into two: Intervention (n = 9) that subjects who get moderate-intensity physical exercise (HIVA) for 8 weeks and Control (n = 9) as subjects were observed for 8 weeks. Muscles samples were taken from the vastus lateralis muscle biopsies that performed 24 hours after the last physical exercise, immunohistochemical examination with monoclonal antibody anti-Pax7, anti-MyoD1 and anti-myogenin and measurement of thigh circumference.

Results: There were significant increase in regeneration of skeletal muscle in the intervention group (increasing of Pax7, MyoD1 and myogenin) than the control group and significant increase in quadriceps muscles mass as measured by thigh circumference (p < 0.001).

Conclusion: HIVA physical exercise of moderate intensity increases skeletal muscle regeneration in clinical stage II HIV infection through the increasing of Pax7, MyoD1, myogenin expression and followed by an increase of quadriceps muscles mass.

Keyword: HIV, Rehabilitation Exercise, Myogenic Regulatory Factors, Indonesia.

Introduction

Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome (HIV/AIDS) is a health risk for millions of people who are currently a pandemic.

The percentage of HIV infection in Indonesia, the highest reported in the age group 25-49 years (69.1%)¹, leads to reduced life quality due to muscle wasting. Hence, people with HIV / AIDS cannot perform daily activities and participate in society’s social members. People with HIV / AIDS will experience impairment and disability due to their progression ². The impact of HIV infection in the form of muscle-wasting disease progression, increased mortality, loss of muscle protein mass, weakness, impaired function ³,⁴, and decreased aerobic capacity ⁵ were also a predictor of mortality.
The quadriceps muscle is one muscle that is essential to maintaining the function of ambulation, so it needs physical exercise to prevent the progression of muscle wasting and stimulate the regeneration process\(^3\). Vastus lateralis muscle represents quadriceps muscle because the strength is essential for weight-bearing and ambulation. It can show the response to physical exercise intervention and the biopsy’s safest\(^6\).

Skeletal muscle mass represented a person’s physical performance\(^7\). Physical exercise is known to increase muscle mass, improve protein balance through a mechanical signal, chemically maintain or increase muscle mass\(^8\), and increase muscle strength. Skeletal muscle strength is a determinant of a person’s functional capacity, providing a higher level of independence and quality of life\(^9\). In this study, patients with HIV infection clinical stage II performed HIVA aerobic physical exercise (The name of tailored physical therapy is HIVA). This study aimed to compare the effect of HIVA exercise in skeletal muscle regeneration and muscle mass in HIV infection clinical stage II patients through increased expression of Pax7, MyoD, and Myogenin.

**Materials and Methods**

**Participants**

The subjects in this study were male of HIV infection clinical stage II, aged 21-50 years, who came to the outpatient clinic of infectious unit Dr. Soetomo General Hospital. Human Immunodeficiency Virus infection clinical stage II is HIV infection defined by WHO criteria \(^10\). All study subjects get antiretroviral therapy. The ethics committee of Dr. Soetomo General Hospital Surabaya approved the study protocol of this research. (No: 258/Panke.KKE/IV/2015).

All participants in this study provided written informed consent.

**Physical Exercise Protocol.**

The subjects in the intervention group were given a physical exercise as aerobics with a frequency of 2 times per week, intensity 60-70% of maximum heart rate, duration of 23 minutes (warm-up, and stretching 6 minutes, the core exercise 13 minutes and cooling-down 4 minutes) for eight weeks. The time adjusted with an intensity of 60-70% maximal heart rate.

**Vastus lateralis muscle biopsy procedure.**

Materials needed in this study are: muscle tissue biopsy results of vastus lateralis, anti-TNF-α monoclonal antibody clone 52B83, Novus Biological product (NB600-1422), anti-calcineurin monoclonal antibody clone 2G8 Novus Biological product (H0005530-M03), anti-Pax7 monoclonal antibody clone 1E12 Novus Biological product (H00005081-M05), anti-NF-κB p65 monoclonal antibody clone 112A1021 Novus Biological product (NB100-56712), anti-MyoD1 monoclonal antibody clone 5.8A Dako product (M3512),

At the point of the bottom third of the line connecting the SIAS-patella performed muscle biopsy. Local anesthesia by injection of Lidocaine 2%, skin sterilized with povidone-iodine. Muscle biopsy using a reusable Medcore gun and Unicore needle size 16G. Specimen storage with formalin 10%. Each slide of the vastus lateralis muscle was viewed under light microscopy, 400 times magnification, counted the number of satellite cells showing Pax-7, Myo D, and Myogenin in 10 fields of view, then the averaged per field of view.

**Statistic Analysis**

Statistical analysis is using the SPSS program. To determine the homogeneity of the data in each group, using the one-sample Kolmogorov-Smirnov test and determining the data normality using the Shapiro-Wilk test. Thigh circumference before and after the physical exercise comparison in each group using a paired t-test. The alteration of thigh circumference after the physical exercise among the two groups used the Lavene test. The significance level was at \( p \leq 0.05 \).

**Results**

The characteristics of the subjects showed in Table 1. Before intervention, there were no significant differences between age, Body Mass Index, and Thigh
Circumference between the intervention and control groups.

### Table 1. Characteristics of subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Intervention</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Min</td>
</tr>
<tr>
<td>Age (year)</td>
<td>9</td>
<td>27.00</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>9</td>
<td>19.40</td>
</tr>
<tr>
<td>TC (cm)</td>
<td>9</td>
<td>42.00</td>
</tr>
</tbody>
</table>

BMI: Body Mass Index, TC: Thigh circumference, SD: Standard Deviation, N: Number of subjects, kg: kilogram, m²: meter square, cm: centimeter

Table 2. Showed thigh circumference by measuring the thigh loop at the distal third of the line connecting the SIAS with the mid patella in-unit cm. Before and after intervention there was significant difference of thigh circumference p≤0.001.

### Table 2. The thigh circumference before and after intervention

<table>
<thead>
<tr>
<th>Thigh circumference</th>
<th>Intervention mean (SD)</th>
<th>Control mean (SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre (cm)</td>
<td>46.7 (3.7)</td>
<td>38.2 (7.5)</td>
<td>0.008</td>
</tr>
<tr>
<td>Post (cm)</td>
<td>50.9 (4.0)</td>
<td>38.5 (7.6)</td>
<td>-</td>
</tr>
<tr>
<td>Alteration (cm)</td>
<td>4.2 (1.5)</td>
<td>0.3 (0.)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>0.247</td>
<td></td>
</tr>
</tbody>
</table>

Note: significance level at p <0.05, cm: centimeter

SD: Standard Deviation,

Table 3. Showed Pax7, MyoD1, and Myogenin expression in satellite cells before and after intervention, there were a significant different of myogenesis factors before and after intervention p≤ 0.05.

### Table 3. Pax7, MyoD1, and Myogenin expression in satellite cells

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention (SD)</th>
<th>Control (SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pax7 (mean cells/field of view)</td>
<td>0.34 (0.14)</td>
<td>0.11 (0.10)</td>
<td>0.002</td>
</tr>
<tr>
<td>MyoD1 (mean cells/field of view)</td>
<td>0.30 (0.13)</td>
<td>0.10 (0.10)</td>
<td>0.010</td>
</tr>
<tr>
<td>Myogenin (mean cells/field of view)</td>
<td>0.53 (0.21)</td>
<td>0.25 (0.15)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Note: significance level at p <0.05, SD: Standard Deviation
Figure 1. Showed the result of Pax7, MyoD1, and myogenin expression of the intervention group obtained from the right vastus lateralis muscle biopsies after HIVA physical exercise followed for eight weeks, and the control group after eight weeks of observation.

**Discussion**

Skeletal muscle regeneration is a highly regulated process, very complicated. It involves many factors in the process, among other specific muscle genes, satellite cells, and extracellular matrix was instrumental in muscle fibers reconstruction. The synthesis and degradation of proteins unbalanced also leads to the loss of muscle mass. Research shows that more than a standard protein diet: 0.8 g/kg/day reduces skeletal muscle mass and the condition of negative protein balance. Malnutrition in HIV infection causes a decrease in the immune response and progression of HIV. The state of malnutrition causes endocrine changes, which led to the mobilization of amino acids, especially skeletal muscles that affect skeletal muscle regeneration.

Skeletal muscle regeneration is mediated by satellite cells, although generally in calm conditions. Satellite cells become active due to muscle damage, subsequently undergo proliferation, differentiation, and fusion to form new muscle fibers, resulting in tissue regeneration and restoration to normal function. Satellite cells closely associated with Pax7, and together with MyoD, determine the fate of satellite cells. Pax 7 expression on calmsatellite cells, and the muscle injury, will stimulate satellite cells to proliferate and rapidly induces the expression of MyoD (Pax7+/MyoD+). Most satellite cells active, differentiated, ensued repression Pax7 and increased expression of Myogenin, and MRF4, a small portion back to the conditions (Pax7+/MyoD-) who responded the next muscle injury, and repair. Muscle regeneration via the activation of many signaling pathways. Activation of Notch signaling increase satellite cells to self-renew and inhibits differentiation by suppressing the expression of MyoD. Notch and Wntsignaling play an essential role in myogenesis, and Wntsignaling stimulates the expression of Pax7.

Physical exercise as a non-pharmacological therapy in people living with HIV in Indonesia has not become standard therapy. There is no formal physical training for people living with HIV on the intensity, duration, frequency, and type. The treatment given to people living with HIV in this study is regular physical exercise as aerobics HIVA, 2x per week frequency, duration of 23 minutes of physical exercise, moderate-intensity 60-70% of maximal heart rate, type of training with weight-bearing activities, for eight weeks or 16 times. The profile of HIV infection is continually changing, and some people regard it as a progressive disease and lethal.
Most people living with HIV who get antiretroviral therapy showed a chronic illness and treatable. Chronic conditions reflect an increase in disability in people living with HIV\textsuperscript{18}, but physical exercise is a crucial strategy for overcoming disabilities.

Paired box transcription factor (Pax7) activate transcription and controls the expression of a myogenic regulatory element in the quiet and active satellite cells \textsuperscript{19}. Paired box transcription factor (Pax7) expression increased within 24 hours after muscle injury and when the satellite cells have been heterogeneous. Research McKay et al. \textsuperscript{20} showed that satellite cell humans have always shifted in the cell cycle, from the phase G\textsubscript{0}/G\textsubscript{1} to S and G\textsubscript{2}/M within 24 hours after contractions that cause muscle injury. In the first 24 hours after muscle contraction, obtained Pax7 increase (36\% in phase G\textsubscript{0}/G\textsubscript{1}), then Pax7 + (59\% in S phase), and the next satellite cells obtained Pax7 + (202\% in G\textsubscript{2}/M phase). These data indicate that the development of satellite cells in the cell cycle lasted 24 hours until at least 72 hours after muscle injury\textsuperscript{21}. This condition means that the active satellite cells did not coincide but in varying cell cycle phases\textsuperscript{20}. In adult muscle under physiological conditions, satellite cells express Pax7. Satellite cells are ready to respond to molecular stimuli from physical training, injuries, and diseases. They can self-renew, extend themselves, proliferation as myoblast or myogenic differentiation for fusion, and restore muscle damage. Satellite cells are the primary contributors to the growth, maintenance, and repair of skeletal muscle after birth \textsuperscript{22, 23}. Some studies in mice have shown that Pax7- will lose satellite cell lineage in all muscle groups\textsuperscript{16, 24} by contrast, research Lepper et al. \textsuperscript{25} reported that Pax7 does not need the normal function of satellite cells anymore after adolescence.

Increased Pax7 expression in this study because muscle contractions produce IGF-1, which stimulates anabolic and myogenic processes and plays a role in modulating muscle growth’s size. Insulin-like growth factor-1 stimulates the metabolism of protein in muscle fibers and increases myonuclei’s number through the proliferation, differentiation, and fusion of satellite cells\textsuperscript{26}. Liu et al.\textsuperscript{27} show that muscle activity and weight-bearing were influential in increasing the level of IGF-1 muscle.

This study indicated that satellite cells’ activation increased significantly in the intervention group (Pax7, \(p = 0.002\), and MyoD1, \(p = 0.010\)). Biopsy in this study was conducted 24 hours after the last physical exercise. Each marker of active satellite cell Pax7 and MyoD1 showed an increase that is not the same. These data concluded the phase difference, some satellite cells in a phase of activation and are already experiencing proliferation. Increased expression of Myogenin showed differentiation and fusion. The myogenesis process is dynamic so that the steps of activation, proliferation, differentiation, and fusion are always changing. These data showed the most significant increase in myogenin expression, which means there is already a fusion of new muscle fibers in the biopsy 24 hours after moderate-intensity HIV-A physical exercise for eight weeks.

Myogenic differentiation factor D (MyoD) is a protein that plays a vital role in regulating muscle differentiation \textsuperscript{28}. MyoD is one of the early markers of myogenic commitment, expressed at low levels in quiet satellite cells and satellite cell fusion. This study showed that the expression of MyoD1 in the intervention group is higher than in the control group. These results are consistent with Charge and Rudnicki’s \textsuperscript{22} research, which shows an increased expression of MyoD in satellite cells and muscle fibers mature in cell proliferation and differentiation for subsequent cell regeneration hypertrophy.

Several myogenic regulators could examine muscle regeneration evaluation after the physical exercise in the vastus lateralis muscle. Satellite cell activation is an essential key in muscle regeneration. During regeneration, satellite cells undergo proliferation, differentiation, and fusion to form new muscle fibers, and it is necessary to compensate for muscle hypertrophy\textsuperscript{29, 30}. The MRF controlled Muscle regeneration (MyoD, Myogenin, and MRF5). MRF plays a role in the regulation of muscle response to changes in physical exercise or activity.
This study increased MyoD1 and Myogenin after HIVA physical exercise as monitor satellite cell activation to physical activity. Myogenic differentiation D (MyoD) is an important regulator involved in skeletal muscle adaptation to HIVA exercise. Research by Kosek et al. shows increased expression of MyoD after physical activity. On activation of satellite cells, MyoD increased within 12 hours of activation or physical exercise. Increased MyoD until more than 36 hours, and would be significantly reduced after one week. In this study, increased expression of MyoD has occurred 24 hours after the last physical exercise. Pislander et al. also show similar results that find an increase in temporary (transient) in the expression of MyoD in response to muscle strengthening exercises.

Contrary to the role of MyoD, Myogenin plays an essential role in muscle differentiation, consistent with a subsequent regulatory function in the process of particular types of muscle fibers. Myogenin expression increased significantly from the results of the biopsy were performed after eight weeks of physical exercise. This research shows that Myogenin increased within one week after physical therapy; these data are similar to those reported by Carson and Booth.

Physical exercise can influence the muscle fibers’ function, modify the structure, metabolism, and increase growth factors. Other signals are paracrine for the activation of satellite cells. These data increased expression of Pax7 and MyoD1 so that the HIVA physical exercise, going on satellite cell activation causes an increase in skeletal muscle regeneration. Increased Pax7, MyoD1, and Myogenin mean that the provision of HIV A physical exercise repeatedly improved the regeneration of skeletal muscle indicated with activation and proliferation (increased expression of Pax7 MyoD1) and satellite cell differentiation (increased expression of Myogenin).

Conclusions

Moderate-intensity HIVA physical exercise in a clinical-stage II HIV infection increases skeletal muscle regeneration. The addition of muscle mass to people living with HIV prevents HIV progression of muscle wasting.

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The authors have no conflicts of interest to declare

Source of Funding: Self

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Professional Socialization among Nursing Students

Sruthi M1, Aleena Baiju2, Swathi Krishna T P2
1Lecturer, Department of Mental Health Nursing, 2B.Sc. Nursing Students, Amrita College of Nursing, Amrita Viswa Vidyapeetham, Kochi, Kerala, India

Abstract

Objective: The objectives of our study is to determine the professional socialization among nursing students, to find out the association between professional socialization and demographic characteristics and to find the subgroup comparison of level of professional socialization among outgoing batch students and first year B.Sc. nursing students

Methodology: A descriptive study was conducted among all four years of nursing students of a private nursing college. Nursing students who meet the inclusion criteria (357) were selected using purposive sampling.

Results: The majority of the students are ‘Experts’ in maintaining professional socialization. But in the subgroup comparison of level of Professional socialization among the final year students with first year showed a 1.1% less in ‘Expert’ category. The results showed a significant association of level of professional socialization with ‘Gender’, ‘Mode of Admission’ and ‘Reason for selecting nursing as a profession’

Conclusion: The need for developing professional socialization has to be considered at the most priority. The results of this study shows the need for introducing new plans to make sure that the students level of socialization

Keywords: Professional Socialization, Autonomy, Competence, Commitment, Group participation, Nursing Students.

Introduction

Socialization is the process during which people learn the roles, statuses and values necessary for participation in social institution. Socialization is a lifelong process that begins with learning the norms and the roles of the family and subculture and making self-concept as individual grow older and join new groups and assume new roles, they learn new norms and redefine their self concept1.

Corresponding Author:
Sruthi M
Lecturer, Department of Mental Health Nursing
Amrita College of Nursing,
Amrita Viswa Vidyapeetham Kochi, Kerala, India
Email ID: sruthilohith@gmail.com
Tel No.+919544290900

Nursing as the largest part of professional personal in forefront line of delivering services in health care systems have multiple and expanded roles and duties. The roles of nursing are multiple and complex and nursing as a professional work require responsibility, attention and caution2. Development of the concept of socialization in nursing literature dates back to before 19853 and socialization is described as a major issue in nursing4.

Socialization is a process of acquiring individual identity in which he learns required values, norms and skills for serving his social task as an effective person his unique position5. Socialization a basic concept in nursing that is due to interaction among interpersonal, intrapersonal and work functional relationships6. These relationships give members the opportunity to organize themselves and their resources and solve their problems
by cooperation. In fact, professional socialization is a learning process of the professional roles. It consists of anticipatory, accommodation, and role management. The process to achieve required professional norms, values, and skills for professional survival is not well known.

A study was conducted in Ilam University of Medical Sciences in 2016 for assessing the level of professional socialization in nursing students. 130 nursing undergraduate students were studied, in that 21.4% of them have average socialization level and 78.6% were at high level, it was assessed based on the developed questionnaire.

A similar study was done on concept analysis of professional socialization in nursing. And they were able to identify that professional socialization varies depending on time, context, and different disciplines. The result of the study describes professional socialization as a complex, inevitable, diverse, dynamic, continual, and unpredictable process. In addition, they found that for individuals to be successfully socialized into their profession, measures such as the provision of comprehensive educational programmes, competent role model, supportive clinical and educational structures, and opportunity for field experience and constructive feedback are some determining factors.

Another study which was conducted among the students and graduate nurses and the results show that the most significant time of stress for student and graduate nurses is when in the clinical practice environment, new comers express that learning how to behave appropriately in the workplace is more difficult than bridging the gap between the theory and practice.

Professional attitude and commitment is seen decreased as compared to the older times among nursing professionals. Social behavior must be built among nurses from the student period onwards. Proper identification and processing of the determinants of professional socialization like knowledge, skills, and attitude in early stage of nursing can acquire professional identity, adapting with the professional role, professional, and organizational commitment and hence improve the overall nursing care quality and interpersonal relationship.

A qualitative research study on professional socialization was conducted among undergraduate nursing students and registered nurses with work experience with 8 to 25 years. The finding shows that nursing profession still struggles with problems that can alter professional identity and there should be an increase in providing educational experience to improve professional identity.

The results of all these research studies show the significance of studying about the professional socialization in undergraduate nursing students and hence to provide interventions to improve socialization in nursing profession.

As the nursing profession is in need to improve in their socialization with their co-workers and with the patients and relatives, the significance of studying professional socialization is increasing. Thus, this study is needed to assess the professional socialization of nursing students and to improve the nursing care quality.

**Methods and Materials**

The aim of the study was to assess the professional socialization among undergraduate nursing students which is developed during the nursing undergraduate period.

We started data collection on 3/10/2020. The consent and the data were collected through google forms. Students were given the google form links after 4 pm. From the purposive sampling the students who didn’t meet the inclusion criteria and those who were not willing to participate in the study are not included in the research study.

The study questionnaire was developed from previous research study on professional socialization, whose result had indicated some variables that influence the professional socialization process. These questionnaires were validated by different educators.
The study questionnaire was consisted of 5 parts: demographic data, professional autonomy, professional commitment and belonging, group participation, and professional competence. The study was conducted in 357 undergraduate nursing students through developed questionnaire.

The result scoring are based on Benner’s stage on clinical competence. Total we have 40 questions and the minimum score is 40 and the maximum score is 280. According to the Benner’s stage of clinical competence, the results could be divided into 5 stages: 40-88 novice, 89-137 advanced beginners, 138-184 competent, 185-232 proficient, and 233-280 expert.

Descriptive statistics and inferential statistics is done for data analysis. The tests used were frequency, percentage, mean and Standard Deviation for finding the distribution of personal characteristics. Independent t-test and Analysis of Variance (Anova) for finding the association between variables. SPSS software 18 is used for analysis and the significance level considered was $p \leq 0.05$.

### Results

**Table 1. Personal Characters of Nursing Students**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 to 18 years</td>
<td>12</td>
<td>3.36%</td>
</tr>
<tr>
<td>19 to 20 years</td>
<td>195</td>
<td>54.62%</td>
</tr>
<tr>
<td>21 to 22 years</td>
<td>134</td>
<td>37.54%</td>
</tr>
<tr>
<td>23 and above</td>
<td>16</td>
<td>4.48%</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>348</td>
<td>97.48%</td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>2.52%</td>
</tr>
<tr>
<td><strong>Religion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hindu</td>
<td>273</td>
<td>76.47%</td>
</tr>
<tr>
<td>Christian</td>
<td>80</td>
<td>22.41%</td>
</tr>
<tr>
<td>Muslim</td>
<td>2</td>
<td>0.56%</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
<td>0.56%</td>
</tr>
<tr>
<td><strong>Year of Study</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd year A batch</td>
<td>47</td>
<td>13.17%</td>
</tr>
<tr>
<td>2nd year B batch</td>
<td>48</td>
<td>13.45%</td>
</tr>
<tr>
<td>3rd year A batch</td>
<td>48</td>
<td>13.45%</td>
</tr>
<tr>
<td>3rd year B batch</td>
<td>44</td>
<td>12.32%</td>
</tr>
<tr>
<td>4th year A batch</td>
<td>53</td>
<td>14.85%</td>
</tr>
<tr>
<td>4th year B batch</td>
<td>45</td>
<td>12.61%</td>
</tr>
<tr>
<td>Outgoing batch</td>
<td>72</td>
<td>20.17%</td>
</tr>
<tr>
<td><strong>Mode of Admission</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Through merit quota</td>
<td>285</td>
<td>79.83%</td>
</tr>
<tr>
<td>Through management quota</td>
<td>72</td>
<td>20.17%</td>
</tr>
</tbody>
</table>
Cont... Table 1. Personal Characters of Nursing Students

\[ n = 357 \]

<table>
<thead>
<tr>
<th>Reason for selecting nursing as a profession</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compulsion from the family</td>
<td>44</td>
<td>12.32%</td>
</tr>
<tr>
<td>like to work in the service sector</td>
<td>198</td>
<td>55.46%</td>
</tr>
<tr>
<td>Dream / Passion</td>
<td>106</td>
<td>29.69%</td>
</tr>
<tr>
<td>Didn’t get admission for any other course</td>
<td>9</td>
<td>2.52%</td>
</tr>
<tr>
<td>Extra Curricular Activities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>229</td>
<td>64.15%</td>
</tr>
<tr>
<td>No</td>
<td>128</td>
<td>35.85%</td>
</tr>
<tr>
<td>Academic results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not attended any university exam</td>
<td>86</td>
<td>24.09%</td>
</tr>
<tr>
<td>Passed all subjects</td>
<td>240</td>
<td>67.23%</td>
</tr>
<tr>
<td>Attending ongoing supplementary exams</td>
<td>31</td>
<td>8.68%</td>
</tr>
</tbody>
</table>

The personal characteristics shows that majority (54.62%) of the samples belongs to the age group of 19 to 20 years and 348 of them (97.48%) where females. The Majority 76.47% of the samples were Hindus 27.46% were from the third year batch. The best part (79.83%) were admitted through merit quota, who liked to work in service sector (55.46%) and who have passed all subjects in the university exam (67.23%)

Professional socialization of nursing students

Table 2: Level of professional socialisation among nursing students

\[ n = 357 \]

<table>
<thead>
<tr>
<th>Scoring</th>
<th>Frequency</th>
<th>Percent</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competent</td>
<td>2</td>
<td>0.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proficient</td>
<td>65</td>
<td>18.2%</td>
<td>246.08</td>
<td>17.14</td>
</tr>
<tr>
<td>Expert</td>
<td>290</td>
<td>81.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>357</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to Benner’s Clinical Competency Scale, the structured tool for assessing professional socialization was graded as Novice (40 – 88), Advanced Beginner (89 – 137), Competent (138 – 184), Proficient (185 – 232) and Expert (233 – 280). From the study, the examined samples (n=357) showed a result that a lion’s share (81.2%) were Experts in Professional socialization were as the outvoted were only (2%). This is an inarguable result that clearly emphasizes how the samples taken from the particular college is keeping a high level of socialization in nursing profession. The mean is 246.08 and SD is 17.14. There are no students in the categories Novice and Advanced beginner.
Association between professional socialization and personal characteristics

Table 3: Association between professional socialization and personal characteristics

n = 357

<table>
<thead>
<tr>
<th>Personal Characteristics</th>
<th>Levene’s test for equality of variances</th>
<th>T – test for equality of Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>Sig.</td>
</tr>
<tr>
<td>Age</td>
<td>0.755</td>
<td>0.52</td>
</tr>
<tr>
<td>Gender</td>
<td>0.309</td>
<td>0.579</td>
</tr>
<tr>
<td>Religion</td>
<td>1.086</td>
<td>0.355</td>
</tr>
<tr>
<td>Year of study</td>
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<td>0.699</td>
</tr>
<tr>
<td>Mode of admission</td>
<td>0.25</td>
<td>0.618</td>
</tr>
<tr>
<td>Reason for selecting nursing</td>
<td>3.22</td>
<td>0.023</td>
</tr>
<tr>
<td>Extracurricular achievements</td>
<td>2.44</td>
<td>0.119</td>
</tr>
<tr>
<td>Academic results</td>
<td>1.678</td>
<td>0.188</td>
</tr>
</tbody>
</table>

*Significant at the level < 0.05

Table 3 narrates the association of level of professional socialization and personal characteristics which shows that there is a significant association between gender, mode of admission, reason for selecting nursing and level of professional socialization at p ≤ 0.05 interval.

Sub group comparison of level of Professional socialization among students of fourth year and first year BSc nursing
Table 4: Subgroup comparison of level of Professional socialization

<table>
<thead>
<tr>
<th>Year of study</th>
<th>Level of Professional Socialization</th>
<th>Total</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Percentage</td>
<td>Frequency</td>
</tr>
<tr>
<td>First year A &amp; B batch</td>
<td>1</td>
<td>1.1%</td>
<td>19</td>
</tr>
<tr>
<td>Fourth year</td>
<td>1</td>
<td>1.4</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 4 represents the subgroup comparison of level of professional socialization of first year B.Sc. Nursing and fourth year B.Sc. Nursing students shows that 78.9% of first year students shows expert socialization whereas 77.8% of fourth year shows expert socialization. The mean value of samples in first year is 244.06 with a SD 19.03 and that of fourth year is 245.26 with a SD of 18.02.

Table 5: Independent t-test results

<table>
<thead>
<tr>
<th>Levene’s test for equality of variances</th>
<th>T – test for equality of variances</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>Sig.</td>
</tr>
<tr>
<td>Equal variances assumed</td>
<td>0.15</td>
</tr>
<tr>
<td>Equal variances not assumed</td>
<td>-0.278</td>
</tr>
</tbody>
</table>

Table 5 illustrates the results of independent t-test, clearly depicts that there is no significance at the level of p <0.05 because the Sig.(2 – tailed) is 0.783 which is > 0.05 p value. Hence there is no significance in the level of professional socialization of first year and fourth year B.Sc. nursing students when they are compared.

Discussions

Nursing is a health care profession which differ from other healthcare providers by their approach to patients and scope of practice. They identifies the issues in each patients individually and plans care, implements it with interdisciplinary collaboration. Hence nurses
needs to collaborate with other health care providers throughout their care and here is the importance of studying Professional Socialization among student nurses. The objectives of the study were to assess the level of professional socialization among the samples, to find its association with personal characteristics and to find subgroup comparison of level of professional socialization with first year and fourth year B.Sc. Nursing students. The study was conducted among 357 samples out of 375 total students of a nursing college who were selected by purposive sampling.

The assessment of level of professional socialization among the students shown that the majority of the students came under the category of experts (81.2%) and only 0.6% came as competent. Also it has to be noted that no students came in the first two levels, ie; novice and advanced beginner, which is an excellent result that shows the students are fit enough to be in nursing profession.

A study done by Nebhinami M Kumar and et.al on Stress and Coping Strategies among undergraduate students, in Western Rajasthan says that students are facing lot of stress during their academic period and active coping was used as the most common coping strategies. This result is quoted here to exclaim that his study samples also may be using such coping strategies for overcoming stress and this helped the majority to keep the professional socialization at its maximum.

The association of level of professional socialization and personal characteristics shown that there is a significant association with ‘gender’, ‘mode of admission’ and ‘reason for selecting nursing as a profession’. A study done by Cherkil S and et.al on coping styles and its association, done in medical students also have a similar association. The study results shown a positive results on ‘self-expectation’ and stress score. This result can be read along with the results of present study that ‘reason for selecting nursing as a profession’ and ‘self-expectation’ brings up the same meaning at the end.

When the third objective, subgroup comparison of level of professional socialization, was done with first year and fourth year B.Sc. Nursing students, the analysis gave the result that there is no significance in the comparison. The results also shown a 1.1% more experts in first year than fourth year. This difference need to be addressed. A few studies will give a support to this result. One study was done by Raghunathan D et.al among dental students on prevalence of depression and the other study was done by Sreedevi P A et.al in nursing students of the same college on correlation of assertiveness and self-esteem of undergraduate students. The first study shows the result of a few protective factors and a few risk factors and concludes with the call for implementing interventions for those at high risk of developing depression. The second study which was done on nursing students came out with the result of need for including assertiveness training in nursing education programme. Both the above said studies shows how the extraneous variables are affecting the students on their academics and on their mind. Farrell SM et.al had done a study on wellbeing and burnout in medical students and the study concludes that majority of the samples experience burnout and this needs to be addressed.

All the above quoted studies gives the picture that even though the research results are near to the accepted ones, the unnoticed variable areas surrounding the samples also need to take into account.

**Conclusion**

Nursing is one among the precious profession which is emerging day by day, with the advancement in science and technology and with the increasing needs of human population. The need for developing professional socialization has to be considered at the most priority. The results of this study shows the need for introducing new plans to make sure that the students level of socialization is keeping its needed status and the students are able to practice this in their professional life also.

**Ethical Clearance:** Taken from Institutional Ethical Committee of Amrita Institute of Medical Sciences, Kochi
Source of Funding: Self

Conflict of Interest: Nil

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11. Ladanzarshenas, farkhondeh sharif,[..] and abbasebadi professional socialization in nursing; content analysis.

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Case Report

Management of Urolithiasis in Cat with Special Diets

Steven Taufic Leo¹, Lita Rakhma Yustinasari², Juliano Mwenda Ntoruru³

¹Veterinary Practitioner, Sunset Vet Clinic, Kuta Bali, Indonesia, ²Lecturer, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia, ³Research Assistant, Meru University of Science and Technology, Kenya

Abstract

Introduction: The current study is focused on the treatment of a cat with urolithiasis. The main goal is to help provide a greater understanding of how to treat the cat with special diets.

Case Description: Struvite urolithiasis was found in a 3-year-old female domestic short-haired cat with the complaint of straining for a week during urination. Ultrasonographs indicated the existence of two calculi in the bladder that were also detected by radiographic examination (7 mm in diameter each). Bladder stones were getting smaller by dissolution diets therapy. In this case, the cat was treated by melox 1.5 mg/ml (dosage 0.1 mg/kgBW) for 14 days, tramadol for 28 days (2mg/kg BW) and also special diets (struvite).

Conclusion: After a month treatment, the stones were almost cannot be seen radiographically. This report describes a cat with urolithiasis case which was successfully treated with special diets for struvite without any surgery.

Keywords: cat, struvite, dissolution diets therapy, urolithiasis

Introduction

Urolithiasis in cats can result in morbidity and even mortality for affected animals; the most common uroliths in cats (more than 80-90%) are struvite (magnesium ammonium phosphate) and calcium oxalate. Diet is very important for both treatment and prevention of urinary calculi, and some specifically formulated diets can promote struvite stone dissolution and reduce its recurrence. Most experts agree that struvite stones should be medically dissolved (unless contraindicated) with dissolution foods and/or medication. Struvite uroliths in cats appear to be between 2 and 7 years of age and female cats appear to have higher increased risk. The knowledge gained about urolithiasis is an outstanding achievement towards the treatment and control of the disease. However, eradication of the disease is the most challenging as it requires total examination of all the factors that are responsible for the formation of uroliths.

Case History and Clinical Observation

A 3-year-old female domestic short-haired cat weighing 15 kg was presented to Bali Vet Clinic, Kuta Bali, Indonesia with the history of straining since a week during urination and after urination she seems like dragging her bum on the floor. The cat was housed indoors and fed a commercially dry food with ad libitum drinking water. The cat was vaccinated periodically. Physical examination of the animal revealed pale mucous membrane with gingivitis and some plaque as well, tartar grade 2/5. Other vital signs such as blood pressure, body temperature, heart and respiratory rate were normal.
Abdominal palpation was detected the pain in belly area and a sensation was felt when the bladder is palpated. Struvite crystal and no bacteria were found on sediment examination. Urinalysis was not being evaluated because the cat’s owner agrees for sediment examination only. The cat was suggested to do a complete radiographic examination of the tract; radiopaque calculi about two stones (7 mm each in diameter) were visible inside the bladder. Ultrasonographic examination showed a thickened bladder wall. A cystotomy was not performed because the cat’s owner refused a surgery.

**Clinical Evaluations and Management**

A diagnosis of urolithiasis was made based on the radiographic and urine sediment examination. We offered two options: doing surgery or treatment with special diets for struvite. The cat’s owner decided to try medication and also food s/d (struvite) first. We suggested to observe the cat’s condition in a month and come for ultrasonograph and another radiographic as well. If the stones not getting smaller then may need surgery. The cat was properly treated with melox 1.5 mg/ml (dosage 0.1 mg/kgBW) for 14 days, tramadol for 28 days (2mg/kg BW) and also special s/d food (struvite) at home. We need transition periods over at least 1 week from the normal diet to the dissolution diet. We reminded owner that possibility for blockage (because this stone could go to urethra and stuck there). Reradiograph cats on dissolution diets was done after 30 days. The stones almost cannot be seen due to tiny on (Fig. 1). Ultrasonographic showed the stone’s size becomes < 3 mm in diameter. We suggested to the cat’s owner for maintaining her condition with special food (for struvite calculi) until the stones were no longer visible radiographically.

![Fig. 1. Radiograph image before treatment (A) showed a several calculi inside the bladder; after 30 days treatment with dissolution diets (B) showed that calculi almost could not be seen.](image)

**Discussion and Conclusion**

Urolithiasis is a nutritional disease that attacks domestic carnivores. Three main contributing factors which important to mechanisms involved in urolith formation are matrix, crystallization inhibitors, and precipitation crystallization factor. Uroliths are not produced unless sufficiently high urine concentrations of urolith-forming constituents exist and transit time of crystals within the urinary tract is prolonged. For struvite stones, proper pH must also exist. These criteria can be affected by urinary tract infection, diet, intestinal absorption, urine volume, frequency of urination, therapeutic agents, and genetics.8
Nutritional management is the best preventive strategy against urolithiasis. As such it may not replace the surgical procedures but may surely help in decreasing the recurrence rate of uroliths, since long-term pharmacological therapy and its potential side effects often lead to subsequent failure. Struvite dissolution diets are magnesium-reduced, acidifying diets. Struvite dissolution diets do not dissolve non-struvite uroliths and are less effective if a persistent UTI is present or the cat is fed anything in addition to the dissolution diet. The rate at which uroliths dissolve is proportional to the surface area of the urolith exposed to the undersaturated urine. Cats fed with low-magnesium, urine-acidifying dry foods had successfully reduction in urolith size at 2 weeks.4,9

In conclusion, dissolution diets therapy is safe and effective in management of sterile struvite calculi in cats. The key goals of dissolution diets include manipulation of urine pH < 6.0 and reduction of dietary magnesium. Early detection of recurrence may allow nonsurgical therapies to be used. Ultrasonography and radiography are complementary to each other where prediction can serve as an alternative to distinguish urolith mineral composition whenever performing surgery is difficult to retrieve the urolith in an unstable patient to choosing medical treatment.12

Conflict of Interest: All authors declared that there is no conflict of interest.

Funding: Self-funding.

Ethical Clearance: Compliance with ethical standards.

References


Electro-acupuncture Combined with Medical Treatment for Successful Management of Legs Paralysis in a Dog

Steven TauficLeo¹, LitaRakhma Yustinasari², Juliano Mwenda Ntoruru³

¹Veterinary Practitioner, Sunset Vet Clinic, Kuta Bali, Indonesia, ²Lecturer, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, UniversitasAirlangga, Indonesia, ³Research Assistant, Meru University of Science and Technology, Kenya

Abstract

A 5-year-old Siberian Husky female dog was referred with concerned maggots and legs paralysis. The dog was examined clinically and radiographically. She was positive of E. canis and Anaplasma. Major clinical sign showed the dog unable to lift the body. Neurological exam was performed, deep pain on all limbs were delayed. The dog got wound treatment and electro-acupuncture through perpendicular insertion into acupuncture points: BL-23, BL-26, BL-40, BL-60, ST-36, and LIV-3 for 20 minutes/day, 70 Hz and 5A, continuously, for 15 days and then reduce to 3 times/week. Significant improvements were observed, finally the dog being recovered. This case showed favorable therapeutic response by alternative treatment.

Keywords: canine, leg paralysis, acupuncture, alternative treatment

Introduction

Peripheral nerve damage is often the cause of leg paralysis. While some conditions can be remedied through surgery, others may not be able to be treated and will require supportive, lifelong care as the limb will not be functional. A variety of research studies have confirmed the efficacy of acupuncture for neurological disorders in veterinary medicine.¹,²,³,⁴,⁵

Case History and Clinical Observation

A female Siberian Husky, approximately 5 years old, came with concerned maggots and paralyzed on all legs. The owner has put her inside cage for 3 years and never go out. She started to be paralysis a week before brought to Sunset Vet Clinic, Bali. Physical examination was normal, mostly laying down, and could not lift her body. She has maggot accumulation on both lateral of pelvic area and both elbow. Blood test was performed, HCT was decreased, biochemistry was normal and she got E. canis and slightly positive of Anaplasma. Neurological exam was performed as well and all proprio were negative, deep pain on all limbs were delayed, all withdrawal was negative. Both plain and myelograph X-ray were performed and no abnormality found, she just laid down laterally and unable to move at all.

Treatment and Discussion

The patient got wound treatment with Sangobion, Doxycycline, and Clindamycin for a week. Her condition was improved, she started to move her head. Two weeks after treatment, her both front limb started to be responsive and she started to lift her head and tried to change her position to ventral position. We explained that what we can do now trial with acupuncture since we do not have CT Scan/MRI for further checkup while see her response.

Electro-acupuncture treatment technique was used, with identifying of the meridian known as responsible for leg paralysis and adjacent regions affections. It may be a simple method of indirectly applying an electrical stimulation to deep tissue. Considering the very small

Corresponding author:
Lita RakhmaYustinasari
E-mail:lita-r-y@fkh.unair.ac.id
(about 0.2mm) diameter of an acupuncture needle used for this purpose together with the shape of its tip, electro-acupuncture will cause only minimal tissue damage. In our case, the major meridian was the Bladder channel (BL), Stomach channel (ST), and Liver channel (LIV) with a total of 6 acupuncture points: BL-23, BL-26, BL-40, BL-60, ST-36, LIV-3 (Table 1).

As technique, it was used an electro-acupuncture device and sterile acupuncture stainless steel needles (0.25 x 25 mm) for single use perpendicularly inserted into the acupuncture points. BL-40 (He–sea point) was chosen as primordial acupuncture points for master point for the caudal back and coxofemoral joints, further acupuncture points being selected on the basis of individual response. The electro-acupuncture treatment session duration was 20 minutes/day, 70 Hz and 5A, and lasted continuously, for 15 days and then reduced to three times a week. Electro-acupuncture treatments using high frequencies (80-120 Hz) induced a stronger local analgesic effect than electro-acupuncture treatments using low frequencies (20 Hz). The duration of acupuncture treatment accorded to individual response, which was evaluated by continually monitoring symptoms as they evolved during treatment and in the subsequent weeks.

<table>
<thead>
<tr>
<th>Acupoints</th>
<th>Depth (CUN)</th>
<th>Attributes and indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL-23</td>
<td>1</td>
<td>Shen-shu; Back-shu association point for the kidney; kidney Yin and Qi deficiency, renal diseases, urinary incontinence, impotence, edema, auditory dysfunction, thoracolumbar intervertebral disk disease, pelvic limb weakness, coxofemoral joint osteoarthritis.</td>
</tr>
<tr>
<td>BL-26</td>
<td>1</td>
<td>Guan-yuan-shu; Gate of Yuan-source Qi; kidney Yang and Qi deficiency, impotence, urinary incontinence, diarrhea, abdominal pain, lumbosacral pain.</td>
</tr>
<tr>
<td>BL-40</td>
<td>1</td>
<td>He–sea point (earth); master point for the caudal back and coxofemoral joints; dysuria, urinary incontinence, coxofemoral joint and thoracolumbar intervertebral disk disease, autoimmune disease, vomiting, diarrhea, pelvic limb paresis or paralysis.</td>
</tr>
<tr>
<td>BL-60</td>
<td>0.5</td>
<td>Kun-lun; Jing-river (fire); epistaxis, intervertebral disk disease, cervical pain, thoracolumbar pain, hock pain, epilepsy, dystocia, hypertension.</td>
</tr>
<tr>
<td>ST-36</td>
<td>1.5</td>
<td>He–sea point (earth); master point for GI tract and abdomen; nausea, vomiting, stomach pain, gastric ulcer, food stasis, general weakness, constipation, diarrhea</td>
</tr>
<tr>
<td>LIV-3</td>
<td>0.5</td>
<td>Tai-chong; Hu-stream point (earth), Yuan-source point; liver Qi stagnation, abnormal cycle, fetlock pain, paralysis of hind limb.</td>
</tr>
</tbody>
</table>

In the first treatment, the patient responded positively. However, she did not show any sensitivity and did not support at all the pelvic limbs. After a week, significant improvements were observed, mainly related a gradually increased sensitivity of all limbs, which proprioceptive on both front limb are better than both hind limb. Unfortunately, the total support hindquarters were accomplished yet only for a very short period of time (10-15 seconds), not yet strong enough to stand up by herself. After eleventh treatment, she was stronger on both front limb and she started to use both hind limb, proprioceptive on both hind limb changed from negative...
to delayed. Right hind limb was stronger than left hind limb. She also got physiotherapy and massage. After a month, she can stand up after lay down by herself. Following four electro-acupuncture sessions settling the animal’s entire recovery.

![Figure 1](image1.png)

**Fig 1.** The case with leg paralysis. (a. Two weeks after medical treatment, the dog’s front limb started to be responsive and she started to lift her head; b. A week after acupuncture treatment, the dog not yet strong enough to stand up by herself; c, d. A month after acupuncture treatment, the dog can stand by herself and walk again)

Electro-acupuncture combined with standard conventional medical treatment were more effective for leg paralysis in dog. Treatment consists in the use of local acupuncture points around the affected nerve, combined with corresponding distant meridian points. The success rate of acupuncture is likely to be inversely proportional to the degree of the nerve damage. From a Traditional Chinese Veterinary Medicine (TCVM) point of view, the treatment of acupuncture is aimed to induce and promote the flow of Qi and blood to the extremity. Electro-acupuncture can deliver an electric current of low frequency via needle electrodes. It can be used to apply electric currents directly to deep tissue, and mainly used for pain control. By improving the circulation, the nerve tissue of the affected area can be nourished, thereby restoring the nerve function and reducing pain.

**Conflict of Interest:** All authors declared that there is no conflict of interest.

**Funding:** Self-funding.

**Ethical Clearance:** Compliance with
ethical standards.

References


Comparison in Synergetic Effect for Silver Nanoparticles that Produced from *P. aeruginosa* and *P. luteola* with Oil Extract of *Cymbopogon citraus* on Different Types of Pathogenic Bacteria

Suaad Ali Ahmed¹, Hussam Mahmood Hasan¹

¹Researchers/ Department of Biology/ College of Science/ University of Baghdad/ Iraq

**Abstract**

Silver nanoparticles have been found to possess both anti-bacterial and anti-inflammatory properties, In addition Many reports refered to the antibacterial activity of lemongrass oil against different organisms. Two species of Gram negative bacteria *Psuedomonas* (*P. auroginosa* and *P. luteola*) were tested for the ability of bearing silver nanoparticles by using LB medium, the two isolates of bacteria were appeared brown color when mixed the supernatant of bacterial culture with AgNO3 solution, that referred the biosynthesis of Silver nanoparticles (Ag NPs). UV–visible spectrophotometer was utilized for estimation of (Ag NPs). The antimicrobial activity of silver nanoparticles arranged from the two isolates was tested against different types of pathogenic bacteria. lemongrass oil was extracted and three different concentrations (v/v) of lemongrass oil with dimethyl sulfoxide (DMSO) 0.5%, 5% and 10%, were prepared to determin the MIC by using well diffusion method. The synergetic effect was demonstrated by combining the (Ag NPs) that produced from , *Pseudomonas aeruginosa* and *Pseudomonas luteola* with MIC of lemongrass oil (0.5%) against same types of clinical bacteria.

**Keywards:** *Pseudomonas luteola*, *Cymbopogon citraus*, *LB medium*

**Introduction**

Human being are often infected by different microorganism like bacteria, molds, yeasts, and viruses, present in their living environments, the development of resistant or even multi-drug resistant pathogens has become a major problem(1). Nanotechnology refers to an emerging field of science that includes synthesis and development of various nanomaterials (2). Recently, different metallic nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate and silver (3). Silver ions and there derivative compound are highly toxic to microorganism. (3) Silver is safe inorganic antibacterial agent used for centuries and it has the ability of killing different type of diseases causing microorganisms (4). Additionally, when compared with other metals, silver presents the lowest toxicity for animal cells (5) Silver nanoparticles have been found to possess both anti-bacterial and anti-inflammatory properties that can promote faster wound healing. (6). Biological synthesis of nanoparticles in spot light due to the fact that it is eco-friendly as compared to other methods of nanoparticle synthesis (7) . Nanoparticles have been successfully synthesized from bacteria, actinomycetes, algae, sugar, plants, and from many more substrates (8). Lemon grass (Cymbopogon Citraus) is a native aromatic tall, it is a perennial herb widely cultivated in the tropics and sub-tropics, is a great interest due to its commercially valuable essential oils and widely used in food technology as well as in traditional medicine (9). Many reports refered to the antibacterial activity of lemongrass oil against different organisms comprising gram positive and gram negative bacteria, yeast and fungi (10) . In this work, (Ag NPs) were synthesized by two types of bacteria, *Pseudomonas aeruginosa* and *Pseudomonas luteola*. The antibacterial activity of
synthesized silver nanoparticles with Lemon grass oil extract on different kinds of bacteria was explored.

**Materials and Method**

**Organisms and Lemon grass oil extraction**

Two species of *Psuedomonas* (*Psuedomonas auroginosa* and *Psuedomonas luteola*) were used in this work. They were cultivated on brain heart infusion broth for 24h at 37 °C, then purified. For extraction of lemongrass oil, 25 g of lemongrass was weighted and cut it into small pieces. 300 ml of distilled water was added to the flask containing the grass and set the apparatus for distillation. The mixture was boiled and collected the distillate until no more oily drops can be seen passing over. The distillate was extracted with hexane, dried them at 45° C, yellow colored oily liquid with fresh lemon like tone was obtained.

**Biosynthesis and characterization of silver nanoparticles**

*Psuedomonas aeruginosa* and *Psuedomonas luteola* were cultured in LB medium. The culture flasks were incubated on shaker at 37°C and agitated at 200 rpm for 24 hours, then harvested and centrifuged at 10000 rpm for 10 minutes. The supernatant was collected for silver nanoparticles biosynthesis, supernatant (10 ml) was mixed with 5ml of silver nitrate solution (10 mM) and incubated at 30°C for 24 h in dark. The silver nanoparticles (AgNPs) were purified by centrifugation at 10,000 rpm for 5 min twice, and collected for further using. (Ag NPs) was estimated by using UV–visible spectrophotometer (300–900 nm), the colored nanoparticle solution shows a peak ~400 nm.

**MIC Determination of lemongrass oil and antibacterial action of (Ag NPs)**

Lemongrass oil MIC and the antibacterial activity of (Ag NPs) prepared from *P.aeruginosa* and *P.luteola* were calculated as procedure of well diffusion method. Three different concentrations (v/v) of lemongrass oil with dimethyl sulfoxide (DMSO) 0.5%, 5% and 10%, were prepared. different types of bacteria including *Staph. aureus, Enterococcus faecalis, Strep.agalactiae, Klebsiella pneumoniae, E. coli, Acineto.bomannii* (clinical isolates) were used in this work. The plates were incubated for 24 h, the diameters of inhibition zones for the Lemongrass concentrations and (Ag NPs) extract when used alone and together were measured.

**Results and Discussion**

**Producing organisms**

Among the noble metals, silver is the best choice metal in the field of biological system. Selection of eco-friendly nontoxic agents are the most important issues which must be considered in green synthesis of nanoparticles. Two species of genus *Psuedomonas* (*P.aeruginosa* and *P.luteola*) were obtained from Department of Biology, College of Science, University of Baghdad used in this work after purification on MacKkonkey agar and Nutrient agar. Bioproduction of (Ag NPs) was investigated by mixing the bacterial supernatant with (AgNo3) solution. The two bacterial isolates showed the brown reported in another organisms like *Bacillus methylotrophicus* and in fungi as *Fusarium semitectum, Aspergillus fumigatus*.

**Estimation of silver nanoparticles**

The biosynthesis of silver nanoparticles for the two bacterial isolates was done by UV.Spectrophotometer at a wavelength range of 200-900 nm. This method has been appeared to be appropriat sensitive way to check the intense surface plasmon resonances of (Ag NPs) in the range of waves 300–600 nm. The results apppeared that high absorption peak at 371 nm was observed from *P. aeruginosa* while *P.luteola* perceived the high absorption peak at 369 nm. Wavelengths 300 – 800 nm are generally used for characterizing metallic nanoparticles ranging in size from 2 nm up to around 100 nm. The exposed to the AgNo3 solution exhibit the wide spectrum range 390 to 410 nm. The presence of the broad resonance refers the aggregation of the silver nanoparticles in the solution, 370 nm indicated to the transverse plasmon vibration in silver nanoparticles.
MIC of Lemongrass oil

Treatment using plant-based medicine appears to be an alternative approach because of the adverse effects associated with the use of synthetic drugs (9). The MIC of lemongrass oil was determined against different types of pathogenic bacteria. The MIC for all bacterial isolates was 0.5% (Figure 1, Table 1). Lemongrass essential oil is applied for its medicinal value to cure acne, oily skin, scabies, flatulence, headaches, blood circulation problems and excessive perspiration due to its antimicrobial and antibacterial activities. It has also been used as carminative, stimulant, emmenagogue, diuretic and antiseptic (16).

Table (1) : The MIC of lemongrass oil on different clinical bacteria

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>0.5%</th>
<th>5%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph.aureus</td>
<td>14 mm</td>
<td>16 mm</td>
<td>18 mm</td>
</tr>
<tr>
<td>Strep.agalactiae</td>
<td>12 mm</td>
<td>14 mm</td>
<td>18 mm</td>
</tr>
<tr>
<td>Entero.faecalis</td>
<td>11 mm</td>
<td>13 mm</td>
<td>14 mm</td>
</tr>
<tr>
<td>E.coli</td>
<td>14 mm</td>
<td>17 mm</td>
<td>19 mm</td>
</tr>
<tr>
<td>Kleb.pneumoniae</td>
<td>16 mm</td>
<td>30 mm</td>
<td>40 mm</td>
</tr>
<tr>
<td>Acineto.baumannii</td>
<td>15 mm</td>
<td>20 mm</td>
<td>30 mm</td>
</tr>
</tbody>
</table>

Figure(1): MIC of Lemongrass oil extract on Mullar Hinton Agar
Antimicrobial activity of silver nanoparticles

Silver is a nontoxic, safe inorganic antimicrobial agent adopted for centuries and it has the inclination of killing different type of diseases motive microorganisms. Silver has been published to be a sturdy antibacterial, antifungal and antiviral agent. The antimicrobial action of biosynthesized (Ag NPs) from two bacterial isolates were investigated facing a span of pathogenic microorganisms by applying well diffusion method. The diameter of inhibition growth zone is monitored in (Fig 2 and Table 2). The results appeared that the antibacterial activity of (Ag NPs) from *P. aeruginosa* was higher than (Ag NPs) from *P. luteola* against *Staph.aureus, Strep. agalactiae and Entero.faecalis*, while the antibacterial activity of (Ag NPs) from *P. luteola* was higher against *E.coli*. The antibacterial action of (Ag NPs) from the two species was equal against *Kleb.pneumoniae and Acinetobbaumannii*. Most of the antibiotic resistance mechanisms are irrelevant for nanoparticles (NPs) because the mode of action of NPs is direct contact with the bacterial cell wall, without the need to penetrate the cell; this raises the hope that NPs would be less prone to promoting resistance in bacteria than antibiotics. Therefore, attention has been focused on new and exciting NP-based materials with antibacterial activity (17).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>AgNPs of <em>P.aeruginosa</em> (mm)</th>
<th>AgNPs of <em>P.luteola</em> (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph.aureus</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Strep.agalactiae</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Entero.faecalis</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>E.coli</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Kleb.pneumoniae</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Acinetobbaumannii</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

Figure(2): Antibacterial activity of (AgNPs) from two isolates on Mullar Hinton Agar

Synergetic effect of combined (AgNPs) from *P. aeruginosa and P. luteola* with MIC of Lemongrass oil extract

Several studies regarding the synergistic activity of nano-Ags in combination with other compounds have been reported (18). The result showed increasing in antibacterial activity when combined AgNPs that produced from two species of *Pseudomonas* (*P. aeruginosa and P. luteola*) with Lemongrass oil extract against all clinical types of bacteria that used in this study, but the synergetic effect from *P. aeruginosa* was more than *P. luteola* against all types of clinical bacteria except in bacteria *E.coli* the synergetic effect of *P. luteola* was the highest (Figure 3 and Table 3). Synergistic activity was done by using lemongrass (*Cymbopogon citratus*) essential oil associated with silver nanoparticles (AgNP) synthesized by green synthesis against *Candida albicans* (19). Other combinational activities of AgNPs...
with antibiotics and detergents were also seen\(^{(18)}\).

Table (3) : The synergetic effect of combined (AgNPs) with MIC of Lemongrass oil extract

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>P.auroginosa</th>
<th>P.luteola</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph.aureus</td>
<td>18 mm</td>
<td>15 mm</td>
</tr>
<tr>
<td>Strep.agalactiae</td>
<td>22 mm</td>
<td>20 mm</td>
</tr>
<tr>
<td>Entero.faecalis</td>
<td>38 mm</td>
<td>22 mm</td>
</tr>
<tr>
<td>E.coli</td>
<td>15 mm</td>
<td>17 mm</td>
</tr>
<tr>
<td>Kleb.pneumoniae</td>
<td>23 mm</td>
<td>20 mm</td>
</tr>
<tr>
<td>Acineto.baumannii</td>
<td>18 mm</td>
<td>17 mm</td>
</tr>
</tbody>
</table>

Figure(3): Synergetic effect of (AgNPs) from two isolates with Lemongrass oil extract against different clinical bacterial types on Mullar Hinton Agar
Conclusion

Silver nanoparticles which are zerovalent, can be a valuable alternative to ionic silver. Nano-Ags are a non-toxic and safe antibacterial agent for the human body. In addition, nano-Ags are also reported to possess antifungal activity, anti-inflammatory properties, antiviral activity and anti-angiogenic activity (18). AgNPs that produced from two species of Pseudomonas (P. aeruginosa and P. luteola) showed increasing in antibacterial activity when combined with MIC of Lemongrass oil extract against different clinical types of bacteria. The synergetic effect from P. aeruginosa was more than P. luteola against most types of clinical bacteria.

Source of Funding : Self

Conflict of Interest : Nil

Ethical Clearance- Taken from Department of Biology/ College of Science/ University of Baghdad committee

References


Predisposition Factors in Exclusive Breastfeeding in Infants
(Literature Review)

Subagyo1, Suparji1, Nana Usnawati1, Vilia Tri Erlina2
1Lecture, 2College Student Jurusan Kebidanan, Politeknik Kesehatan Kementerian Kesehatan Surabaya, Indonesia

Abstract
Background. Nutritional intake in the first thousand days of a child’s life up to 2 years old is very important for growth and development. The low number of exclusive breastfeeding is still to be concern of the Indonesian government. Based on data from Magetan District Health Office in 2019, the coverage of exclusive breastfeeding in the Poncol Puskesmas was 48.4%. The purpose of this systematic literature review is to systematically review some of the results of recent research on the drivers that influence the success of exclusive breastfeeding, namely the factors of knowledge, education, employment, and attitude. The type of research used is systematic literature review (SLR) using the Preferred Reporting Items for Systematic Review and Meta Analysis (PRISMA) approach. The database used are DOAJ and SINTA (Garuda), the last 5 years publication limits, analytical research, cross sectional research designs, full text articles, research areas are Indonesia. From the research results found 546 journals and after screening obtained 9 relevant journals. Regarding education, 3 out of 4 journals stated that there was a relationship between education and exclusive breastfeeding. On the employment factor, 4 out of 5 journals stated that there was a relationship between work and exclusive breastfeeding. In the knowledge factor, all of journals stated that there was a relationship between knowledge and exclusive breastfeeding. While in the attitude factor, 3 out of 4 journals stated that there was a relationship between attitude and exclusive breastfeeding. The majority, of journal stated that there is relationship between education, knowledge, employment, and attitude of mother’s breastfeeding. It is hoped that the results of this review will be able to bring change to readers, especially health workers who have a very important role in providing education and promotion regarding exclusive breastfeeding.

Keywords: Knowledge, Employment, Education, Attitude.

Introduction
One of the priorities for health development in Indonesia is to improve and improve the health of mothers and babies. A healthy baby is supported by several factors, one of which is nutrition. Nutritional intake in the first thousand days of life of children up to 2 years of age is very important for growth and development.

Breastmilk (ASI) is a very good intake in the first thousand days of birth. According to Pitaloka, mothers often lack information about the benefits of exclusive breastfeeding and the impact of not being exclusively breastfed. The low rate of exclusive breastfeeding is still a concern of the Indonesian government.

The Indonesian government has a target of achieving exclusive breastfeeding of 80%2. Based on data obtained from the results of the National Riskesdas in 2018, the achievement of exclusive breastfeeding in Indonesia is only around 37.3% of the expected target. The East Java government stated in 2016 that the target for exclusive breastfeeding was 80%. However, in fact, the results of Riskesdas reported that exclusive breastfeeding
coverage in the East Java region only reached 40.05% \(^2,^3\). According to data from the Magetan District Health Office, the achievement of exclusive breastfeeding in 2019 was 69.4%, which has met the expected target of 50%. However, there are 3 working areas of the Community Health Center that still do not meet the expected target for achieving exclusive breastfeeding, one of which is the Poncol Community Health Center. Based on data obtained from the Poncol Community Health Center 2019, the number of babies (0-6 months) in a period of 1 year is 157. Of these, 76 babies (48.4%) get breastfeeding exclusively, while the rest a total of 81 babies (51.6%) were not exclusively breastfed\(^4\).

Breastfeeding behavior is a mother’s action based on her knowledge and experience in breastfeeding. According to Green’s theory in Notoatmodjo 2012, a person’s behavior is driven by several factors, including predisposing factors, enabling factors, and reinforcing factors\(^5\). Based on Rizky’s research in 2017, predisposing factors that cause the low achievement of exclusive breastfeeding are the lack of knowledge and experience of mothers, low education, and reasons for work that cannot be left behind\(^6\). A part from that, it is also supported by the fact that there are facilities and an exclusive breastfeeding campaign at the time of antenatal care. Lack of family support and the influence of the mass media on advertisements for infant formula milk also influenced mothers not to exclusively breastfeed\(^7\). The impact of babies who are not exclusively breastfed can suffer from vomiting and diarrhea, so that death due to vomiting increases 23.5 times and diarrhea is 17 times more frequent in infants who are given formula milk\(^8\). Haryono & Setianingsih say that babies who are not given ASI have lower endurance than babies who are breastfed\(^9\).

Efforts to increase the use of breast milk have become a worldwide goal. Every year on August 1-7 is the world breastfeeding week \(^2\). Exclusive breastfeeding program in Indonesia is a top priority where the government participates in exclusive breastfeeding by stipulating PP RI No. 33 of 2012. Provision of breastfeeding facilities in the workplace has also become a concern of the government in order to increase motivation to provide exclusive breastfeeding \(^2\). In addition, to support the realization of the exclusive breastfeeding program, the Magetan Regency Government is also promoting MAYANGSARI cadres who are expected to provide information to mothers about the provision and benefits of breastfeeding\(^2,^4,^10\).

**Materials and Methods**

The type of research used in this research is systematic literature review (SLR) or in Indonesian it is called a systematic literature review. According to Kitchenham and Charters 2007 systematic literature review is a literature review method that identifies, assesses, and interprets all findings on a research topic to answer predetermined research questions\(^11\). SLR is a term used to refer to a particular research or research methodology and development carried out to collect and evaluate research related to a particular topic focus\(^12\).

In this literature review, the research design used is the PRISMA (Preffered Reporting Items for Systematic Review and Meta Analysis) approach. The databases used are Sinta (Garuda) and DOAJ (Directory of Open Access Journals) with publication limits for the last 5 years. from 2015-2020, full text articles, research areas are in Indonesia. The keywords used in the search were the factors of exclusive breastfeeding mothers’ education, knowledge of exclusive breastfeeding mothers, occupation of exclusive breastfeeding mothers, and attitudes of exclusively breastfed mothers.

**Results**

The search results according to the keywords obtained as many as 536 articles and after being selected according to the inclusion criteria, 9 articles were obtained. The following is a discussion of each of the factors that will be reviewed, namely the factors of education, work, knowledge, and attitudes.
### Table 1. Results Research articles on predisposing factors affecting exclusive breastfeeding

<table>
<thead>
<tr>
<th>No</th>
<th>Author and Year</th>
<th>Method (Design, Samples, Variables, Instruments, Analysis)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Utami, T. Rizky, dkk (2015)6</td>
<td>D : Cross sectional, S : A total of 56 respondents. VI : Work, Attitude, VD : Exclusive Breastfeeding, I : Questionnaire, A : Chi Square test</td>
<td>There is no effect of mother’s work on exclusive breastfeeding behavior (p value = 0.374). There is an effect of mother’s attitude on exclusive breastfeeding behavior (p value = 0.022).</td>
</tr>
<tr>
<td>2</td>
<td>Iqmy A. Ledy. (2017)13</td>
<td>D: Cross sectional, S: A total of 42 mothers with babies aged 6-12 months. VI: Knowledge, education, occupation, VD: Exclusive breastfeeding, I: -, A: Chi Square test</td>
<td>There is an effect of mother’s knowledge on exclusive breastfeeding (p value = 0.000). There is an effect of maternal education on exclusive breastfeeding (p value = 0.000). There is an effect of mother’s work on exclusive breastfeeding (p value = 0.31).</td>
</tr>
<tr>
<td>3</td>
<td>Yulita, Defi. (2018)14</td>
<td>D: Cross sectional, S: A total of 38 mothers with babies aged 6-12 months. VI: Knowledge and employment status, VD: Exclusive breastfeeding, I: Questionnaire, A: Chi Square test</td>
<td>There is an effect of the level of knowledge on exclusive breastfeeding (p value = 0.000). There is an effect of work on exclusive breastfeeding (p value = 0.036).</td>
</tr>
<tr>
<td>4</td>
<td>Mariza, Ana (2015)15</td>
<td>D: Cross sectional, S: A total of 39 mothers with babies aged 6-12 months. VI: Education and work, VD: Exclusive breastfeeding, I: -, A: Chi Square test, logistic regression test.</td>
<td>There is an effect of maternal education on exclusive breastfeeding (p value = 0.000). Mothers who are highly educated have 24.70 times the opportunity to provide exclusive breastfeeding compared to mothers with low education (OR = 24.700). There is an effect of work on exclusive breastfeeding (p value = 0.003). Mothers who work have a 11.05 times chance of not giving exclusive breastfeeding compared to mothers who do not work (OR = 11.050).</td>
</tr>
<tr>
<td>5</td>
<td>Oktavianisya, Nelyta (2017)16</td>
<td>D: Cross sectional, S: A total of 40 mothers with babies aged 6-12 months. VI: Occupation and Attitude, VD: Exclusive Breastfeeding, I: Questionnaire, A: Chi Square Test</td>
<td>There is an effect of mother’s work on exclusive breastfeeding (p value = 0.000). There is an effect of attitude towards exclusive breastfeeding (p value = 0.012).</td>
</tr>
<tr>
<td>7</td>
<td>Haurissa B.G, dkk. (2019)17</td>
<td>D: Cross sectional, S: A total of 79 mothers with babies aged 6-12 months. VI: Knowledge, Attitude VD: Exclusive Breastfeeding, I: Questionnaire, A: Rank Spearmen Correlation Test.</td>
<td>There is an effect of maternal knowledge on exclusive breastfeeding (p value = 0.01). There is no influence of mother’s attitude towards exclusive breastfeeding (p value = 0.134).</td>
</tr>
<tr>
<td>8</td>
<td>Chaitom, D, dkk. (2019)18.</td>
<td>D: Cross sectional, S: A total of 63 mothers with babies aged 6-12 months. VI: Attitude, VD: Exclusive Breastfeeding, I: Questionnaire, A: Chi-square test</td>
<td>There is an influence of attitude on exclusive breastfeeding (p value = 0.028).</td>
</tr>
<tr>
<td>9</td>
<td>Refi Lindawati (2019)19</td>
<td>D: Cross sectional, S: A total of 42 mothers with babies aged 6-12 months. VI: Knowledge, Education, VD: Exclusive breastfeeding, I: Questionnaire, A: Chi-square test</td>
<td>There is an effect of knowledge on exclusive breastfeeding (p value = 0.028). There is an effect of education on exclusive breastfeeding (p value = 0.028).</td>
</tr>
</tbody>
</table>
Discussion

The Effect of Breastfeeding Mother’s Education Level on Exclusive Breastfeeding

The relationship between education and exclusive breastfeeding. After reviewing several studies, there were different results, namely 3 out of 4 journals stated that there was a relationship between education and exclusive breastfeeding. The three journals are research by Ana Mariza 2015, Ledy Octaviani Iqmy 2017, and Refi Lindawati 2019. Meanwhile, the journal from Diah Ayu Pitaloka, Rumaidhil Abrory, Ayu Deni Pramita 2018 states that there is no relationship between education and exclusive breastfeeding. Of the five journals, the majority of breastfeeding mothers do not exclusively breastfeed their babies. The research sample taken was mostly mothers who had low or basic education. The education level classification of the research respondents is different. In one of the classifying journals of the level of education based on RI Law no. 20 of 2003 concerning the National Education System, divided into 3 levels, namely primary education, secondary education, and higher education. While the other four journals, the classification of education is divided into 2 levels, namely low / basic education and higher education. Overall, the five journals are easy to understand, starting from the abstract, introduction, research methods, data collection, discussion, and delivery of research results. However, in the journal Ana Mariza 2015 and Ledy Octaviani Iqmy 2017, the research instrument is not clearly explained. Whereas in the journal Ledy Octaviani Iqmy 2017, the title used is too long so it is not persuasive. According to Barker and Schutz (1972), the length of the research title is a maximum of 20 words, made short, and eliminating unnecessary words.

According to Nursalam in Refi Lindawati, 2019, the higher level of education will facilitate the process of finding and receiving information so that the knowledge they have is also more and more. One’s education improves cognitive abilities (knowledge), affective (attitude determination), psychomotor (ability to perceive). According to Notoadmodjo in Yulita Defi 2018, someone with a higher education is usually more receptive to new things and is more open about their health. In addition, mothers who have higher education usually have great curiosity and try to find information or experience. This is able to increase knowledge for the mother so that it is hoped that the mother will be able to overcome the problem and can improve the health of themselves and their families. Through this learning process, a person has the awareness to make changes in an effort to improve health. Education also affects the response to something that comes from outside because it is able to think more rationally. Mothers with low education ability to think rationally are not as good as mothers with higher education. The ability to understand something new is a little difficult or there is even a misunderstanding of capturing the information. The low level of maternal education results in a lack of knowledge of mothers in dealing with problems, especially in exclusive breastfeeding. However, if mothers with low education are given a good understanding by health workers so that there is no misunderstanding in receiving information, it is hoped that the mother will be able to provide breastfeeding exclusively.

The Influence of Type of Breastfeeding Mother’s Occupation on Exclusive Breastfeeding

According to journals that have been analyzed by researchers, 3 out of 5 journals state that there is a relationship between maternal work and exclusive breastfeeding. The three journals are research by Ana Mariza 2015, Defi Yulita 2018, and Nelyta Oktavianisya & Sri Sumarni 2017. Meanwhile, 2 journals from Rizky Tri Utami, et al. (2015) and Ledy Octaviani Iqmy 2017, state that there is no relationship between maternal occupation and exclusive breastfeeding. In the journal Ledy Octaviani Iqmy 2017, states that in processing data using the chi square statistical test, the results obtained p value = 0.31 with the interpretation that there is no relationship between maternal occupation and exclusive breastfeeding. The result of p value => 0.05, then H0 is accepted with the interpretation that there is no relationship between maternal occupation and exclusive breastfeeding.
breastfeeding. In the five journals, the majority of mothers who do not provide exclusive breastfeeding are mothers who work13,14,15. According to Nursalam in Ana Mariza (2015), work is a necessity that must be done, especially to support the life of her family. The dual role of caring for children and working outside the home often makes it difficult for mothers to manage time due to the large number of working hours and also the distance between work and home is far. By working, mothers cannot have full contact with their babies. As a result, mothers tend to give formula milk and it is given by bottle. This causes the frequency of breastfeeding to decrease and milk production to decrease. This situation will cause the mother to stop breastfeeding. Working mothers who provide exclusive breastfeeding must have high commitment and seriousness. Because it takes a lot of time and is a big challenge for breastfeeding mothers14,15.

According to the authors, working mothers should be able to exclusively breastfeed their babies. Work is not an absolute factor that causes mothers to not be able to provide exclusive breastfeeding. If the mother has good knowledge about the importance of exclusive breastfeeding, how to express and store breast milk, and exclusive breastfeeding, it can increase exclusive breastfeeding. In addition, support from the closest people and the work environment can influence mothers to continue to provide exclusive breastfeeding. As stated in Law no. 36 of 2009 concerning Health, that during breastfeeding, the family, government, and society must fully support the mother by providing special time and facilities held in the workplace and public advice13,15.

**The Effect of Breastfeeding Mother’s Knowledge Level on Exclusive Breastfeeding**

The results of the journal search found 4 journals about knowledge and the entire journal stated that there was a relationship between maternal knowledge and exclusive breastfeeding. The five journals include Defi Yulita (2018), Rizky Tri Utami, et al. (2015), Ledy Octaviani Iqmy (2017), Refi Lindawati (2019), and Theafilia Golda Beatriks Harisa, Iyam Manuwe, Kusmiyati (2019). According to Reber (2010), knowledge is a collection of information that a person has that is generated from any process, whether born from innate or achieved through experience. Knowledge is a determining factor in how humans think, feel and act (Oemarjoedi in Defi, 2018). Knowledge can be obtained through seeing, hearing, experiencing real events, or through formal and informal education. Mothers who have low knowledge will have limited knowledge. Knowledge is one of the predisposing factors that influence a person’s behavior. Mothers who do not receive information about exclusive breastfeeding during pregnancy will influence the mother’s behavior in giving exclusive breastfeeding to her baby later (Suhartono in Refi Lindawati, 2019). Correct knowledge about exclusive breastfeeding will encourage a further response from the mother, namely in the form of exclusive breastfeeding13,14,19,22,23.

Currently there are still many mothers who do not know about the benefits of providing exclusive breastfeeding for babies and for mothers, besides that mothers are still influenced by the hereditary tradition of the family that the mother’s milk will not be sufficient to be given to the baby so that the baby must be given additional food (milk), formula, honey, team porridge, etc.) before the baby is 6 months old. Therefore, the role of health workers is very important in providing understanding and promoting exclusive breastfeeding, not only to mothers but also to families and the wider community. Even though the mother has good knowledge about exclusive breastfeeding, if it is not supported by health facilities and health workers, then the mother will find it difficult to realize this behavior (Yulita Defi, 2018). According to Refi Lindawati (2019), there are also mothers who have good knowledge but do not apply exclusive breastfeeding behavior. This can be influenced by several factors, for example because mothers are lazy, mothers are busy, there is no support from health workers and their families, the absence of supporting facilities, the influence of the promotion of formula milk from the mass media. In addition, if the information provided is inaccurate, the information received will also be wrong or cause misunderstanding14,19.
The knowledge factor has an important role in providing influence as an initial impetus for someone to behave. Behaviors that are based on knowledge will be more durable than behavior that are not based on knowledge. Mothers who have high knowledge about the benefits of exclusive breastfeeding will give breastfeeding exclusively to their babies after giving birth compared to mothers who have low knowledge. This is because mothers who have high knowledge about exclusive breastfeeding generally know the various benefits of exclusive breastfeeding (Ledy, 2018). From the journals above, the results presented are very clear about the importance of providing good knowledge or information to mothers from the time of pregnancy. Health workers have an important role in providing counseling and promotion to increase maternal knowledge about exclusive breastfeeding22,24.

The Effect of Breastfeeding Mother’s Attitude on Exclusive Breastfeeding

According to the results of the search for journals that have been conducted, 3 out of 4 journals have found that there is a relationship between mother’s attitude and exclusive breastfeeding. The journal is the result of another research by Rizky Tri Utami, et al. (2015), Nelyta Oktavianisya & Sri Sumarni (2017), and Chintya D.C, Adisti A.R, Ardiansa A.T.C (2019). Whereas 1 journal that has different research results is research from Theafilia Golda Beatriks Harisa, Iyam Manueke, Kusmiyati (2019). According to Secord and Backman (in Nelyta, 2017), attitude is a certain order in terms of feelings (affection), thoughts (cognitive), and predisposition of one’s actions (konasi) to an aspect of the surrounding environment. Attitudes that become an evaluative statement towards an object will further determine individual actions towards something. Attitudes can be divided into: (1). A positive attitude, namely an attitude that shows or shows, accepts, agrees to the prevailing norms where the individual is located, and (2). Negative attitude, which shows rejection or disagreement with the prevailing norms in which the individual is located16,18,22,24. A person will do an action if he has a positive view of the action and believes that other people want him to do it. This belief affects a person’s attitude and behavior to do an action. This belief can come from the experience concerned or be influenced by information obtained about this behavior25.

The realization of this positive attitude in order to become real action requires a factor of support from certain parties, such as health workers, family or closest people. The surrounding environment plays an important role in influencing this. The best decisions are influenced by the immediate environment. In addition, self-desire is not strong enough as a determining factor for breastfeeding if it is not conditioned by other driving factors, enabling factors, and reinforcing factors. In the four journals, the majority of mothers have a positive attitude towards exclusive breastfeeding and give exclusive breastfeeding to their babies. In the research of Theafilia Golda Beatriks Harisa, Iyam Manueke, Kusmiyati (2019), the discussion section states that the results of the Spearmen Rank Correlation Test regarding the relationship between attitudes and exclusive breastfeeding behavior obtained a value of p = 0.134> α (0.05), this shows no The relationship between maternal attitudes and exclusive breastfeeding behavior, however, the data obtained shows that more mothers have positive attitudes towards exclusive breastfeeding, so there is a conclusion, the researchers concluded that there is a relationship between maternal attitudes and exclusive breastfeeding17.

Conclusion

Conclusions from the results of a review of journal articles on predisposing factors for exclusive breastfeeding, currently there are still many mothers who do not know about the benefits of providing exclusive breastfeeding for babies and for mothers, besides that mothers are still influenced by the hereditary tradition of the family that the mother’s breast milk will not be sufficient. to be given to babies so that babies must be given additional other foods (formula milk, honey, team porridge, etc.) before the baby is 6 months old. Therefore, the role of health workers is very important in providing understanding and promoting exclusive
breastfeeding, not only to mothers but also to families and the wider community. Even though mothers have good knowledge about exclusive breastfeeding, if it is not supported by health facilities and health workers, then mothers will find it difficult to realize exclusive breastfeeding behavior.

**Conflict of Interest:** None

**Source of Support:** Self

**Ethical Clearance:** Because this article is not the result of research and is the result of a literature review, permission is not required from the ethical composition.

**References**

Screening of Healthy Women Using Visual Inspection with Acetic Acid For Detection of Cervical Cancer

Suchitra A Rati1, Jayashree Pujari2
1Prof. Vice Principal, HOD, 2Associate Professor Department of Community Health Nursing, BLDEA’S Shri B M Patil Institute of Nursing Sciences-Vijayapur

Abstract

Background: Cervical cancer is the fourth most common cancer in women worldwide and second most common cancer in developing and under developing Nations. The higher incidence of cervical cancer in developing countries is mainly because of lack of resources, lack of effective screening technologies and poorly organized health care delivery in the rural area.

Objective: To determine the prevalence of cervical precancerous lesion among healthy women.

Materials and Methods: The study was conducted for 600 Healthy women who were selected by using purposive sampling technique. The prevalence of precancerous cervical lesion was assessed by Visual Inspection with acetic acid and the data was analyzed by using inferential and descriptive statistical methods.

Result: It was also observed that prevalence of cervical Precancerous lesions in healthy women was 5.0%. It means that 30(5.0%) out of 600 healthy women had risk of cervical precancerous lesions in this present study. Among the study samples who suspected to be positive, off them 10(3.3%) had two or less quadrant involved, and 5 (1.7%) had three quadrants involved. About action taken 30(5.0%) were referred for confirmation, 230(38.3%) followed up after 3 years, 340(56.7%) had referred medical treatment and VIA test after six months. The study result shows that Reveal that prevalence of cervical Precancerous lesions was highly associated with visual examination findings such as cervicitis, white discharge, and abnormal findings.

Conclusion: The study results conclude that VIA test can be used as an alternative screening technique for early diagnosis of precancerous lesion among women at low resource setting.

Key words: Healthy women, precancerous lesion, VIA Test

Introduction

Death Rate from Cervical Cancer Higher Than Thought

Cervical cancer is a major public health problem in developing countries such as India and it has lead to the greatest economic and social impact on the population. It mainly affect the women in reproductive age group. Cervical cancer is a taboo issue in many places as it is linked to sexual transmission. Unless women come forward and break this stigma and come forward for the health check up, we contribute to larger number of mortality and morbidity related to oral cancer.

Cervical cancer is the fourth most common cancer in women worldwide and second most common cancer in women living in less developed regions, which preferably effects the women within the age group of 18 to 45 years and it is commonly caused by human papilloma virus. The higher incidence of cervical cancer in developing countries is mainly because of lack of resources, lack of effective screening technologies and poorly organized health care delivery in the rural area.

India with a population of 436.76 million women aged 15 years and above are at the risk of developing oral cancer. It has been estimated that every year 122844 are diagnosed with cervical cancer and 67477 people die.
with cervical cancer as per the statistics of 2012.  

According to World Health Organization (WHO) Report estimated 530,000 new cases of cervical cancer globally (estimations for 2012), with approximately 270,000 deaths (representing 7.5% of all female cancer deaths). More than 85% of these deaths occurred in low- and middle-income countries.

Cancer preferably occurs in the middle aged women in the age group of 40-50 years and mostly occurs in people with low socio economic status who are incapable to undergo regular health check up because of financial inadequacy, 80% of the new cervical cancer cases occur in developing countries, like India which contributes about one fourth cases of the globe every year. It is estimated that about 9 million new cancer cases are diagnosed every year and over 4.5 million people die from cancer each year in the world.

INDIA: The estimated number of new cancers in India per year is about 7 lakhs and over 3.5 lakhs people die of cancer each year. Out of these 7 lakhs new cancers about 2.3 lakhs (33%) cancers are tobacco related. There would be about 1.5 lakhs cancer cases at any given time in Karnataka and about 35,000 new cancer cases are added to this pool each year.

Many studies have highlighted that the main reason for poor coverage of screening campaign of cancer of cervix in India is due to lack of information, unavailability of resources even various preventive methods are available in India. The Study depicts that none of the woman have received HPV Vaccine and unaware of benefits of it. Almost eighty five percent of females and had poor knowledge and expressed community clinical outreach services helps in enhancing and active participation in such screening programmes.

Many advanced evidence based screening programmes are available for detecting precancerous lesions if these are treated effectively by simple treatment can prevent preceding into invasive cervical cancer. Periodical implementation of population based screening programmes in developed country has been reduced 85% of incidence and mortality related to Ca of cervix. Whereas only 5% of women were screened in developing countries annually with expenditure of 5% resources leading into diagnosing at 3rd or 4th stage with estimation of 60 to 80% of cases.

Revolution in lifestyle and socio-demographic characteristics are pleasuring variety of iceberg diseases among pupils located in middle income countries acquiring second most carcinoma of cervix among famines alone. Many of the research studies have highlighted lack in accessibility of material and badly structured health care units are important reason for elevation in occurrence of new cases. The popularized cancer center TATA Memorial uttered that alertness and utilization of simple screening methods of precancerous lesions of cervix at peripheral area by help of VIA or VILI might bring down incidence of cervical cancer.

A Study by Awasthy S et.al on awareness and practice of Screening related to CA of cervix among female residing in rural area of Kerala reveals that majority were unacquainted about risk factors of Cervical cancer. Even though 3/4th study participants were aware about screening test for early detection only around 7% have undergone pap test. Two studies of Tamilnadu and Maharashtra reveals that only 2.6% coverage of screening among women in age group of 18-69 at India.

Objectives Stated and objectives achieved

- To determine the prevalence of cervical precancerous lesion among healthy women
- To find out the association between the precancerous lesions with selected demographic lesions

Hypothesis

H_0: There is no significant association between the prevalence of precancerous lesion with selected demographic variables

H_1: There is significant association between the prevalence of precancerous lesion with selected demographic variables
Materials and Methods

Research Variable: Cervical Precancerous Lesions

Baseline Variables: Age, Occupation, Marital status, Age at Marriage, Number of pregnancy, last menstruation, Miscarriage, income of family, Nature of discomfort

Source of data: Data is collected from Healthy woman from selected rural area of Vijayapur District

Research design: Descriptive Research design

Setting: Study was conducted in selected Community health center of Vijayapur District

Sample: In this study the sample consist of Healthy women

Sampling Technique: In this Study the sampling Technique used was Purposive sampling Technique

Sample size: The sample size for the present study consist of 600 healthy women

Result and Discussion

Table No 1: Association between cervical Precancerous lesions with selected demographic variable.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Demographic variables</th>
<th>Precancerous lesions</th>
<th>Chi-square value</th>
<th>d.f</th>
<th>P-value</th>
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<td></td>
<td>18-24</td>
<td>94</td>
<td>04</td>
<td>3.06</td>
<td>3</td>
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<td></td>
<td>24-30</td>
<td>128</td>
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<td></td>
<td>30-36</td>
<td>96</td>
<td>06</td>
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<td>≥ 36</td>
<td>252</td>
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<td>4</td>
<td>Last Menstruation</td>
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Cont... Table No 1: Association between cervical Precancerous lesions with selected demographic variable.

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<th>Less than 15 days</th>
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<td>74</td>
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<td>Hindu</td>
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<td>8 Occupation</td>
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<td>02</td>
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</table>

It was clear that cervical Precancerous lesions were not associated with any of the selected demographic variables & does not have its influence on occurrence of cervical Precancerous lesions among healthy women. A Cervical visual inspection with acetic acid as an alternative screening test for cervical cancer detection VIA and Pap smear tests were performed on 468 women in Iran. The result shows that 43 patients had positive VIA and 23 patients had positive Pap smear results.
Sensitivity, specificity, positive predictive value and negative predictive value of VIA were 66.7%, 55.1%, 18.6% and 91.5%, respectively and for Pap smear test they were 75%, 82.1%, 39.1% and 95.5%, respectively. Test accuracy for VIA and Pap smear were 56.7 and 81.1, respectively. It concludes that VIA has high sensitivity but low positive predictive value. By considering the low price of this test and its availability, it can be proposed as a screening method in cervical cancer.7

The study conducted by Bhattacharyya et.al, reveals that the incidence of CIN is more[30%] in Poor economical condition with enhancement of cervical cancer upto 3% in the similar group with reason of lack of personal hygiene, low standard of living conditions, early age sexual contact, lack of education and multiple relationship.13

<table>
<thead>
<tr>
<th>SLNO</th>
<th>Visual Examination</th>
<th>Precancerous lesions</th>
<th>Chi-square value</th>
<th>d.f</th>
<th>P-value</th>
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<tr>
<td></td>
<td></td>
<td>Negative</td>
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<td>18</td>
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<td></td>
<td>Cervicitis</td>
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<td>22</td>
<td>3.3</td>
<td>0.06(NS)</td>
</tr>
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<td></td>
<td>Present</td>
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<td>08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vaginitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>464</td>
<td>30</td>
<td>3.3</td>
<td>0.06(NS)</td>
</tr>
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<td>0</td>
<td></td>
<td></td>
</tr>
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<td></td>
<td>White Discharge</td>
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</tr>
<tr>
<td></td>
<td>Absent</td>
<td>570</td>
<td>18</td>
<td>116.3</td>
<td>0.0001(S)</td>
</tr>
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<td>12</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Others</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>244</td>
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<td>0.70</td>
<td>0.40(NS)</td>
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<td>Present</td>
<td>41</td>
<td>1</td>
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</tbody>
</table>

Table no 2, Reveals that prevalence of cervical Precancerous lesions was highly associated with visual examination findings such as cervicitis, white discharge, and abnormal findings. Although the Pap smear remains the most common screening test for cervical cancer, many less developed countries do not have adequate resources to implement cytology-based prevention programs. An alternative, low-cost test, visual inspection using acetic acid (VIA), has emerged for use in low-resource settings where it can be performed by auxiliary health professionals. VIA is similar to colposcopy in that acetic acid is applied and any acetowhite lesion is visualized9. By considering the low price of this test and its availability, it can be proposed as a screening method
in cervical cancer.

A study by A Omole–Ohonsi recommends that VIA can be adopted as alternative screening technique especially at poor resource setting with hopes for better services to needy women.

**Recommendations**

- In northern part of Karnataka the data related to prevalence of cervical cancer is not available. By considering the low price of VIA test, it can be proposed as a screening method in cervical Precancerous lesions.
- Screening helps for early treatment and prevents further complications. Hence it can be used as alternative screening modality for cervical cancer in low resource locations.
- A similar study can be conducted on a large sample may help to draw more definite conclusions and make generalization
- A similar study can be undertaken for sensitization of women to undergo screening of precancerous lesion

**Conclusion**

Presently cervical cancer has become most common among women dragging second position among mortality among females. Many evidence based low resource setting screening techniques are available still many of the health centers had delayed in implementation leading into severity of it among women this can be resolved by adopting simple low cost technique that is VIA

**Acknowledgement:** We acknowledge Rajiv Gandhi university of health sciences for the grant for conducting this research study and also extend gratitude to Principal, medical officer, ASHA worker and participants for providing conducive environment for successful completion of the study

**Conflict of Interest:** None Declared

**Ethical Clearance Certificate:** Ethical clearance certificate were obtained from institutional ethical clearance committee

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Evaluate the Biological Activities of Biosynthesized ZnO- Nanoparticles Using Escherichia Coli

Suhad H¹, Neihaya HZ¹, Raghad AL¹
¹Scholar Researcher, Dep. of Biology, College of Science, Mustansiriyah University, Iraq

Abstract

About 50 isolates of E. coli were identified (83% from 60 stool sample, and 30 test bacteria which formed biofilm. ZnO- NPs synthesis by Escherichia coli and a white cluster pellet appeared, and absorption peak of UV-Vis. spectroscopy observed at 268 nm. XRD pattern showed the lattice planes of (100), (002), (101), (102), (110), (103) and (112) agreed to the 2θ values of 32.45°, 34.73°, 36.56°, 47.70°, 55.86°, 62.12° and 63.10° respectively, and the diffraction peaks are assigned with the hexagonal phase, while SEM images exhibited that size of the particles ranged between (31.55−45.9) nm. ZnO NPs displayed antibacterial potentiality against pathogenic bacteria, and inhibition zone around ZnO NPs as follows 5, 4, 2, 2, and 2mm for P.aeruginosa, S.aureus, A.baumannii, K.pneumoniae, and E. coli respectively. Also, ZnO NPs was able to decrease biofilm, and results showed that inhibition percentage were (18.6, 27.7, 39.4, and19.6) % against S.aureus, P. aeruginosa, A. baumannii, and K. pneumoniae, respectively after 48 hours of incubation. A549 cells viability was decreased with increasing the concentration of the ZnO NPs, and the IC₅₀ values of the A549 and WRL cells were 105.8 and 167.3 µg/mL respectively. In this study, the synthesized ZnO NPs using nonpathogenic E.coli showed antibacterial, antibiofilm and anticancer activities against studied pathogenic bacteria. So, these nanoparticles can be further used in biomedical, pharmaceutical and other applications as an effective antimicrobial and anti-cancerous agent.

Key words: Biosynthesized ZnO NPs, E.coli, Antibacterial, antibiofilm, Anticancer.

Introduction

Nanoparticles (NPs) is one of the roads to nanotechnology that is related with nanoscale materials through very small particles size ranging between 1 to 100 nm. NPs display distinctive properties due to their very small size also high surface area to volume ratio, which have attributed to the significant differences in the properties over their bulk counterparts (1). Zinc is an important nutrient in living organisms (2). In addition, ZnO has been registered as “Generally Recognized as Safe” (GRAS) by the US Food and Drug Administration (3) owing to its non-toxic properties (4). Researches showed that ZnO NPs possess a great possibility in biological applications, such as the antimicrobial agents (5). Moreover, many studies have been described on the efficacy of ZnO- NPs in preventing the growth of broad-spectrum of pathogens (6), which possibly could substitute the conventional antibiotic (7). The effect of zinc oxide on highly resistant biofilms, distinguishing degrees of operation for treatment depending on the bacteria used (8). ZnO- NPs also have been widely used in cancer therapy and have been reported to stimulate selective cytotoxic effects on cancer cell proliferation. Zinc oxide nanoparticles may be the most cytotoxic to cancer cells than adipocyte cells (9), these findings indicated that zinc oxide nanoparticles may selectively induce cancer cell apoptosis, which could be promising candidates for cancer care (8). This study aims to

Corresponding author:
Neihaya HZ
Email: neihaya1@gmail.com, dr.neihayahz@uomustansiriyah.edu.iq
biosynthesis ZnO-NPs from nonpathogenic *E.coli*, and used these nanoparticles as antibacterial, antibiofilm against multidrug resistant isolates, in-addition to that used as anticancer against human epithelial alveolar cells (A549).

**Materials and Methods**

**Collection of *E.coli***

Sixty stool samples were collected from non-infectious persons to obtain of *E.coli* bacteria, and ages ranged from (6-60) years old.

**Test bacterial isolates**

About 30 isolates of test bacteria (*P.aeroginosa, A.baumannii, K.pneumoniae, E.coli*, and *S.aureus*) were obtained from AL-Mustansiriyah university laboratories.

Detection of Biofilm production

Congo red test

Pathogenic bacteria were screened to detection their ability to biofilm production as in (10).

**Microtiter plate method**

To explore the susceptibility of bacteria to biofilm formation using the Microtiter plate method according to (11).

**Biological synthesis of ZnO- Nps**

Method of biosynthesis was according to (12), and the creation of a white precipitate at the bottom of the flask is an indication of the formation of nanoparticles (13).

**Characterization of biosynthesized ZnO nanoparticles**

Morphological study of ZnO NPs was identified using SEM (TESCAN-VEGA/USA). While, Optical properties were studied using UV-Vis. spectroscopy (Metertech sp. 8001) at (200-900) nm. Also, FTIR (4000-400) cm$^{-1}$ (Shimadzu/Japan) was used to determine the functional groups, and XRD (7000- Shimadzu Maxima) to determine the crystalline structure of ZnO NPs.

Determination of shelf life of biosynthesized ZnO-NPs

 Biosynthesized ZnO-NPs were stored at 4°C, 25°C, and 37°C for 7 months, then UV-Vis. spectrophotometer and FTIR were performed.

**Minimum inhibitory concentration of ZnO NPs**

MIC of the synthesized ZnO nanoparticles was evaluated by the microtiter plate dilution method according to (14) with modification.

**Effect of ZnO-Nps on Biofilm Formation**

**Congo red agar method**

ZnO NPs used in different concentration (100, 50, and 25 mg/ml) and the method as in (15) with modification.

**Microtiter plate method**

The procedure was used as described by (16) with modification, and the inhibition of biofilm formation was calculated as equation described by (17).

\[
\% \text{ Inhibition of biofilm formation} = \frac{OD \text{ control} - OD \text{ treatment}}{OD \text{ control}} \times 100
\]

Anticancer effect of ZnO NPs

Cancer cells (A549) and normal cells (WRL68) were used and the concentration ZnO NPs (25, 50, 100, 200, 400 µg/mL). Statistical analysis was performed to calculate the IC50 according to the following equation:

\[
\text{Viability (％)} = \frac{\text{optical density of sample}}{\text{optical density of control}} \times 100
\]

**Statistical analysis**

All statistical analysis were carried out using one way analysis of variance ANOVA(Duncan), and the statistical significance was fined as ≤ 0.05. The data was determined using Graph Pad Prism version 6 (Graph Pad Software Inc.,La Jolla, CA).
Results and Discussion

Isolation and Identification of *Escherichia coli*

About 50 isolates of *E. coli* were identified (83\%) from 60 stool sample, and documentation as described by (18). *Escherichia coli* under acidic environment eosin Y is precipitated and formed an amide bond among eosin Y and methylene blue in the medium (19).

Biofilm Formation

Result exhibited that biofilm formed by (3) isolates of *S. aureus*, while (2) isolates of *P. aeruginosa*, (3) isolates of *E. coli*, (4) isolates of *K. pneumoniae*, (4) isolates of *A. baumannii* by Congo red agar method.

Results of Microtiter plate method showed that 4 isolates of *P. aeruginosa*, *A. baumannii*, *E. coli* and *K. pneumoniae* produced strong biofilm, while 2 isolates produced moderate biofilm and 2 isolates weak biofilm production, and the 3 isolates of *S. aureus* produce strong biofilm.

Duarte *et al*, (20) observed that 74.7\% of *A. baumannii* isolates were capable to produce biofilm. Karigoudar *et al*., (21) showed 69\% of *E. coli* were able to produce biofilm, and Murugan *et al*., (22) reported that *P. aeruginosa* and *S. aureus* has a very high capacity for biofilm production. Nirwati *et al* (23) observed that 85.63 \% of *K. pneumoniae* isolates were able to produce biofilm.

Biosynthesis of ZnO Nanoparticles

ZnO- NPs synthesis by *Escherichia coli* as white cluster pellet, and the dry weight was evaluated. The biosynthesized of NPs regulated by general conditions such as metal ions are restriction in the microbial cells or on the microbial surface in the presence of enzymes, thereby reduced to form NPs (24).

ZnO-Nps Characterization

An absorption peak observed at 268 nm refer to the effective biosynthesis of ZnO NPs. Ifeanyichukwu *et al*., (25) reported that absorption peak of ZnO NPs synthesized from pomegranate leaf at 284 nm. While FTIR gave information about functional group related with the synthesized nanoparticles. XRD pattern observed lattice planes of (100), (002), (101), (102), (110), (103) and (112) agreed to the 2θ values of 32.45°, 34.73°, 36.56°, 47.70°, 55.86°, 62.12° and 63.10° respectively with the hexagonal phase of ZnO.

SEM images showed the size of ZnO NPs particles ranged between (31.55–45.9) nm, and aggregated as uneven round structure, which is similar to that reported by (26) (Figure 1).

![Image](image-url)
The Zinc Oxide nanoparticles stored for 7 months at different temperatures and remained stable without change in color. The results in Figure (2) illustrates the transmittance spectrum of the ZnO NPs as a wavelength (263 nm) and the band at 434 cm\(^{-1}\) is confirmed the stretching vibration of ZnO NPs.

![Figure (2) A- UV-visible transmittance spectrum, B- FTIR result of ZnO NPs](image)

Similar results were also reported by (27). The good stability of ZnO NPs because of the free amino and carboxylic groups and it is interacted with the surface of ZnO NPs, and the amide group obtained from the protein acted as capping agent of the ligands of the ZnO NPs (28).

**Minimal Inhibitory Concentration of ZnO nanoparticles**

Results showed that the MIC of biosynthesized ZnO NPs was found to be 12.5 mg/ml against each *E.coli*, *A.baumannii*, and *K.pneumoniae*. While the MIC against *P.aeruginosa* was 50 mg/ml, and 25mg/ml for *S.aureus*. The diameter of inhibition zones around the filter paper saturated with sub MIC ZnO NPs as flows (5, 4, 2, 2, and 2) mm for (*P. aeruginosa*, *S. aureus* *A.baumannii*, *K.pneumoniae*, and *E.coli*) respectively.

The variation in the antimicrobial activates of ZnO NPs as manifested by MIC of nanoparticles probably results from the differences in the tested genus and species (29). The mechanism of antimicrobial activity of ZnO NPs may include release of Zn\(^{2+}\) and generation of ROS and damage to cell membrane (30).

**Antibiofilm on Congo red method**

Pink colonies in the presence of Zinc oxide nanoparticles implied a loss of biofilm formation capability in all pathogenic bacterial isolates of the study (*A.baumannii*, *P.aeruginosa*, *K.pneumoniae*, and *S.aureus*), (Fig 3).

![Figure (3) Antibiofilm activity of ZnO NPs. A–control B–Antibiofilm of 25mg/ml ZnO NPs, C–Antibiofilm of 50mg/ml ZnO NPs D– Antibiofilm of 100mg/ml](image)
Antibiofilm on Microtiter plate method

ZnO NPs was able to decrease biofilm formation from MDR bacterial isolates. Results showed that inhibition percentage of biofilm were (18.6, 27.7, 39.4, and 19.6) % against *S.aureus*, *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae*, respectively after 48 hours of incubation Table (1).

The resistant properties of biofilm, lead to eradication of biofilm related disease is challenging (31) ZnO nanoparticles have bioactivity properties like regulator of biofilms formation according to the concentration of this nanoparticles, as it has been as promising antibacterial agents than conventional antibiotics (31).

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Biofilm inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(3) S.aureus</td>
<td>18.6</td>
</tr>
<tr>
<td>(1) P.aeruginosa</td>
<td>27.7</td>
</tr>
<tr>
<td>(6) A.baumannii</td>
<td>39.4</td>
</tr>
<tr>
<td>(7) K.pneumoniae</td>
<td>19.6</td>
</tr>
</tbody>
</table>

Cytotoxicity test of ZnO NPs:

The cells viability was decreased with increasing the concentration of the ZnO The IC$_{50}$ values of the A549 and WRL cells were 105.8, 167.3 µg ml$^{-1}$ respectively as shown in figure (4). The results showed that adding ZnO NPs decrease the cell viability of A 549 cells, and this decreasing related strongly with the concentrations significantly ($P < 0.05$). The percentage of decreasing viability was (42.3 ± 2.6, 54.1 ± 1.3, 68.2 ± 0.9, 81 ± 1.8 and 93 ± 2.3) in concentration (400, 200, 100, 50, 25) respectively, while adding the same concentration to the WRL 68 cells did not show significant effect of viability rate ranged between (62.6 ± 4.4, 71.3 ± 7.1, 88.3 ± 6.2, 87.6 ± 5.2 and 94.2 ± 1.7) as shown in table (2).

![Figure (4) Cytotoxicity of ZnO NPs with A549 and normal WRL 68. Each point is the mean value of three replicate](image)
Table (2) Flow cytometric analysis of C549 and WRL68 cells after treated with ZnO-NPs.

<table>
<thead>
<tr>
<th>Concentration of ZnO NPs(mg/ml)</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A 549</td>
</tr>
<tr>
<td>400</td>
<td>42.3±2.6</td>
</tr>
<tr>
<td>200</td>
<td>54.1±1.3</td>
</tr>
<tr>
<td>100</td>
<td>68.2±0.9</td>
</tr>
<tr>
<td>50</td>
<td>81±1.8</td>
</tr>
<tr>
<td>25</td>
<td>93.1±2.3</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SD of three experiments.

Reddy and Srividya (30) studied the cytotoxicity effect of ZnO NPs against (A549, HEK) human cell lines, and revealed the dose dependent cytotoxicity of zinc oxide nanoparticles using tested cell cultures. The biosynthesized ZnO NPs due to its semiconducting nature are reported to induce cytotoxicity in cancer cells by the generation of reactive oxygen species on the surface of the particle, the released Zn\(^{2+}\) ions are dissolved in culture media indicating direct interaction of NPs with a membrane of cancer cell resulting in oxidative stress thereby leading to the ultimate death of cancer cells (34).

**Conclusions**

From the results of this study, the biosynthesized ZnO NPs using nonpathogenic *E.coli* showed antibacterial, antibiofilm and anticancer activities against studied MDR bacteria. So, these nanoparticles can be further used in many biotechnological applications as an effective antimicrobial, biofilm disinfectant and anti-cancerous agent.

**Conflict of Interest:** None

**Funding:** Self

**Ethical Clearance:** Not required

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A Study on Patient Satisfaction in a Tertiary Care Teaching Hospital Mysore

Sujay Mugaloremutt Jayadeva, Harshith.N

1Assistant Professor, Department of Health System Management Studies, JSS Academy of Higher Education & Research, Sri Shivarahreshwara Nagara, Mysuru, Karnataka, India

Abstract

As it’s effortless to enumerate the physical values of the company’s products, as well as the services, even so the perceptions of the customerare something that really resolves a company’s brand appraisement in a marketplace. Patient satisfaction finds to be crucial for any hospital it not only brings good image to the hospital, but also gives a strong satisfaction and trust for the patient towards the hospital, this research was carried out mainly to assess the perceptions of healthcare satisfaction among the patients. 100 patients were taken as sample size in this study and the study was carried out. The data were obtained from the patients by collecting their reviews based on the questionnaire. Results suggested that a majority of the patients required customer services as their first preference and also there were only 6 patients who gave the scale of 10 for satisfaction. So the study concludes that there should be measures taken by the hospital in order to increase the patient satisfaction towards the hospital services.

Key Words: Patient satisfaction, perceptions, satisfaction, hospital, questionnaire

Introduction

As it’s effortless to enumerate the physical values of the company’s products, as well as the services, even so the perceptions of the customer are something that really resolves a company’s brand appraisement in a marketplace. Accordingly, the potentiality of the healthcare marketer’s in building the value of a brand and disseminate such value in a brand along with disseminating that built value towards target audience through numerous strategies of marketing and advertising, such activities are fundamental to achieve enduring achievements in his/her medical practices.

Patient’s Perceptions are about, deliberations of potential and existing patients on your medical practices. Such like the Perceptions results in a new patient acquisition, along with the potentiality to sustain the Quality Relationship with the existing and potential patients of your hospital.

The patients desire for quality healthcare practices, nevertheless the patients also desire to have the knowledge about whether they are acquiring the finest values. Such like values cannot be determined by just pricing of a company’s product/services, but it can be determined through the possibility & level of the care provided to the patient. Having brand awareness is just not sufficient. Patient this time additionally desires of having good senses on their healthcare providers.

Materials and Methods

The research methodology of this study is to investigate the relationship among hospital brand image,
service quality, patient satisfaction/perception and loyalty. The study begins with setting an objective to investigate the patient’s perceptions on the hospital’s brand equity and what all factors help in building up a better brand and what concepts, influences patient’s decision making towards selection of brand with meeting the needs of the patients.

**Data Collection Method**

A questionnaire study was conducted to assess the patient’s perception of selected corporate hospital’s brand equity. Responses have been collected from the 100 patients of Tertiary care teaching hospital for collecting the data required for the study. The data were obtained from the patients by collecting their reviews based on the questionnaire.

**Study Area**

The study was conducted in the tertiary care Teaching Hospital, Mysore

**Results and Discussion**

The present study indications depend on personal particulars of respondents’ perceptions on brand equity of a hospital for building up a better brand. Total 100 samples were obtained to get the satisfactory results for the study.

<table>
<thead>
<tr>
<th>TABLE NO 01: GENDER</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL No</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

**Figure 1: Graphical representation: Gender**

According to the Table Number 01, and Figure Number 01 it referred the number of respondents with respect to “Gender”, the majority of the respondents are Males with 63% to Females of 37%.

<table>
<thead>
<tr>
<th>TABLE NO 2: CUSTOMER TESTIMONIALS INFLUENCE IN DECISION MAKING</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of respondent</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
</tbody>
</table>
According to Table Number 2 and Figure Number 2. This study the patient’s reviews on “how much influence does customer testimonials have on patients’ decision making” the respondents reveals, doesn’t influence for 5% of respondents, 6% respondents as influence very little, 16% respondents as influence moderately, 38% reacts as influences and 35% respondents reveals it as influences a lot. For majority of the respondents customer testimonials Influences in their decision making influence.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Attributes</th>
<th>Respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Brand and reputation</td>
<td>42</td>
</tr>
<tr>
<td>02</td>
<td>Customer service</td>
<td>81</td>
</tr>
<tr>
<td>03</td>
<td>range of services provided</td>
<td>51</td>
</tr>
<tr>
<td>04</td>
<td>Proximity to your location</td>
<td>40</td>
</tr>
<tr>
<td>05</td>
<td>Size and perceived stability</td>
<td>11</td>
</tr>
<tr>
<td>06</td>
<td>Industry and market knowledge</td>
<td>28</td>
</tr>
<tr>
<td>07</td>
<td>Sophistication of technology available</td>
<td>48</td>
</tr>
<tr>
<td>08</td>
<td>Global presence</td>
<td>16</td>
</tr>
<tr>
<td>09</td>
<td>Value for fees charged</td>
<td>59</td>
</tr>
<tr>
<td>10</td>
<td>Loyalty rewards and other similar benefits</td>
<td>28</td>
</tr>
</tbody>
</table>
According to Table 3 and Figure 3, this study reveals that the patient’s reviews on “to what all things mentioned above in the table do the patients give importance” the respondents reveals, brand and reputation for 42% of respondents, 81% respondents as customer services, 51% respondents as range of services offered, 40% reacts as proximity to your location, 11% respondents as size and perceived stability, 28% responds for industry and market knowledge, 48% responds for sophistication of technology available, 16% responds for global presence, 59% responds for value for fee charged, and 28% of respondents reacts for loyalty rewards and other similar benefits. Majority of the respondents reacts for CUSTOMER SERVICE with 81%, VALUE FOR FEE CHARGED with 59%, and RANGE OF SERVICES OFFERED with 51%.

**TABLE NO 4: PATIENT EXPECTATIONS APART FROM DIRECT BENEFITS**

<table>
<thead>
<tr>
<th>Identification with the users of the brand</th>
<th>A feeling of association with the company</th>
<th>To receive loyalty rewards and other similar benefits</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of respondents</td>
<td>37</td>
<td>46</td>
<td>44</td>
</tr>
<tr>
<td>Percentage</td>
<td>37%</td>
<td>46%</td>
<td>44%</td>
</tr>
</tbody>
</table>
According to Table Number 4 and Figure Number 4 This study the patient’s reviews on “apart from the direct benefits of a service, what else do the patients’ look for in it” the respondents reveals, identification with the users of the brand for 37% of respondents, 46% respondents as A feeling of association with the company, 44% respondents as to receive loyalty rewards and other similar benefits, 5% reacts as Others (the 4% of the respondents says NO and the 1% says Quality and expertise. For majority of the respondents “A feeling of association with the company” is the benefit they are look for apart from direct benefit.

**TABLE NO 5: PRICE SENSITIVITY OF PATIENTS ON A SCALE OF 1-10**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of respondent</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>10</td>
<td>21</td>
<td>37</td>
<td>16</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Percentage</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>3%</td>
<td>10%</td>
<td>21%</td>
<td>37%</td>
<td>16%</td>
<td>7%</td>
<td>6%</td>
</tr>
</tbody>
</table>
According to Table Number 5 and Figure Number 5, this study’s reviews on “On a scale of 1-10, how price sensitive the patients are” the respondents reveals 0% of respondents for a scale of 1, 0% respondents for the scale of 2, 0% respondents for scale of 3, 3% reacts on 4, 10% respondents on 5, 21% responds for 6, 37% responds for 7, 16% responds for 8, 7% responds for 9 and 6% of respondents reacts for 10. Majority of the respondents reacts for “7”, according the majority the patients ranks themselves at 7 on the of 1-10.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of respondent</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>8</td>
<td>25</td>
<td>42</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Percentage</td>
<td>0%</td>
<td>1%</td>
<td>2%</td>
<td>0%</td>
<td>5%</td>
<td>8%</td>
<td>25%</td>
<td>42%</td>
<td>11%</td>
<td>6%</td>
</tr>
</tbody>
</table>

Figure 6: Satisfaction scale of patients
According to Table Number 6 and Figure Number 6. This study the patient’s reviews on “On a scale of 1-10, how satisfied the patients are with the services received” the respondents reveals 0% of respondents for a scale of 1, 1% respondents for the scale of 2, 2% respondents for scale of 3, 0% reacts on 4, 5% respondents on 5, 8% responds for 6, 25% responds for 7, 42% responds for 8, 11% responds for 9 and 6% of respondents reacts for 10. Majority of the respondents reacts for “8”, according the majority the patients ranks they rank the services at 8 on the of 1-10.

**Conclusion**

Recently there is some problem, including the changing of medical background, the intense competition, the increasing of the hospital choices of patient’s are based on the QUALITY and the REPUTATION of the hospitals. The marketing and Branding departments play vital roles in attracting the patients based on their satisfactions. As the study was conducted to know about the perceptions of the patients towards patient satisfaction. Healthcare management now a days should encounter not only best quality healthcare services, But also must meet the patient’s needs and satisfactions, which leads towards higher patient satisfaction and loyalty towards Brand and also helps in rapid increases in revenue

**Ethical Approval:** Not Required

**Conflict of Interest:** The Author declares that there are no conflicts of interest.

**Funding:** Nil

**References**

Success Rate of Intact Canal Wall Tympanoplasty in Safe Type of Chronic Suppurative Otitis Media’ Patients

Sukma Nisa Janitra1, Artono1

1 Senior Resident, 2 Teaching Staff, Department of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

Abstract

Background: Surgical therapy for chronic suppurative otitis media (CSOM) is by tympanoplasty with or without mastoidectomy. The purpose of surgery is to eradicate the source of infection. Success indicators of tympanoplasty are achieving dry ears without recurrent otorrhea, myringoplasty, and an increase in the hearing threshold.

Objectives: is to know the success rate of intact canal wall tympanoplasty in safe type CSOM patients.

Methods: Retrospective descriptive was used in this study. All safe-type of CSOM patients undergoing intact canal wall tympanoplasty surgery from January to December 2018 at Dr. Soetomo General Hospital were used as the participant data.

Results: There were 32 participants, divided into a higher proportion of males than females (ratio 1.1:1). The characteristics of the participants included; the most age group were 21-30 years (37.50%), origin outside Surabaya (59.37%), and high school education level (62.50%). Most perforations were subtotal (40.63%) and hearing loss was conduction (62.50%). Apparently, 56.25% were patent Eustachian tubes, 90.63% were sclerotic mastoid Schuller photographs, and 62.50% were granulation tympanic cavity findings. Additionally, 78.13% were performed tympanoplasty type 1. The results of postoperative eradication showed dry ears (84.38%) and successful of myringoplasty (71.88%). The mean increase in the hearing threshold for AC, BC, and ABG after surgery was 15.98 dB, 4.57 dB, and 11.40 dB.

Conclusion: The results of eradication after intact canal wall tympanoplasty were mostly dry ears, successful myringoplasty, and an increase in the mean hearing threshold of AC, BC, and ABG.

Keywords: Chronic Suppurative Otitis Media, Safe Type, Intact Canal Wall Tympanoplasty, Intact Canal Wall Up Mastoidectomy

Introduction

Chronic suppurative otitis media (CSOM) is a chronic middle ear infection that lasts more than two months. It was characterized by a perforation of the tympanic membrane and continuous or intermittent discharge of secretions from the ear.1 Chronic suppurative otitis media (CSOM) is an ear disease inflammation commonly found in developing countries. The data from WHO (World Health Organization) shows that the prevalence of CSOM in developing countries such as Malaysia, the Philippines, and Thailand is still relatively high, namely 2-4% compared to developed countries in Europe such as Australia, England, Denmark and Finland which is around 0.4%.1,2 The survey conducted in seven provinces in Indonesia was obtained the prevalence of
CSOM was 3.6% of the Indonesian population. The low socio-economic life, slum environment, poor health, and nutritional status are risk factors that form the basis for the increasing prevalence of CSOM in developing countries.  

The management of the safe type of CSOM consists of non-surgery and surgery. The surgical option for safe type CSOM is tympanoplasty with or without mastoidectomy. The purpose of surgical therapy for tympanoplasty is to eradicate the infection from reaching dry ears, while another is to improve hearing process. A number of studies have reported the success factor of tympanoplasty influenced by age, perforation size, location of the perforation, ear condition before surgery, condition of the tympanic cavity during surgery, and Eustachian tube patency. Other factors are also influenced by operating technique, operator experience, previous surgery history, and smoking status. The success indicators of tympanoplasty surgery are achieving dry ears without recurring otorrhea, myringoplasty, and an increase in the hearing threshold. This study purpose is to determine the clinical characteristics of safe type CSOM patients undergoing intact canal wall tympanoplasty.

**Method**

A retrospective descriptive study was used with medical record data for January - December 2018 at out-patient care, Otology division of Dr. Soetomo General Hospital. The study inclusion criteria were patients with safe type CSOM who were undergoing intact canal wall tympanoplasty surgery. The study exclusion criteria included safe-type CSOM patients who underwent revision surgery, congenital anomalies, and incomplete medical record data. There were 136 patients with a diagnosis of safe type CSOM and 56 patients (41.18%) of whom underwent intact canal wall tympanoplasty surgery. The data that met the inclusion criteria were 32 patients. Participants were identified based on demographic data, clinical findings, plain Schuller mastoid radiograph, Eustachian tube patency test, operative findings, and type of tympanoplasty. The postoperative evaluation consisted of postoperative eradication results, myringoplasty, and pure tone audiometry 3 months postoperatively. The data distribution was analyzed by using the SPSS version 16.0 program.

This study has gone through a research protocol and received approval from the Health Research Ethics Committee of Dr. Soetomo General Hospital, Faculty of Medicine, Universitas Airlangga, Ref. No. 0131 / LOE / 301.4.2 / X / 2020.

**Results**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Amount</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>53.13%</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>46.88%</td>
</tr>
<tr>
<td>Age’s Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-20 years</td>
<td>11</td>
<td>34.38%</td>
</tr>
<tr>
<td>21-30 years</td>
<td>12</td>
<td>37.50%</td>
</tr>
</tbody>
</table>
In this study, the male group was more than the female (1.1:1). The most age group was in the population of 21-30 (37.50%) with ages between 13-58 years. Then, most of the patients were coming from outside Surabaya (59.38%). The highest education level was SMA (62.50%) and half of the participants were unemployed (50%) (Table 1).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Amount</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Perforation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>11</td>
<td>34.38%</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>25.00%</td>
</tr>
<tr>
<td>Subtotal</td>
<td>13</td>
<td>40.63%</td>
</tr>
<tr>
<td>Types of hearing loss</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A conductive deafness</td>
<td>20</td>
<td>62.50%</td>
</tr>
</tbody>
</table>
Based on the table above, most types of perforation were subtotal type (40.63%), while most types of hearing loss were conduction deafness (62.50%). The tubal patency test of this study showed that the majority of patients (56.25%) and plain radiographs of Schuller mastoid were mostly sclerotic (90.63%) (Table 2).

The most pathology finding of the tympanic cavity during surgery was granulation tissue (62.50%), while the most type of tympanoplasty often performed was tympanoplasty type 1 (78.13%) (Table 3).
The results of postoperative eradication showed dry ear conditions (84.38%) and the success of myringoplasty was 71.88% (Table 4).

The mean preoperative Air Conduction (AC) hearing threshold increased from 56.09 dB to 40.12 dB postoperatively, so that the mean increase in AC hearing threshold was 15.98 dB. The mean result of preoperative Bone Conduction (BC) hearing threshold increased from 18.91 dB to 14.34 dB postoperatively, so that the mean increase in BC hearing threshold was 4.57 dB. The results of the preoperative mean Air Bone Gap (ABG) increased from 37.19 dB to 25.79 dB postoperatively, so that the mean increase in ABG was 11.40 dB (Table 5).

### Table 4. Characteristics of intact canal wall tympanoplasty surgery

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Amount</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results of postoperative eradication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>27</td>
<td>84.38%</td>
</tr>
<tr>
<td>Wet</td>
<td>5</td>
<td>15.63%</td>
</tr>
<tr>
<td>Myringoplasty</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth</td>
<td>23</td>
<td>71.88%</td>
</tr>
<tr>
<td>Failed</td>
<td>9</td>
<td>28.13%</td>
</tr>
</tbody>
</table>

Research on hygiene categories showed females have higher hygiene standards than males. This psychological predisposition was related to the incidence of exposure to allergens, bacteria, and parasites.6

The population obtained in this study that most productive age was between 21-30 years (37.50%)
and the number decreased in the fourth to fifth decade. Research by Kumar, et al., found that the largest age group was 21-30 years and decreases in the age group over 60 years. The safe type of CSOM patients who come to an outpatient unit of Dr. Soetomo General Hospital was 59.38% came from outside Surabaya. The majority come from small cities to rural areas in East Java. As reported by Parmar, et al., the difference in the incidence of CSOM among elementary school students in the village (5.11%) was higher than elementary school students in the cities (2.32%). High incidence in small cities or rural areas is associated with a lack of education, education level, awareness of the disease, poor hygiene, and limited health services. Half of the participants were unemployed (50%) and the highest level of education was high school (59.26%). Kumara, et al., showed that CSOM is more common among people with lower socioeconomic status (39.4%). Sufferers of Chronic suppurative otitis media (CSOM) are often found in families with low socioeconomic status and income. Inadequate socioeconomic conditions, malnutrition, poor hygiene, recurrent respiratory infections, and inadequate health care centers were the factors in the occurrence of CSOM.6

The most perforation type in this study was the subtotal type (40.63%). Thakur, et al., obtained similar results (43.6%). The posterosuperior quadrant has more vascularization than other areas so if an inflammatory process occurs, it is susceptible to cell exposure. Repeated inflammation and negative intratympanic pressure effects tympanic membrane retraction until membrane rupture occurs, especially in areas with weaker structures. The most common type of hearing loss in this study was the conductive type (62.50%). Similar results were reported by Kumar, et al.,7 The results of chronic inflammation of the middle ear makes the formation of secretions, perforation of the tympanic membrane, and breaking the ossicular chain. This condition inhibits the transmission of sound waves from the middle ear to the inner ear causing conductive-type hearing loss.12

Mostly, Eustachian tube patency test showed a patent results (56.25%). Kumar, et al., reported that patent tubes in safe type of CSOM patients was 76%. Additionally, the inflammatory process of CSOM could change the pathology of tubal mucosa so it interferes with the function of the Eustachian tube. The inflammatory reaction causes mucosal epithelium hyperplasia, metaplasia, and edema to form granulations or polyps. The distribution of goblet cells and glandular increases in the bone and isthmus so it has an impact on tubal patency, especially the function of ventilation and drainage.11 The plain Schuller mastoid radiograph showed mostly the sclerotic features (90.63%). Roy, et al., obtained the same results with the largest picture being sclerotic (78.85%). The inflammatory process in CSOM affects mastoid pneumatization so the air cells mastoid tend to decrease. The duration is also believed to affect the degree of pneumatization of the mastoid air cells.13

The most common pathology of the tympanic cavity was granulation (62.50%). The same results were obtained in the study by Thakur, et al., which stated that Granulation is formed due to an inflammatory process in the middle ear and causes bone erosion in both the ossicular chain and the tympanic cavity wall. The tympanoplasty technique used in this study was based on the Wullstein classification. Most types of tympanoplasty were type 1 (78.13%). Similar research results were obtained by Antony, et al.,14 The purpose of tympanoplasty are to close the canal perforation, eradicate germs and improve hearing.10 Evaluation of the radiation results that carried out 3 months after the surgery was mostly dry ears (84.38%). The same result was reported by Puspitowati.15 The purpose of surgery for CSOM is to eradicate the source of infection and achieve dry ear conditions.4

Postoperative myringoplasty was mostly successful (71.88%). Yurtas, et al., showed myringoplasty were not much different (75%). Eustachian tube patency was one of the factors that play a significant role as a prognostic
for the success of safe type CSOM surgery because it is considered to provide an image of aeration in the middle ear. Patients with normal tubal function show a better ratio of myringoplasty growth outcomes than abnormal tubal function. Several studies reported that the success of tympanoplasty formation also influenced by age, perforation size, location of the perforation, preoperative ear conditions, and the condition of the tympanic cavity during surgery. Surgical technique, operator experience, previous surgical history, and smoking status are also factors in the success of tympanoplasty.

In this study, the means postoperative AC hearing threshold increased by 15.98 dB. The mean postoperative BC hearing threshold increased by 4.57 dB and ABG increased by 11.40 dB. Additionally, Similar results were reported by Dangol and Shrivastav with AC hearing threshold was an increase in postoperative by 11.45 dB and ABG by 8.9 dB. Post tympanoplasty hearing improvement can be affected by the degree of ocular bone damage. The increase in the hearing threshold appears to be more significant in good ossicle conditions.

**Conclusion**

The results of postoperative eradication were mostly obtained as a dry ear by 84.38%. The myringoplasty was successful by 71.88%. There was an increase in the mean postoperative hearing threshold of AC by 15.98 dB, BC hearing threshold by 4.57 dB, and ABG by 11.40 dB.

**Conflict of Interest:** None.

**Source of Funding**

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**Ethical Clearance:** Taken from Health Research Ethics Committee of Dr. Soetomo Teaching Hospital Surabaya.

**References**


Estimation of Height from Foot Measurement in Kalaburagi District

Sunilkumar C.A 1, Santosh Garampalli 2

1 Assistant Professor Dept of Forensic Medicine KBNIMS Kalaburagi, Karnataka, 2 Associate Professor Dept of Forensic Medicine MRMC Kalaburagi, Karnataka

Abstract

Estimation of height of a person plays an important role in identification of a person which is necessary in criminal cases and as well as in mass disasters where only a portion or a part of body may be seen. The present study was carried out on the measurement of foot length and body height of 342 students (170 males and 172 females) of 05 to 30 years of age. Anthropometric measurements were taken by using anthropometric instruments in centimeter to the nearest millimeter. All the measurements were taken in a well lighted room. Obtained data was analysed for correlation coefficient and to derive a regression formula between foot length and height of an individual. A medium correlation of height was observed with foot length and it was statistically significant. The present study would be useful for anthropologists and forensic experts

Key words: Co-relation coefficient, Forensic Anthropology, Foot Length, Regression Equation, Stature

Introduction

The science of measuring the body parts constitutes Bertillon’s system or anthropometry.

Historical aspect of anthropometry as a means of establishing human identity is pretty interesting. It is also viewed as a traditional and basic tool of biological anthropology 1, but it has a long tradition of use in forensic sciences. Relationship that exists between different parts of the body and height has been of great interest to anthropologists, artists, scientists, anatomists, and medico-legalists for a long time. Height estimation by measurement of various long bones has been attempted by several workers with variable degree of success 2,3,4. Ossification and maturation in the foot occurs earlier than the long bones and therefore, during adolescence age, height could be more accurately predicted from foot measurement as compared to that from long bones 8. There are a lot of variations in estimating stature from limb measurements among people of different region and race. Hence there is a need to conduct more studies among people of different regions and group so that stature estimation becomes more reliable and identification of an individual is easily established. The aim of our study is to help in the identification of victims of mass deaths due to natural calamities or manmade disasters from fragmentary and dismembered human remains, identification of criminals from foot print & to find if there is any sex and racial differences of height when compared to foot length. The study also aims to find out any correlation of height with foot length among male and female population of kalaburagi district of

Corresponding author:
Dr Santosh Garampalli
Associate professor Dept of Forensic medicine MRMC kalaburagi,K B.N Medical College Dist: Kalaburagi, Karnataka
Karnataka state & to predict and estimate height from foot length by deriving a regression equation.

**Materials and Methods**

This observational cross sectional study using convenient sample technique was done over a period of 1 year from March 2019 to February 2020. The present study was carried out in the department of forensic medicine and toxicology at KBNIMS Kalaburagi. A total of 342 subjects were included in the study, out of which 170 males and 172 females within age group of 05 to 30 years. The subjects included in study were healthy individuals free from any apparent skeletal deformity. The left foot was taken for the measurement as per the recommendation of the International agreement for paired measurements at Geneva. Besides the above measurements, stature of each subject was also recorded. All measurements were taken in well lighted room. The measurements were taken using standard anthropometric instruments in centimeters to the nearest millimeter in following manner.

**Anthropometric Measurement**

**Foot length:** It is the distance from the most prominent part of the heel backward to the most distal part of the longest toe (2nd or 1st).

**Instrument:** Vernier Calliper.

**Technique:** The measurement was made on standing subject. The calliper was horizontally placed along the medial boarder of the foot. The fixed part of the outer jaw of the calliper was applied to the pternion and the mobile part of the outer was approximated to the acropodian and measurement were taken. In the same way measurement of the other side were taken.

**Stature:** It is the vertical distance between the point vertex and the heel touching the floor (ground surface).

**Technique:** The subject was made to stand in erect posture against the wall with the feet axis parallel or slightly divergent and the head balance on neck and the measurement was taken. The data was collected, analysed and subjected to statistical analysis using statistical package for social sciences (SPSS) to know the correlation of the stature with the length of feet and simple linear regression formulae were derived for various combinations. The reliability of estimation of stature from the lengths of feet was determined with the help of standard error of estimation (SEE).

The exclusion criteria were:

1. Major ailments in the past affecting foot.
3. Congenital or acquired deformity of foot, or spines.
4. Past histories of generalized disease affecting height like rickets, osteomyelitis, gigantism, dwarfism, achondroplasia, cretinism etc.

**Results and Analysis**

The mean age of males was 17.27 years with standard deviation (SD) of 5.424 whereas the mean age of females was 15.85 years with standard deviation (SD) of 6.069.

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Sex</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>female</td>
</tr>
<tr>
<td>5 to 9</td>
<td>22(12.9%)</td>
<td>42(24.4%)</td>
</tr>
<tr>
<td>10 to 14</td>
<td>24(14.1%)</td>
<td>18(10.5%)</td>
</tr>
<tr>
<td>15 to 19</td>
<td>40(23.5%)</td>
<td>51(29.7%)</td>
</tr>
<tr>
<td>20 to 29</td>
<td>84(49.4%)</td>
<td>61(35.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>170(100%)</td>
<td>172(100%)</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>17.27±5.424</td>
<td>15.85±6.069</td>
</tr>
</tbody>
</table>
We have seen that mean height was found to be 159.21±18.64cm in male subjects and 145.22±18.02cm in female subjects. The mean foot length was found to be 24.62±2.75cm in male subjects and 22.24±2.46cm in female subjects. The mean foot breadth was found to be 9.92±1.07cm in male subjects and 8.99±0.90 cm in female subjects. The differential trends as assessed by mean of t-test reveal highly significant sex differences (p<0.000) for Stature, for foot length and for foot breadth. The following Regression formulæ were derived.
Regression formula from foot length for Males:
Stature (y) = 6.52 foot length - 1.30

Regression formula from foot length for
Females: Stature (y) = 6.82 foot length - 6.45

Regression formula from foot length for both
Genders: Stature (y) = 6.52 foot length + 0.78

Regression formula from foot breadth for
Males: Stature (y) = 14.75 foot breadth + 12.82

Regression formula from foot breadth for
Females: Stature (y) = 17.24 foot breadth - 9.76

Regression formula from foot breadth for both
Genders: Stature (y) = 15.66 foot breadth + 4.08

Discussion
Patel et al (2007)10 carried a study in Gujarat region and found the correlation coefficient between height and foot length as +0.65 in males and +0.80 in females; also derived regression equations to calculate the height from foot length as: H = 75.45 + 3.64FL for males and H=75.41 + 3.43 for females (H= Height, FL= Foot Length). Jaydip Sen et al (2008)11 conducted a study to estimate sex from foot length, foot breadth, and foot index among 350 living adult (age range: 18–50 years) Rajbanshi individuals (175 men and 175 women). They concluded that foot dimensions show significant sex differences and higher correlation between foot length and stature [correlation coefficient r=0.623 for males and 0.682 for females] than that between foot breadth and stature [correlation coefficient r=0.523 for males and 0.387 for females]. Mohanty & Agrawal12 studied a population of 300 students (M=206, F=94) aged 18—25 years of Odisha and developed a regression equation that could calculate the height of an individual from his foot length. They found that as height increases foot length of both male and female also increases. Krishnan (2008)13 examined the relationship of stature to foot size of 1040 adult male Gujjars of North India (age 18 to 30 years) and found that the highest correlation coefficient were shown by the toe length measurements (0.79 – 0.86). Agnihotri et al (2007)14 developed a relationship between the foot length and stature using linear and curvilinear regression analysis on a study group comprising of 250 medical students (125 males and 125 females) aged 18–30 years. It was concluded that general multiple linear regression model was highly significant (p<0.01) and validated with highest value for the coefficient of determination R2 = 0.769 and multiple correlation coefficient r = 0.877.

Conclusion
Simple regression equation derived from this study can be used to estimate height of individuals. This fact will definitely be useful in medicolegal investigations and in anthropological and archeological studies where total height of an individual can be calculated if foot dimension is known.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Taken from KBNIMS ethical committee

References


Phylogenetic Analysis Related to Hepatitis B Virus based on Region Surfaces in Tuban, East Java Province, Indonesia

Supiana Dian Nurtjahyani1, Retno Handajani2,3
1Faculty of Teaching and Education Science, University of PGRI Ronggolawe, Tuban, East Java, Indonesia, 2Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia, 3Institute of Tropical Disease, Universitas Airlangga, Surabaya, East Java, Indonesia

Abstract
Hepatitis B virus (HBV) is a virus cause liver disease. This virus could be transmitted through blood product, and it becomes the major problems in developing countries. Genotype distribution of HBV depends on geographical characteristics. Here, we reported HBV genotyping analysis of blood donors with positive Hepatitis Surface Antigen (HbsAg) at Indonesian Red Cross Foundation in Tuban, East Java. Total 150 sera were collected from blood donors at Indonesian Red Cross Foundation in Tuban, East Java. Enzyme-Linked Immunosorbent Assay (ELISA) was conducted by PRISM® HBsAg kit (ABBOTT) according to the manufacture’s instruction, to determine sample that has positive HbsAg. The genotyping analysis was done using polymerase chain reaction and sequencing method from 12 samples. Genetyx ver.10 software was used for identification of HBV type. Analysis of HBV genotype revealed three different types of HBV. HBV type B was the major HBV (50%). Interestingly, there was one unidentified HBV type suggested as a novel subtype of HBV. Further genotyping analysis is needed to confirm novelty of the unidentified HBV type. Analysis of HBV genotype revealed three different types of HBV. HBV type B was the major HBV (50%). Interestingly, there was one unidentified HBV type suggested as a novel subtype of HBV. Further genotyping analysis is needed to confirm novelty of the unidentified HBV type. This study was the first evidence of blood donor’s genotyping analysis in Tuban.

Keywords: blood donors, genotyping analysis, hepatitis B virus, region surface, Tuban.

Introduction
Hepatitis B virus (HBV) is a major virus causing both acute and chronic liver diseases that are spread widely in Indonesia. Chronic infection of HBV has a risk to develop become to carcinoma and liver cirrhosis. Indonesia is included in group of countries that have high prevalence of HBV with blood Hepatitis B surface antigen (HbsAg-emia) approximately 3-20%. Therefore, HBV infection still becomes the main health problem.

To date, there are 10 HBV genotypes already identified all over the world coded from A to J. These types are classified based on genomic properties of HBV. Classification is mainly observed from the genomic divergence, divergence with percentage >8% in nucleotide sequences from HBV whole genomes or >4% from region surface (S) genes.

Several regions in Indonesia have different prevalences of HBV infection. Lombok Island is a hyper endemic region in which about 10.6%-20.3% individuals in suburban area become a carrier of this infection. Infection transmitted through blood product remains high. It is reported that the highest evidence of blood donors with positive HBV is found in Papua province, about 10.5%. In addition, it also explained the evidence...
of blood donors with positive HBV at 11 big cities in Indonesia ranging from 2.1% to 9.5%.4

Tuban is one of the districts in East Java, Indonesia. This district is located near Central Java boundaries; therefore, it becomes one of the regions that connects East Java and Central Java. Based on data from Indonesian Ministry of Health in East Java region in 2007, Tuban has relatively high prevalence of hepatitis with frequency of 0.24% and ranked seventh among all 38 districts and cities in East Java province. That frequency might increase because from the recent data, the frequency of hepatitis prevalence increased significantly from 2007 to 2013 in East Java province2,3,4. Besides that, it has been suggested that the geographical position of Tuban could support the increased frequency of hepatitis prevalence. Here, we reported the first evidence of HBV genotyping analyses based on region surface gene from blood donors with positive HbsAg at Indonesian Red Cross Foundation in Tuban, East Java, Indonesia.

**Materials and Methods**

**Sample preparation**

Sample used in this study was 150 sera from blood donors at Indonesian Red Cross Foundation in Tuban, East Java. Enzyme-Linked Immunosorbent Assay (ELISA) was conducted by PRISM® HbsAg kit (ABBOTT) according to the manufacture’s instruction, to determine sample that has positive HbsAg. Genomic DNA was extracted from positive samples of human blood by Gentra Puregene Blood kit (QIAGEN) according to the manufacturer’s instructions prior to genotyping analysis.

**Genotyping Analysis**

Samples with positive HbsAg were continued for genotyping analysis. HBV genotyping analysis was performed using nested polymerase chain reaction (PCR) and DNA sequencing. Nested PCR was done using two different primers, external and internal primers. Sequences of external primers used in the first step are 5’- GTG GTG GAC TTC TCT CAA TTT TC – 3’ (P7) and 5’-CGG TAW[^A/T] AAA GGG ACT CAM[^A/C]GAT-3’ (P8). And sequences of internal primers used in the second step are 5’-CAA GGT ATG TTG CCC GTT TG-3’ (HBS1) and 5’-AAA GCC CTG CGA ACC ACT GA-3’ (HBS2).13,14

DNA fragment resulted from PCR was used for sequencing after purification step. Purification was performed by QIAquick Gel Extraction kit (QIAGEN) according to the manufacturer’s instruction. Purified DNA was then mixed with one of the primers and Big Dye Terminator kit reagent. Sequencing was conducted using ABI PRISM-310 Sequencer Machine (Applied Biosystems Inc.).

**Identification of HBV genotype**

The identification of HBV genotype was analysed by multiple alignments between sequencing results from sample and gen bank (NCBI) were performed using software Genetyx-Win Ver.10. Phylogenetic tree was made to determine the HBV genotype.

**Results and Discussion**

ELISA analyses from 150 samples of sera resulted in 13 samples that have positive HbsAg. DNA Sequence analysis was performed only in 12 samples. One sample failed to produce a good sequencing result. We used primers that amplified region surface gene in our study for genotyping analysis. The genotyping analysis showed that 12 samples could be included in two major groups. HBV type B was the be included in all groups of HBV type already identified.
Study conducted by Telenta et al. showed the usage of primers targeting the region of surface gene could showed genotype analysis results and analysis using whole genome of HBV. Multiple alignments were conducted between sequencing results from 12 samples and sequence from previous studies already described. Furthermore, phylogenetic tree was made from the alignment analysis. The genotyping analysis showed that 12 samples could be included in two major groups. HBV type B was the dominant type identified in six samples, thus frequency was 50%. This was followed by HBV type C identified in five samples, thus frequency was 41.7%. One sample with code VHB-Tbn-39 (frequency 8.3%) could not be included in all groups of HBV type already identified. HBV type C was considered prior to hepatocellular carcinomas and higher frequency of mutation at HBV core promoter region that related to susceptibility to chemical drugs, compared to HBV type B.

In general, our results showed a similarity with previous study conduct at other areas in Indonesia, whereas HBV types B and C are more dominant than other types. Although Lombok and Papua are well known as regions with the highest prevalence of Hepatitis, HBV type B and C are also dominant in both regions. But in Tuban, both types are almost equal in frequencies. Several Asian countries have similarities in dominant types of HBV such as China and Japan. Compared with other regions such as America and Africa, there were predominant other types of HBV such as types A, D, and E.
Several factors were related to distribution of HBV such as: 1) the effects of mutation prior to drug resistances and vaccination to patient with HBV; 2) individual sex behaviour in those regions\textsuperscript{18}; and 3) poor infection control at dental services\textsuperscript{19}. Basically, social life was closely related to the HBV evidence. Therefore, although there was similarity in predominant HBV types among Indonesian regions but it might be possible that the clinical characteristics among patients in different geographic regions were also different, as suggested in previous study by Orito \textit{et al.}\textsuperscript{20}. Thus, it is possible that the distributions of HBV are different worldwide.

**Conclusion**

In summary, since there is no clinical study related to genotyping analysis in Tuban, at least in part, the data resulted from this study seemed to be an important preliminary data. In our case, one sample was found to be significantly different from any HBV type already known. Further research with large numbers of sample, clinical feature observation, randomized, placebo-controlled study may be needed to confirm the predominant HBV type in Tuban, East Java, Indonesia. In addition, more sensitive genotype analysis should be carried out to reveal one sample that could not be identified.

**Conflict of Interest** : The authors declare that they have no conflict of interest.

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**Ethical Approval** : This study had been approved by local ethics committee Medical Faculty, University of Airlangga, Surabaya, East Java, Indonesia.

**References**


Management of Patient With Caustic Ingestion Injury: A Case Report

Supriadi1, Budi Widodo2, Brinna Anindita3
1Senior Resident, Department of Internal Medicine, 2Gastroenterohepatologist, Division of Gastroentero-Hepatology, Department of Internal Medicine, 3Internist, Department of Internal Medicine, Faculty of Medicine, Airlangga University-Dr Soetomo General Hospital, Surabaya

Abstract

The ingestion of caustic substances, both accidental and voluntary, determines a complex syndrome, characterized by severe, often irreversible, visceral lesions, with still remarkable mortality in highly compromised patients. A 36-year-old woman came to Emergency-Room with the chief complaints of blackish vomiting about 4 times after drinking liquid to clean the bathroom floor. Patients with findings of grade 2B esophageal injury, pangastritis and gastric ulcer on endoscopy have high the risk of perforation and complications. Endoscopy done within 12 hours and no later than 24 hours following caustic ingestion to classify mucosal injury subsequent to caustic ingestion is useful to determine the severity of injury, particularly in suicidal cases, and thus helpful in predicting outcomes. Patient threat with rehydration with normal saline, total parenteral nutrition, pump omeprazole, injection of methylprednisolone, antibiotic, and sucralfate. Consultation with the Psychiatry department has been conducted psychoeducation and psychotherapy.

Keywords: caustic ingestion injury, endoscopy, suicidal cases.

Introduction

The ingestion of caustic substances, both accidental and voluntary, determines a complex syndrome, characterized by severe, often irreversible, visceral lesions, with still remarkable mortality in highly compromised patients. The most severe oesophageal and gastric damage is notoriously related to voluntary and pseudo-voluntary ingestion of high amount or concentration of strong acids or alkali compounds.1) Corrosive substances can be acidic or alkaline.

Mucosal damage caused by corrosive material can appear one to two months after the contact.2)

Ten-percent of patients sustaining caustic mucosal injury-(CMI) will experience immediate complication. The most common serious immediate complications after caustic material ingestion therefore include: perforation, bleeding, but late findings include fistula formation. Reported mortality approaches 10–20%. Among those sustaining caustic injury in a suicide attempt, mortality may approach 75%. The delayed complications include stricture formation leading to malnutrition and long term risk of developing malignant transformation.1)

We report a young adult woman with caustic ingestion injury. The aim of this case report is endoscopy following caustic ingestion to classify mucosal injury subsequent to caustic ingestion is useful to determine the severity of injury, particularly in suicidal cases, and thus helpful in predicting outcomes.

Case Description

A 36-year-old woman came to Emergency-Room with the chief complaints of blackish vomiting about 4 times after drinking liquid to clean the bathroom floor. When in the ER patient continued to vomit about 3 times. According to her husband, the patient did this to commit suicide due to their domestic problems. On physical examination general condition alert and weak.
Blood pressure 160/80mmHg, pulse 112x/minute, regular-rhythm, respiratory-rate 28x/minute, axillary-temperature 36.7°C. On abdominal examination, contour was flat, supple on palpation, no tenderness.

Laboratories tests showed Hb 13.5--g/dL, leucocyte--112210/mm3, thrombocyte 414000/mm3, Natrium 145--mmol/L, kalium 3.4-mmol/L, chloride 113-mmol/L, blood-glucose 89-mg/dl, AST 14-U/L, ALT 11-U/L, albumin 4.64-g/dL, urea 7-mg/dL, creatinine 0.64-mg/dL, HbsAg non-reactive. Chest-X-ray and BOF: within normal limit.

Based upon all finding we assessed the patient with intoxication of caustic substance and tentamen suicide, therapy with rehydration with normal saline, total parenteral nutrition, pump omeprazole 8mg/hour, injection of methylprednisolone 62.5mg/8hours intravenously, injection of ceftriaxone 1gram/12hours intravenously, orally sucralfate 10ml/8hours. Consultation with the Psychiatry department has been conducted psychoeducation and psychotherapy.

On 2nd day of treatment, cito endoscopy was performed on patients and grade IIB esophageal injury, pangastritis, and gastric ulcer were obtained. On 3rd day of treatment the patient feel better, she and her family refused further hospital care due to cost problems.

Fig.1 Multiple erythema appeared in the cavum oris, erythema and lacerations in the oropharynx and hypopharynx, Epiglottis looks edematous

Fig.2 The proximal to distal esophagus appeared hyperemetic and lacerations. Grade2B ulcers.

Fig.3 Gaster Mucousa appeared to be covered with blood clots, including multiple erythema, lacerations, and ulcers, with necrosis in the pre-pyloric region

Discussion

Ingestion of acid or alkaline caustic substances may cause serious injuries in the esophagus and stomach. The degree of injury is determined by the nature of the substance, the amount consumed or its concentration and state and the time of contact with the gastrointestinal mucosa.3) Mechanism of action of corrosive agents: Alkali ingestion: Causes liquefaction necrosis. This process includes protein dissolution, collagen destruction, fat saponification, cell membrane emulsification, submucosal vascular thrombosis and cell death. Acid ingestion: Causes coagulation necrosis. In this process, hydrogen (H+) ions desiccate epithelial cells producing an eschar. This process leads to edema, erythema, mucosal sloughing, ulceration and necrosis of tissues. Both acids and alkalis cause fibrosis and cicatrization (stricture formation).4)

Hemorrhage, thrombosis, and inflammation with oedema are the dominant processes during the first 24hours following ingestion. The clinical presentation
of caustic ingestion is diverse and do not always correlate with the degree of injury. Symptoms mainly depend on the location of damage. Hoarseness and stridor are signs that are highly suggestive of an upper respiratory tract involvement, particularly the epiglottis and larynx. Presence of these findings may signal a potentially life-threatening respiratory event. The upper gastrointestinal tract, on the other hand, may present as dysphagia or odynophagia for esophageal injury and hematemesis or epigastric pain for gastric involvement. The chief complaints of this patient is blackish vomiting about 4 times after drinking liquid to clean the bathroom floor.

Esophagogastroduodenoscopy is a sophisticated method for diagnostic evaluation of acute corrosive poisonings and injuries of the upper gastrointestinal tract. According to the latest controlled studies the most optimal timing for esophagogastroduodenoscopy is the first 12–24 hours following corrosive ingestion while according to other authors it may be safely performed within the first 96 hours following corrosive ingestion. Endoscopy, as part of the instrumental techniques usable in the acute phase, is the mainstay of diagnostic evaluation and staging, as it allows you to check: (a) the presence of lesions; (b) the severity of lesions; (c) the extent of the lesions by considered area (for example the esophagus); (d) the topographical distribution in the upper digestive tract (from the pharynx to the duodenum); (e) the presence of objective evidences correlated to the risk of perforation. Endoscopy should be always performed in all patients in whom the ingestion of caustic in large amount or strong concentration is sure or at least suspected, such as intentional or pseudovoluntary ones. About 20% of them could have visceral lesions without any oral pathological finding.

The findings on upper GI endoscopy are based on Zargar’s modified endoscopic-classification of burns due to corrosive ingestion into six grades: 0 (normal mucosa), 1 (erythema/hyperemia), 2a (superficial ulcer/erosion/friability/hemorrhage/exudates), 2b (findings in 2a deep discrete/circumferential ulcers), 3a (scattered necrosis, black/grey discoloration), 3b (extensive/circumferential necrosis of mucosa).

The results of this study confirm Zargar’s endoscopic-classification of mucosal injuries post caustic ingestion in relation to clinical outcome. The tensile strength of healing tissues in the first 3 weeks is low due to an absence of collagen. New collagen formation does not begin until the second-week after injury. Thus, it is advocated that endoscopy should be avoided from 5 to 15 days after caustic ingestion. Currently, EGD evaluation within 12 hours and no later than 24 hours after caustic ingestion is considered safe. EGD is not recommended from 2 to 3 days up to 2 weeks after caustic ingestion as a result of wound softening. On 2nd day of treatment, cito endoscopy was performed on patients and grade-2b esophageal injury, erosion of pangastritis, and gastric ulcer were obtained.

Short-term complications include perforation and death. Perforation of the esophagus or stomach can occur at any time during the first 2 to 3 weeks of ingestion. A sudden worsening of symptoms or an acute deterioration of a previously stable condition should warrant a thorough investigation to rule out the possibility of a perforated viscus. Chronic complications of caustic ingestion include stricture formation, gastric outlet obstruction and malignant transformation. Patients with esophageal strictures usually complain of dysphagia and substernal pressure, and ay become symptomatic 3 weeks or later after ingestion.

Management of caustic injury includes immediate resuscitation and evaluation of extent of damage. The cornerstone of all caustic ingestions is airway and hemodynamic stabilization. Since direct exposure of the upper respiratory-tract by the corrosive substance may occur, patients should be evaluated for the need to do immediate intubation or tracheostomy. Patient therapy with rehydration with normal saline, total parenteral nutrition, pump omeprazole 8mg/hour, injection of methylprednisolone 62.5mg/8hours intravenously, injection of ceftriaxone 1 gram/12 hours intravenously, orally sucralfate 10ml/8hours.
Neutralizing agents.

It has now been emphasized that these substances should not be administered due to the additional thermal injury and chemical destruction of tissues these reactions produce.9)

Nasogastric tube.

Routine nasogastric intubation for the purpose of evacuating any remaining caustic material is no longer warranted prior to endoscopic assessment of mucosal injury. This is due to the possibility of inducing retching or vomiting leading to further esophageal exposure by reflux of the remaining intragastric caustic material.10)

Gastric acid suppression and mucosal protection

Upon admission, the patient should be kept fasting. Gastric acid suppression with H2-blockers or intravenous protonpumpinhibitors are often initiated to allow faster mucosal healing and to prevent stress ulcers.5)

Antibiotics

A study in 1992 analyzed the utility of antibiotic together with systemic steroid administration in caustic ingestion. It was concluded that antibiotics with steroids may be useful in preventing strictures in patients with extensive burns.10)

Steroids

Methylprednisolone at a dose of 1 g/1.73-m2 per day for 3-days showed benefit in reducing stricture development. However, another study showed that prednisolone at a dose of 2g/kg intravenous did not provide any benefit in preventing stricture development.11,12)

Triamcinolone

Intralesional steroid such as triamcinolone (40-100mg/session) has long been known to augment the dilatation of caustic-induced esophageal strictures although results from most studies are still conflicting.5)

Conclusion

An 36-year-old woman with findings of grade-2B esophageal injury, pangastritis and gastric ulcer on endoscopy have high the risk of perforation and complications due to caustic ingestion injury. Endoscopy done within 12 hours and no later than 24 hours following caustic ingestion to classify mucosal injury subsequent to caustic ingestion is useful to determine the severity of injury, particularly in suicidal cases, and thus helpful in predicting outcomes.

Conflict of Interest: The authors report no conflict of interest.

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Ethical Clearance: Not required for a case report.

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References


Knowledge Regarding Prevention of Urinary Tract Infection In Patients with Indwelling Catheter among Staff Nurses: An Intervventional Study

Surinder Singh¹, Ruchi Kumari², Aarti Thakur³, Sakshi Tomar⁴, Mridul⁴

¹Assistant Professor, ²Nursing Tutor, Department of Medical Surgical Nursing, ³Nursing Tutor, Department of Obstetrics and Gynaecology Nursing, ⁴Clinical Instructor, Chitkara University College of Nursing, Chitkara University, Himachal Pradesh, India

Abstract

The study based on a broad framework with input, throughput, output and feedback first presented by Ludwig Von Bertalanffy. The investigation utilised an evaluative approach. Samples of 50 staff nurses were collected using random sampling by lottery. A structured questionnaire was used for data collection. The overall pre-test knowledge mean score was 14.14±1.629. After administration of the self-instructional module, the post-test mean score increased to 20.56±4.362. The hypothesis H1 expressed that there will be a considerable distinction between pre-test and post-test information scores of nursing officers regarding urinary tract infection. The ‘t ‘ test was discovered to be more prominent than the table worth. Acquired worth was (10.353), and table worth was (2.045). It showed the effectiveness of the SIM. The study was no significant relationship with demographic variables because the chi- square value was less than the table value. Thus, the H2 has been dismissed.

Key words: Effectiveness, urinary tract infections, knowledge, Self Instructional Module.

Introduction

The statement underlines the importance of kidneys to our lives. Satisfactory working of the renal system is essential for the support of a sound body.¹ These are the part of the urinary system, which precisely maintains the body’s chemical environment and performs various excretory and secretory functions of the body. The urinary bladder works as storage, whereas the ureters and urethra can be considered as waste channels for the urological system.² After shaped by the kidneys, when there is a decrease in the outflow of urine, it leads to UTI caused by pathogenic organisms in the urinary tract. It is one of the most common infections treated by primary care providers. The majority of urinary tract infections are brought about by catheterisation.

An indwelling catheter is embedded into the bladder to gather the urine from the bladder. A Foley’s catheter is a standard indwelling catheter. Its component is a balloon attachment toward one side. After addition to the catheter, the balloon is packed with sterile water. It keeps the catheter from leakage.³ Prevalence of urinary tract infections cystitis: 6.2 million grown-ups self-revealed having a bladder infection for every three months within the US 1988-1994.⁴ It is observed that 10-12% of patients hospitalised and 4% of patients in the community area have a urinary catheter in situ at some random time. Hospital-acquired UTIs create 5 percentage of urinary catheterised understandable – patients per day. With related bacteria in 4% and the 4% number of as 80% is an outcome of the urinary catheter. Health care providers play a vital role in the prevention of infection, except in particular circumstances. All the catheters should be administered in a sterile manner. Using non-sterile techniques lead to urinary tract infections.⁴
Need for the study

The kidney filters the body's liquid waste in the form of urine and sends it into the bladder to be stored. Urine contains salts, fluids and waste material; however, bacteria is not usually present in the urine. Microorganisms entering the bladder or kidney can regenerate quickly in the urine, causing a urinary tract infection. UTI are perhaps the most widely recognised hospital-acquired infection. Roughly 40% of all nosocomial infection and 80% of these are related to a urinary catheter's utilisation. The majority of deaths are due to the development of gram-negative bacteria. 33% of these infections are due to the bacteria formed inside the urinary system. 66% to 86% of the diseases happen chiefly due to urinary catheterization.

Nurses are a vital part of in-hospital treatment. They are responsible for taking care of the patients' basic needs throughout the day. It is their responsibility to give the best possible care to prevent any infection during the hospital stay, which is achieved by improving the quality of nursing care.

During the researcher's clinical posting, it was observed that the quality and standard of nursing care given by the staff for a patient with an indwelling catheter was inadequate. Due to that, many patients had developed catheter- associated urinary tract infection. The nursing personnel were consistently answerable for setting and keeping up the urinary catheter framework, including cleaning and different works.

Methodology

This pre-experimental study approved by the Institutional Ethical Committee (IEC-1988 A) was conducted from December 2014 to March 2015 in the hospital of Sri Guru Harkrishan Sahib College, Mohali. Formal permission was taken. Composed assent was sought from the subjects of the investigation.

Study design and setting

The study comprised a "Pre-Experimental One group Pre-test and Post-test Design" in SGHS hospital equipped with 300 beds and an intensive care unit with 60 beds.

Sample and Sample Size

The hospital had one hundred twenty staff nurses working in different units in shift duties. A sample of 50 nurses was selected by the non-replacement simple random sampling lottery method with the following inclusion and exclusion criterion.

Methods and tools of data collection

A questionnaire was used for accurately collecting data, followed by administering a self-instructional module. Based on the goals, the instrument was isolated into two segments.

Selection and development of a tool

A structured questionnaire was developed through reviewing of literature via pubmed, cinhal, experts, guides, co-guides is utilized as an apparatus for present investigation. Apparatus will be chosen in the wake of surveying the literature and after discussing with specialists.

Reviewing literature via pubmed, cinhal, experts, the guides developed a structured questionnaire, and co-guides used it for investigation

Description of tool

Segment I: Demographic variables

Segment II: Structured knowledge questionnaire. The tool consists of 30 items. Segment II: Structured questionnaire.

The questionnaire made in English, contained 30 multiple-choice questions with four options. Each correct response earned one mark. The incorrect response earned no marks.

The questionnaire covered different areas - introduction, definition and types, causes, risk factors,
sign and symptoms, prevention and management.

Procedure of Data Collection

Immediately after that, knowledge development tools were administered, and a pre-test was conducted after that SIM was introduced. After seven days, a post-test was conducted.

Results

Table 1: Frequency distribution of overall knowledge score among staff nurses regarding UTI

<table>
<thead>
<tr>
<th>Criteria on Measurement of Knowledge Score</th>
<th>PRE Frequency (f)</th>
<th>POST Frequency (f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below average</td>
<td>31 (62%)</td>
<td>9 (18%)</td>
</tr>
<tr>
<td>Average</td>
<td>19 (38%)</td>
<td>15 (30%)</td>
</tr>
<tr>
<td>Above average</td>
<td>0 (0%)</td>
<td>26 (52%)</td>
</tr>
</tbody>
</table>

Table 1 illustrates the frequency and percentage distribution of the staff nurses’ knowledge base in the pre and post-test scenarios. The pre-test outcome showed that 62% of participants had below-average knowledge, and 38% had average knowledge. No one qualified the above-average score.

The post-test score demonstrated a distinct improvement in their knowledge. 52% of the nurses had acquired better than expected information, while 30% had the required standard information. However, 18% of the nurses in the post-test continued to have below-average information.

Discussion

This study, based on a sample size of 50 nurses, demonstrated that 62% of the participants had low knowledge, 38% had average knowledge about the sensitivities/criticalities of catheter application. No participant possessed good knowledge in the pre-test scores.

The self-instructional module content on urinary tract infection included introduction, definition, types, risk factors, causes, sign and symptoms, prevention, and management. The self-instructional module was administered to the staff nurses soon after the pre-test. The post-test score was assessed by paired ‘t’ test. The mean of the post-test was 20.56 with SD of 4.362 and a paired value of 10.353, implying that the post-test score had better than the pre-test of staff nurses. The hypothesis (H1) that there was a difference between knowledge of pre-test and post-test scores was thus accepted. Overall, the pre-test mean score of staff nurses was 14.14 (SD+1.628), and the post-test mean score was 20.56 (4.362), implying that the improvement mean was 6.420. The determined ‘t’ test value was 5.79, which was more than the table value, which shows a considerable distinction between the pre-test and post-test scores of nurses concerning urinary tract infection. This also implied that the self-instructional module was practical. The determined chi-square values did not match exactly with the table values, implying no significant association between the demographic variables. Hence the hypothesis (H2) was rejected.

Conclusion

The findings showed that the teaching program effectively enhanced the work knowledge and reduced the occurrences urinary tract infections in patients with indwelling catheters. The study recommended the necessity of conducting regularly, in-service education programs for nursing personal regarding the prevention of urinary tract infections to enhance patient safety and quality of care in hospital.

Ethical Issues: informed written consent was taken No author had any conflict of interest to declare.

Funding

The study did not receive external funding.
References


2. Nurse CTA Campaign Landing Page - CareThrough [Internet]. [cited 2014 Jan 20].


Correlation Luteinizing Hormone (LH)/Follicle Stimulating Hormone (FSH) Ratio with Low Density Lipoprotein (LDL)/High Density Lipoprotein (HDL) Ratio in Polycystic Ovarium Syndrome (PCOS) Patients

Susi Oktaviani¹, Jusak Nugraha², Ashon Sa’adi³

¹Resident, ²Lecturer, Department of Clinical Pathology, ³Lecturer, Department of Gynecological Obstetrics, Faculty of Medicine, Airlangga University - Dr. Soetomo General Hospital, Surabaya, Indonesia

Abstract

The aim of this study is to analyze the correlation between the ratio of LH and FSH levels with LDL and HDL cholesterol in PCOS patients. This study is an observational cross-sectional study. The population in this study were women newly diagnosed with polycystic ovary syndrome who came to RSIA Ferina, RSIA Lombok 22, Elizabeth Clinic and RSIA Putri Surabaya and Clinical Pathology Laboratory of dr. Soetomo Surabaya. Examination of FSH, LH and LDL and HDL cholesterol levels on 27 specimens (stored frozen serum) using the ICT (Accurate Intan Madya®, VEDALAB) and enzymatic colorimetric (Dimension EXL®, Siemens) methods. Statistical analysis was performed using the Spearman and Pearson correlation test, with a significance level of p <0.05. The results showed that there was a moderate positive correlation between FSH and LDL cholesterol (r = 0.491, p = 0.009). Phenotype D of PCOS (non-hyperandrogenic type) had a moderate negative correlation between the LH / FSH ratio and the LDL/HDL cholesterol level ratio (r = -0.487, p <0.05). However, there was no relationship between the LH/FSH ratio and the ratio of LDL/HDL cholesterol levels in all SOPK patients in this study. This study showed that phenotype D PCOS patients (non-hyperandrogenic type) were less likely to have cardiovascular complications than other phenotypic PCOS patients. Insulin resistance has a role in the risk of dyslipidemia in non-hyperandrogenic PCOS patients.

Keywords: PCOS, LH, FSH, LDL, HDL

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder that is most often found in women of reproductive age, with an incidence of 4-18% worldwide. Santoso and Irawan conducted a study in Surabaya to get the prevalence of PCOS in women of reproductive age by 4.5% in 2007. The prevalence of this disease has increased to 8-10% due to changes in people’s lifestyles with a high calorie diet and sedentary life style.¹² The diagnosis of PCOS is determined by the presence of two of the three Rotterdam criteria, namely: oligomenorrhea or anovulation, clinical hyperandrogens, and polycystic ovary morphology on ultrasound evaluation. Many PCOS patients do not have clear clinical symptoms, and experience difficulty in diagnosis, especially in the Asian population. An increase in the ratio of LH/FSH was seen in PCOS patients. A disturbance in pulsatile GnRH causes an increase in LH levels but is not followed by an increase in FSH levels. However, other research states that only 30-90% of PCOS patients experience an increase in the LH/FSH ratio.³
PCOS patients often have a variety of endocrine and metabolic abnormalities. PCOS patients have increased levels of LDL cholesterol (LDL-C) and decreased levels of HDL cholesterol (HDL-C). The increase in LDL occurs due to insulin resistance and hyperinsulinemia which causes an increase in free fatty acids (FFA), and further induces the synthesis of very low-density lipoprotein (VLDL) and LDL-C. The decrease in HDL is caused by insulin resistance and the degradation of Apolipoprotein A1/HDL-C.³

Women with PCOS have a four times greater risk of developing metabolic syndrome than healthy population. According to NCEP (National Cholesterol Education Program) guidelines about 70% of women with PCOS have dyslipidemia. Rizzo’s research reports that women with polycystic ovaries are more likely to have stenotic coronary heart disease detected by angiography than women with normal ovaries. This study aims to determine the correlation between the LH/FSH ratio and the ratio of LDL/HDL cholesterol levels in PCOS patients.⁴,⁵

Materials and Methods

This research is an observational analytic study with a cross sectional study approach. The research specimens were taken from samples of PCOS patients who came to RSIA Ferina, RSIA Lombok 22, Elizabeth Clinic and RSIA Putri from March to July 2019. Inclusion criteria included: PCOS patients diagnosed with 2 of the 3 Rotterdam criteria, age 18-40 years. Exclusion criteria were using hormonal contraception, suffering from heart disease, hypertension, insulin resistance/hyperinsulinemia, and endometriosis, also on statin medication.

Venous blood serum was taken from a population that met the criteria for the main study. The stored serum is used for checking the levels of FSH, LH, LDL-C and HDL-C in 2020. Specimens were stored at -80°C while waiting for the running process. Examination of FSH, LH using the AIM tool (Accurate Intan Medika) with the ICT method. The LDL-C and HDL-C tests used the RXL dimension (Siemens Healthineers, Germany). The data normality test was performed using the Shapiro-Wilk test. Data that is normally distributed are expressed in mean values, while data that are not normally distributed are expressed in the mean value. The correlation of LH and FSH ratios with the ratio of LDL and HDL cholesterol levels in PCOS patients was analyzed by doing the Pearson correlation test (if the data were normally distributed) or the Spearman (if the data were not normally distributed).

Results and Discussion

The number of subjects who participated in this study were 27 patients PCOS. Data on the characteristics of research subjects included age, body mass index and phenotype (Table 1). The results of this study are in line with the research of Wahyuni et al, which shows that obesity is more likely to experience PCOS, namely 50.5%.⁶ Tavares and Barros state that the highest prevalence of patients with metabolic syndrome in each PCOS phenotype in Brazil has a BMI>30 kg/m² (obese). Obesity will cause chronic hyperinsulin or insulin resistance, and can aggravate hormonal and metabolic disorders in PCOS patients. This may be one reason obese patients sought treatment more frequently in this study.⁷

The Rotterdam criteria divides PCOS patients into 4 phenotypes. Phenotype A is PCOS patients with classic symptoms (hyperandrogen triad, anovulation, and polycystic ovary). Phenotype B has symptoms of hyperandrogens and polycystic ovaries. Phenotype C has symptoms of hyperandrogens and anovulation. Phenotype D had symptoms of anovulation and polycystic ovaries without symptoms of hyperandrogens. Wiweko’s research in Indonesia, Daan N, et al in the Netherlands, showed that phenotype A and D populations were the largest phenotypes. The Rotterdam criterion uses the Ferriman-Gallwey score, where a score of more than 8 is used to diagnose hyperandrogens. This score is probably lower in PCOS patients in Asia, so that the sensitivity decreases.⁸,⁹,¹⁰ The combination of the higher incidence of polycystic ovary morphology compared to European patients and the sensitivity of this low Ferriman-Gallwey
score could explain phenotypes A, B, C only slightly compared to phenotype D in this study.

Table 1. Characteristics of PCOS patients (n=27).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n (%)</th>
<th>Mean ± std</th>
<th>Median ± std</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td>29.93 ± 4.323</td>
<td>29 (24 – 39)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27</td>
<td>29.91 ± 3.097</td>
<td>30.1 (21.2 – 35.5)</td>
</tr>
<tr>
<td>Phenotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Rotterdam)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>5 (18.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2 (7.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>20 (74.1%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Description: BMI, Body Mass Index; n, the number of cases.

The age range of research subjects was 24-39 years. This study sample (60%) was obese (BMI >30 kg/m²) with a mean BMI of 29.91 kg/m² (overweight). This study also showed that phenotype D was more than phenotype A and phenotype C.

Table 2. Comprehensive Results of lipid and hormonal profile of PCOS patients (n=27).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± std</th>
<th>Median ± std</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (mIU/mL)</td>
<td>16.23 ± 7.305</td>
<td>16 (5 – 29.5)</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>7.94 ± 2.683</td>
<td>7.3 (5 – 18.3)</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>86.93 ± 21.870</td>
<td>90 (31 – 127)</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>36.04 ± 15.073</td>
<td>35 (4 – 68)</td>
</tr>
</tbody>
</table>

Description: LH, Luteinizing Hormone; FSH, Follicle-Stimulating Hormone; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; n, the number of cases.

The population of this study shows that there is an imbalance in LH and FSH production, as in table 2. Based on the mean of LH and FSH, it is found that LH / FSH > 2. In the past, this ratio served as the gold standard for clinical diagnosis of PCOS in addition to the Rotterdam criteria. However, LH / FSH levels have become controversial after a number of studies have reported a variable prevalence of this ratio (30-90%) among women with PCOS. A disturbance in pulsatile GnRH causes an increase in LH levels but is not followed by an increase in FSH levels. The hyperandrogenic state causes an increase in estrogen levels by the aromatase enzyme activity. Estrogen will inhibit FSH production from the anterior pituitary.
Table 3. Correlation of Lipid and hormonal profile in PCOS patients (n=27).

<table>
<thead>
<tr>
<th>Lipid Profile</th>
<th>LHa r = -0.277; p = 0.161</th>
<th>FSHb r = 0.491; p = 0.009</th>
<th>LH/FSHa</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLa</td>
<td>r = -0.085; p = 0.673</td>
<td>r = -0.172; p = 0.390</td>
<td></td>
</tr>
<tr>
<td>HDLa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL/HDLb</td>
<td></td>
<td></td>
<td>r = -0.232; p = 0.244</td>
</tr>
</tbody>
</table>

*aNormal distribution and Pearson correlation, bNon-Normal and spearman correlation, significant value <0.05* (P-value represents significance from Pearson and Spearman correlation statistical analysis). Description: LH, Luteinizing Hormone; FSH, Follicle-Stimulating Hormone; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; n, the number of cases.

The results of the correlation test between FSH levels and LDL cholesterol levels showed a positive correlation 0.491, the strength of the relationship was moderate, which was significant (p = 0.009) (Table 3). This means that there is a significant relationship between FSH and LDL cholesterol (p < 0.05). The results of the correlation test between FSH levels and HDL cholesterol levels showed a negative correlation of -0.172, which was not significant (p = 0.390). This means that there is no significant relationship between FSH and HDL cholesterol (p > 0.05). These results are in accordance with the results of research by Arshad F, et al. (2019) in Pakistan which showed that FSH had a significant correlation with LDL (p = 0.01, r = 0.36) while FSH had no significant correlation with HDL (r = -0.152, p = 0.323).

Table 4. Correlation of Lipid and hormonal profile in PCOS patients based on phenotypes (n=27).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LDL/HDLa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperandrogen types (Phenotype A+C)</td>
<td>LDL/HDLa</td>
</tr>
<tr>
<td>LH/FSHb</td>
<td>r = -0.357; p = 0.432</td>
</tr>
<tr>
<td>Non-hyperandrogen types (Phenotype D)</td>
<td>LDL/HDLa</td>
</tr>
<tr>
<td>LH/FSHb</td>
<td>-</td>
</tr>
<tr>
<td>Non-hyperandrogen types (Phenotype D)</td>
<td>r = -0.487; p = 0.029</td>
</tr>
</tbody>
</table>

*aNormal distribution and Pearson correlation, bNon-Normal and spearman correlation, significant value <0.05* (P-value represents significance from Pearson and Spearman correlation statistical analysis). Description: LH, Luteinizing Hormone; FSH, Follicle-Stimulating Hormone; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; n, the number of cases.

The correlation of the LH and FSH ratios with the LDL and HDL cholesterol ratios in phenotype D (Table 4) is in accordance with the explanation of several previous studies. Women with hyperandrogenic PCOS differed significantly from women with non-hyperandrogenic PCOS regarding most of the endocrinological and cardiometabolic parameters. Women with hyperandrogenic PCOS have worse
cardiometabolic profiles, including higher BMI, higher LDL cholesterol and lower HDL cholesterol. Another study showed that the significantly lower prevalence of metabolic syndrome (6.5%) and hypertension (11.8%) in non-hyperandrogenic women was consistent with the prevalence previously reported in a population of normal (non-PCOS) women with age, BMI, and the same ethnic background. Overweight or obesity (28.5%) and lipid disorders (increased LDL-C, 52.2%) were relatively common among young women with PCOS phenotype D compared with non-PCOS.9,13

Based on the case finding in this study, it was found that phenotype D was the largest phenotype in the subjects of this study. The subjects of this study were taken consecutively, based on patients who came to several hospitals and clinics in Surabaya. This indirectly illustrates that phenotype D is the most common PCOS phenotype in the East Java region, which turns out to be consistent with research conducted in Asia. The correlation of LH and FSH ratios with the ratio of LDL and HDL cholesterol to phenotype D is in accordance with the explanation of several previous studies, indicating that insulin resistance has a role in the risk of dyslipidemia in non-hyperandrogenic PCOS patients. SOPK patients have impaired tissue insulin resistance. This will trigger the release of excess insulin as a form of compensation. This hyperinsulinemia condition will cause increased activation of the Insulin Growth Factor-1 (IGF-1) receptor. Ovarian theca cell abnormalities in SOPK patients also cause a decrease in aromatase so that estrogen decreases. This decrease in estrogen causes the synthesis of G protein coupled estrogen receptor to decrease, resulting in an increase in circulating LDL-C due to decreased LDL receptors. This imbalance of LDL-C and HDL-C cholesterol causes an increase in the ratio of circulating LDL-C to HDL-C. Lipids that accumulate in these blood vessels will cause atherosclerosis. 14

**Conclusion**

The negative correlation of the LH and FSH ratios with the LDL and HDL cholesterol ratios in phenotype D. Women with hyperandrogenic PCOS have worse cardiometabolic profiles, including higher BMI, higher LDL cholesterol and lower HDL cholesterol. Overweight or obesity and lipid disorders were relatively common among young women with PCOS phenotype D compared with non-PCOS. Insulin resistance still has a role in the risk of dyslipidemia in non-hyperandrogenic PCOS patients

**Conflict of Interest**: The author declare that they have no conflict of interest.

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**Acknowledgements**: We thank Arif Nur Muhammad Ansori for editing the manuscript.

**Ethical Approval**

This research was carried out when it had received ethical clearance from the ethical committee of the Faculty of Medicine, Airlangga University, Surabaya, Indonesia has agreed and stated that this research is ethical, with ethical clearance number 125/EC/KEPK/FKUA/2020. Respondents included in the inclusion criteria determined in the study were given informed consent and a statement to be willing to participate in this study. If the respondent is not willing, it will be excluded and not included in the study.

**References**


The Medical and Psychological Effect of Hate Crimes and Hate Speech on Vulnerable Social Groups

Suvidutt M.S., Aditya Tomer

1PhD Scholar (Law), AIALS, Amity University, Noida; Advocate-on-Record, Supreme Court of India,
2Addl. Director, Amity Law School, Amity University, Noida

Abstract

The present study would be analyzing and interpreting the intricacies of hate speech in two-fold. Firstly, the authors would be meticulously analyzing the legal parlance of the term ‘hate speech’ in the present aeon and its repercussions, i.e., outline the constitutive and consequential harm faced by the people that goes beyond legal texts and judgments and beyond the legal notion of hate speech.

In furtherance, this research article would address the aftereffects of hate speech medically and how it takes a psychological toll on minority bigotry and pervasive gender or ethnic differences by using a plethora of well-known logical research approaches that are unique to analytic philosophy.

Hate speech cases have resulted in bans on campus, legal rulings, and national controversy. The term ‘Free Speech’ and ‘Hate Speech’ has been misconstrued and used misappropriately. The prime reason is the absence of precise clarification and the unbelievably wide usage of the term. The targeted communities experiencing hate speech are of paramount importance but are often overlooked due to the legitimacy of laws pertaining to hate speech.

Keywords: hate crime, hate speech, medical effect, psychological effect, social group

‘A public health approach to bigotry will not eliminate/ eradicate hatred, but may at least mitigate the damage hatred can inflict upon society.’

– Ronald Pies

Introduction

As everyone is aware that there is no standard definition of the term ‘Hate speech’, however, the same has/ is being used at persecuting people by denigrating their religious, ethnic or other racial identities. The term ‘Hate speech’ has always been used to mean violent, disrespectful, threatening, bullying or inciting abuse, hostility or bigotry against vulnerable groups characterized by characteristics such as ethnicity, faith, place of birth, residence, country, language, caste or community, sexual orientation or personal convictions. The balance of society is disrupted by these speeches, and it breaks the multifaceted structure of society.

While a single individual or small community may be the immediate target, through fostering bigotry and intolerance, the harm caused by hate speech can spread to whole populations. Although hate speech has been there for time immemorial, to date, there is no dearth of laws pertaining to the menace of hate speech.
The inclusion of Sections 153A and Section 505C in the Indian Penal Code, 1860 with respect to hate speech only make the substantive law more wholesome. The real need is to overhaul the prevailing laws with proper vigour and efficacy in order to devise ways to combat the injury caused due to hate speech. The authors would like to assert that on account of public order, incitement to violence and State protection, hate speech that has become a preeminent concern must be curtailed.

**Materials and Method**

This research paper is based on doctrinal study. The authors relied on several books and research papers by analyzing and interpreting them to bring a different point of view.

**Discussion**

How Far the Limitation on Free Speech Play a Role?

One among the most cherished rights of a civilized, democratic society is ‘Right to Freedom of Speech and Expression’ which has been enshrined and enunciated under Article 19 (1) (a) of the Indian Constitution. The prime objective of this provision is promoting a plurality of opinion. Per contra, it is of paramount importance to note that in an unjust society, this right will be in contrast with the doctrine of non-discrimination.

Equality of speech, including offensive speech many a time, berates the essence of equality. A question that arises is ‘Whether Freedom of Speech and Expression is absolute, or can there be any restrictions put on this right?’ Post the insertion of Article 19 (2) in the Indian Constitution by relying on the case of *Gitlow v. New York*, reasonable restrictions were imposed on this right. This provision act as a check to the provision that grants the freedom of speech and expression.

However, the authors deem that the same has not been imposed accordingly as the thin line to demarcate Free speech and Hate speech has been ignored that results in infringing the fundamental right of Freedom of speech and expression and misuse of the law. To demarcate a line between free speech and hate speech is a challenge that policy makers, law agencies, judiciary etc. are facing. It is a conundrum that need to be resolved. This is not only the scenario in India but also worldwide.

**An Analysis of Reported Hate Crimes**

Globally, hate crimes are under-reported, offering little details on the incidents. Based on the analysis, it was brought to light that in 2010 alone, there were 3,770 hate crimes reported in Germany, which included not only hate crimes but also incitement to hatred and propaganda offences. In 2014-2015, there were approximately 42,930 racially motivated public order offences in England and Wales. The FBI reported 7,145 hate crimes in 2017. It was found out that the reported hate crimes in 2017 were mainly motivated by hostility based on race/ethnicity (58.1 percent), religion (22 percent), sexual orientation (15.9 percent), gender identity (0.6 percent) and disability (1.6 percent).

To put an embargo on hate speeches and hate crimes, a bill was introduced in the U.S. House of Representatives known as ‘The National Opposition to Hate, Assault, and Threats to Equality Act, 2017,’ to collect more precise hate crime reports by training law enforcement to identify hate crimes and setting up monitoring hotlines. Apart from the introduction of the bill, there was no further action or measure taken on it.

In furtherance, the FBI’s Uniform Crime Reporting (UCR) while submitting the hate crime statistics elucidated that the hate crime information for 2019, submitted by 15,588 law enforcement authorities, offers information about the cases, suspects, perpetrators, and hate crime sites. There were 7,314 cases of hate crime incidents, including 8,559 offences among these organizations that sent incident reports.

The current study on hate crimes relied on hate crimes reported to the authorities, and thus rests not only on the desire of the victims to report but also on their capacity to supply the police officials with ample proof of the hate motivation. This leads to a shortage of knowledge that makes it impossible to measure the
nature of the issue and to take appropriate action to resolve it. Taking this into consideration, the European Commission against Racism and Intolerance (ECRI) proposes that States must offer practical assistance to hate speech and abuse targets. They should be made aware of their right to redress by institutional, civil and criminal action and advised to report the authorities and access legal and therapeutic support.

**Harms to Victims As Well As Perpetrators: A Medical/ Psychological Based Approach**

Based on research study by numerous psychiatrists, it has been noticed that people victimized by violent hate crimes are likely to suffer more psychological trauma than the victims of other violent crimes.\[19\] It takes a psychological toll on the victims, which leads to post-traumatic stress, safety concerns, depression, anxiety and frustration.\[20\]\[21\]

The first step in crime intervention and prevention is the awareness of the mindset of the hate crime perpetrator. Until thoroughly recognizing the reason for the offence, in the sense of social intolerance, bigotry, and discrimination, it is difficult to assess the far-reaching psychosocial impact for the victims or identify the mindset of the perpetrator. After a comprehensive analysis, it was found that most of the hate crimes/hate speeches are consummated by the youngsters. Regarding the diversity of youths, it is during puberty that the core signs of hate impulses become fully visible. To grasp the enormity of evil activities such as hate crimes, youngsters become ‘armed’ with the cognitive and moral apparatus and are thereby therefore motivated to commit them.\[22\]

Genetic theories have revealed that the genes of an individual may predispose the person to choose criminal activity under certain circumstances.\[23\] A genetic factor for illegal and aggressive activity has been proposed in both twin and adoption research.\[24\] It was found that the concept of hate crimes has not been discussed/addressed in any genetic testing.

The psychoanalytical approach of Young-Bruehl linked the lack of satisfaction of essential needs to the commission of hate crimes in many at-risk youths.\[25\] Without a welcoming atmosphere to express the typical anxiety, uncertainties and insecurity of adolescence, there is an incomplete sense of belonging and a fragile intensity of ego among young people. Due to these deficits, adolescents may use prejudices in their community because of these deficiencies to project unacceptable emotions, not to an individual nearby, but to an entire society, including a group outside personal familiarity. The consequence is intolerance of others and resentment at the shortcomings attributed to them. The average youth who acts on his hatred feels rejected and abandoned from friends and members of family and uses hate to make up for feelings of inadequacy. In their recruiting of youngsters, hate groups are effective because their beliefs have a kind of outward superego. The actions of the group offer gratification and an independent teenager may thus be persuaded to adhere to the group and disregard previous principles and convictions entirely.\[26\]

Aaron T. Beck, an American psychiatrist, views hate as a neurological challenge, a disease of thought. Thought drives conduct in this theoretical context, and the violence-prone person, including the perpetrator of hate crime, has a profound weakness in his understanding of social experiences.\[27\] He sees himself as righteous and moralistic, while the enemy is to blame for his troubles. Youths who commit hate crimes exhibit issues with impulse control, thrill-seeking conduct, problems with intimidation, behaviour or hostility, a desire to be professional, or feelings of betrayal and underlying harm.\[28\]

Data on convicted hate crime offenders have demonstrated that many of the most violent types of hate violence are perpetrated by persons with previous criminal backgrounds, those who are socially deprived and those who are susceptible to drug abuse. Furthermore, abusive offenders have displayed characteristics predicting antisocial and repetitive behaviour. In terms of psychological or demographic
status, including variables such as income level, religion, and national origin, the bulk of hate crime offenders are little identified.

It is of paramount importance that if hate speech continues to be unchallenged, over time bigotry towards already marginalized people is intensified, and their increased marginalization and alienation is promoted. Increased social tensions, clashes and, in some situations, armed confrontation will also occur.

Has the Government Taken Sufficient Steps to Combat Hate Speech?

Hate speech manifests itself in different forms to date, along with the rampant advancement of technology. The laws of various countries and international orders also respond to it in varied aspects. While several nations have regarded the malicious use of hate speech as a criminal act and have outlawed it, it is still widespread.

Some of the countries that consider hate speech as an offence and has enacted laws to prevent the same are Public Order Act, 1986 in U.K. has elucidated incitement to racial hatred as an offence; inciting abhorrence against any recognisable group has been considered as an indictable offence under the Canadian Criminal Code; Australia has formulated the Racial and Religious Tolerance Act 2001 that prohibits hatred against any race or religious beliefs etc.

In an article entitled, “Hatred-A Public Health Issue,” Public Health experts Izzeldin Abuelaish and Neil Arya claimed that “Hatred can be conceptualised as an infectious disease, leading to the scaling up of violence, fear, and ignorance. Hatred is contagious, it is capable of crossing hurdles and boundaries”.[29] It has been further enunciated that if hate and bigotry are both negative and contagious, how will this issue be approached by a public health approach? The professionals suggested numerous preventive strategies for the said question which includes promoting knowledge of the harmful health impacts of hatred; improving mental self-awareness and dispute solving skills; building ‘immunity’ against provocative hate speech, and fostering an understanding of mutual interest and human rights. Considering the strategies recommended by the public health professionals may not eradicate hate speech/ hatred, but can at least minimize the harm that hate will cause on society.

Although the hate speech laws are sufficiently broad, however, a greater degree of legal protection is in need in the present aeon that determines hate speech from the viewpoint of the vulnerable communities and also reflects the truly reprehensible nature of the offenders. In furtherance, it is not only that there must be stringent laws concerning hate speech but also must consider the strategies/ recommendations of medical professionals. The concept of hate speech must commence in early childhood. There is currently a lack of vertical and interdisciplinary training. Students, instructors, and administrators all need support to act correctly when they see violence happening in the classroom or on the playground. There is a need for comprehensive study and data collection on hate crime activities, with specific regard on youth offenders.

Conclusion

When we look into the fundamental right of Freedom of Speech and Expression along with the reasonable restrictions, it hasn’t been giving a huge impact on the society. Whatever amendments that have been enacted in the present legal framework with respect to hate speech by numerous countries, the authors believe that it is just to rationalize the existing provisions and an effective or adequate step hasn’t been taken by the government (s) to date to curb hate speech. Any further laws/ programs initiated towards prevention of hate speech/ hate crime must concentrate on fostering empathy and an awareness of diversity among school children.

In furtherance, the key reason for such a debate on hate speech/ hate crime is the lack of definition, which is of utmost importance. Many courts encounter this issue and often fail to provide a clear-cut definition for ‘hate speech’. Hate speech negates the entire possibility of social contact and avoids the continuation of never-ending debate of thoughts and feelings. Beyond the technical complexities in implementing applicable
statutory prohibitions, the difficulty in distinguishing between speech/ expression and actions emphasizes the need to punish varied forms of hate speech addressed to particular persons.

Despite the implementation of various educational programs and interventions, hate crimes are still persisting. Such proactive initiatives and strategies to deter hate before it is expressed in a criminal act can only minimize bias-motivated crimes. It is pertinent to note that as long as schools breed bigotry, hate crimes tend to happen. The authors would like to suggest that programs that encourage awareness for police and victim assistance providers to help hate crime victims coping with trauma are required across the globe. As elaborated earlier, a public health approach is the desideratum to prohibit hate speeches as it not only takes a psychological toll on the individual but on the society at large. In order to prevent such hate crimes against humanity, there needs to be narrowly tailored legislation banning the propagation of mis ethnic stereotypes designed to elicit crimes against outgroups should be implemented within a reasonable timeframe.

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Reference

16. Ibid.


The SNP rs13118928, rs1828591 and rs10519717 in the HHIP Gene are not Associated on COPD Susceptibility in Male Javanese Smokers

Syazili Mustofa1,2, Sutyarso3, Muhartono4, Yandri5, Retno Ariza Soeprihatini Soemarwoto6, Hendri Busman7, Wawan Abdullah Setiawan7

1Student, Doctoral Program, Faculty of Mathematics and Natural Sciences, 2Lecturer, Department of Biochemistry, Molecular Biology and Physiology, Faculty of Medicine, 3Professor, Department of Biology, Faculty of Mathematics and Natural Sciences, 4Professor, Department of Anatomical Pathology, Faculty of Medicine, 5Professor, Department of Chemistry, Faculty of Mathematics and Natural Sciences, 6Lecturer, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, 7Lecturer, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Bandar Lampung, Indonesia

Abstract

Background: Hedgehog Interacting Protein (HHIP) gene polymorphisms have an association on COPD has been carried out in Europe and Asia but in Indonesia there is still very limited study on this type and the largest ethnic group in Indonesia is the Javanese. Objective: To analyze the association between the HHIP gene polymorphism and the incidence of COPD in male Javanese smokers in Lampung, Indonesia. Method: In a case-control study in Javanese male smokers, three single nucleotide polymorphism (SNPs) in the HHIP gene were analyzed by Sanger sequencing method. There were 110 participants in this study which were divided into 2 groups, such as COPD group (55 participants) and control group (55 participants). Three SNPs in the gene (rs13118928, rs1828591 and rs10519717) were selected for genotyping. Genotype distributions were compared between patients and controls. The statistical analysis was carried out with the SPSS program with a chi-square test. Result: The genotypic frequency of the HHIP gene sequence at the SNP position rs1828591, such as AA (52.72%), GG (3.63%) and AG (43.63%) in COPD group, while in the control group such as AA (38.18%), GG (9.09%) and GG (52.72%; p>0.05). The genotypic frequency of the HHIP gene sequence at the SNP position rs13118928 consisted of AA (47.27%) and AG (53.72%) in the control group, while the COPD group consisted of AA (52.72%), GG (1.81%) and AG (45.45%; p>0.05). The genotypic frequency of the HHIP gene sequence at the SNP position rs10519717 consisted of TT (34.54%), CC (14.56%) and CT (50.90%) in COPD group, while controls group consisted of TT (23.63%), CC (16.37%) and CT (60.00%; p>0.05). The genotypic analysis of Three SNPs in HHIP gene were observed but showed no significant difference between case and control groups. Conclusion: Single-nucleotide variants in the HHIP gene are not associated with COPD susceptibility in Javanese male smokers.

Keywords: HHIP, COPD, Javanese, Indonesia, male smokers

Introduction

Chronic obstructive pulmonary disease (COPD) is one of the major health problems that occur worldwide and in Indonesia. This disease is the fourth leading cause of death in the world, causing 3 million deaths of about 80 million people suffering from COPD in 2005. This disease is a major global health problem due to its high prevalence, which is about 10% of the adult population(1). The prevalence of COPD in Asia is estimated at 6.3% with the highest prevalence in Vietnam and the People’s Republic of China. The prevalence of COPD
in Indonesia is not certain. The results of a survey of non-communicable diseases by the Directorate General for the Control of Infectious Diseases & Environmental Health in 5 provincial hospitals in Indonesia (West Java, Central Java, East Java, Lampung, and South Sumatra) in 2004, showed that COPD was the first contributor to morbidity (35%), followed by bronchial asthma (33%), lung cancer (30%) and others (2%). COPD also ranks 6th of the Top 10 Non-Communicable Diseases Causes of Hospitalization in Indonesia in 2009 and 2010(2).

Hedgehog Interacting Protein (HHIP) is an inhibitor of the Hedgehog (HH) signaling pathway and a protein that plays a role in pulmonary tissue remodeling. HH protein is an important morphogen for various developmental processes, including the anteroposterior pattern of the limb and the regulation of left-right asymmetry in embryonic development. The HH signaling pathway plays an important role in pulmonary morphogenesis and cellular response to lung injury. The HH signaling pathway also has implications for cell development, cell repair, and cancer cell development(3). Several cell surface receptors are responsible for transmitting and/or regulating HH signals. HH protein is an important mediator for smoke-induced diseases such as lung cancer and COPD. HH signaling protein expression is regulated by HHIP. This HHIP protein is a highly conserved and specific HH signaling inhibitor in vertebrates(4). Expression of HHIP protects mice from cigarette smoke-induced emphysema by reducing the number and activation of lymphocytes in the lungs and reducing the number and size of lymphoid follicles(5).

The HHIP gene is found on chromosome 4 arm of the p locus 13. This gene is highly expressed in the lungs and brain. Several genomic regions that code for HHIP are associated with COPD susceptibility. Polymorphisms can cause changes in gene expression leading to functional changes and ultimately COPD. A significant reduction in HHIP gene expression on mRNA and protein was found in COPD patients compared to smokers with normal lung function(6). Haploinsufensing the HHIP gene in genetically engineered mice caused an increase in emphysema due to exposure to cigarette smoke. Mice with haploinsufficiency (HHIP<sup>−/−</sup>) are more prone to develop severe emphysema due to exposure to cigarette smoke than wild type mice (HHIP<sup>+/+</sup>)(5).

Study on the polymorphism of the HHIP gene and its association with COPD has been carried out by many researchers, especially in Europe and Asia. A European genome association study has shown that two single nucleotide polymorphisms (SNPs) near the HHIP gene, namely SNP rs1828591 and rs13118928, are associated with the risk of COPD. Both SNPs are significantly associated with the risk of COPD in Caucasians(7). The Asian study involved Han Chinese and Mongolian populations. The HHIP gene is involved in COPD susceptibility in the Han Chinese population. The polymorphism of the HHIP SNP gene rs10519717 is associated with the severity of COPD(8). Smokers with the HHIP variant rs7654947 were associated with the development of COPD and decreased lung function. Smoking and gene susceptibility have a cooperative effect on the risk of COPD and decreased lung function(9). HHIP gene polymorphisms on SNP rs10519717 are associated with COPD susceptibility in Mongolians(10).

The causal association between genetic factors and COPD is still a hot topic of study around the world with varying results. Based on the evidence currently available, it is possible that in the future the role of doctors will shift. Currently the role of doctors is primarily to recognize and manage disease, the main role of doctors in the future is to interpret and apply genetic and genomic information in prevention and therapy(11).

Study on the polymorphism of the HHIP gene and its relationship with the risk of COPD in Indonesia has not been reported. The largest ethnic group in Indonesia is the Javanese, which is estimated to be 41% of the entire population of Indonesia. A preliminary survey at the Harum Melati Respiratory Clinic, Pringsewu Regency, Lampung Province, obtained data on the number of COPD patients in 2019 as many as 161 people from a total of 1600 patients with lung disease. Most of the COPD patients (104 people / 64.5%) came from Javanese ethnicity. Based on the description above,
it is necessary to analyze the relationship between the Hedgehog Interacting Protein Gene Polymorphism and the Chronic Obstructive Pulmonary Incidence in the Javanese Tribe in Lampung, Indonesia.

**Method**

**Participant**

One hundred and ten male Javanese smokers were included in a case-control study. These subjects attended the Harum Melati Respiratory clinic, Pringsewu, Lampung. Case group of this study were patients diagnosed with COPD and were active smokers. The inclusion criteria for the group included being diagnosed with COPD (12) with a stable condition, having a history of smoking >10 pack years on the Brinkman index (13) and Javanese (both parents are Javanese). The COPD group exclusion criteria included COPD patients with comorbidities such as tuberculosis, diabetes mellitus, liver and kidney disorders acquired by history and physical examination, COPD patients in exacerbations, and patients not cooperating with spirometry. Meanwhile, The control group consisted of participants who had the following criteria: healthy male smokers, >40 years old, had a history of smoking >10 pack years on the Brinkman index (13), Javanese (both parents were Javanese), and had no history of smoking. COPD, bronchitis, bronchiectasis, bronchial asthma, pneumothorax, lung cancer, bronchopulmonary allergic disease, and no family history of COPD.

**Design Study**

This study was an observational study with a cross sectional comparative study design. In this study, the participants were divided into 2 groups which included COPD participants who were active smokers and participants who were healthy but active smokers. Sampling of patients was carried out at the Harum Melati Lung Specialist Clinic, Pringsewu Regency, Lampung Province, while the control group was obtained in Pringsewu and Tanggamus districts. Pulmonary function measurements and blood sampling were carried out at the Harum Melati Lung Specialist Clinic, Pringsewu Regency, Lampung Province. DNA extraction and PCR processes for the HHIP gene were carried out at Unit Pelaksana Teknis (UPT) Laboratorium Terpadu dan Sentra Inovasi Teknologi (LTSIT) Universitas Lampung. Sequencing is carried out by a gene sequencing service provider, namely Genetika Indonesia Ltd, Jakarta, Indonesia.

**Pulmonary Function Test Using Spirometry**

Lung function tests were measured by the spirometry method using a flow spirometer (CHESTGRAPH HI 101; Chest MI, Inc, Tokyo, Japan). Spirometry measures two volumes of air, namely the volume exhaled strongly during maximum inspiration or what is called forced vital capacity (FVC), and the volume of air exhaled in the first second of this breath or Forced expiratory volume in one second (FEV1). A comparison between FEV1 and FVC (FEV1/FVC ratio) is calculated. If the FEV1/FVC value after bronchodilator administration is less than 70%, the lung function test results are declared obstructive.

**Genetic analysis**

All of the participants’ blood was collected into Vacutainer ® tubes. Blood specimens were centrifuged with a special solution, namely Pancoll. This solution is in the form of a sugar solution that has a certain specific gravity, which when the blood is placed on top of this solution, then centrifuged will separate the blood into its component components. Initially 4 ml of Pancoll solution was put in a tube, then carefully 3 ml of blood was placed on top of the Pancoll solution with the help of a pipette. Then the tube rotates using a centrifugator with a speed of 5000 rpm for 10 minutes. Note the buffy coat/yellowish layer over the pancoll layer. With a single-use pipette, collect as much of the yellow layer as possible (generally in <0.5mL volume), avoiding taking significant amounts of plasma or RBCs. Transfer the buffy coat to a sterile pour-off tube.

The process of genomic DNA extraction was derived from 500 µL of buffy coat using a DNA extraction device brand Wizard® Genomic DNA Purification
Kit made by Promega in Madison, USA following the extraction protocol from the manufacturer. The quality and quantity of extracted DNA were analyzed using a nanophotometer made by IMPLEN, Munich, Germany.

In this study, three pairs of primers were designed. The primary source was taken from the sequence “Homo sapiens Hedgehog Interacting Protein (HHIP) gene, complete cds” with access code DQ995342.1. The first pair amplifies the SNP rs1828591, the forward sequence is TGAGGTTGAGTTTGGAG and the reverse sequence is 5’-AGAGGTGTTTCATGTTTCCA-3’. The second primary pair amplified SNP rs13118928, with a forward 5’-CCCTTCATACCTCCTTCTC-3’ sequence and a 5’-GGTGGGAAGAAACATTACA-3’ reverse sequence. The third primary pair amplified SNP rs10519717 with the forward sequence 5’-TACGTGATGTTTTGGGCT-3’ and the reverse sequence 5’-GGTGAACAGACTCCAAACTC-3’.

PCR multiplication was carried out with a total volume of 50 µL consisting of 10 nanograms of sample DNA, each 0.2 micromolar forward and reverse primer, 10 parts Taq Buffer, TaqGold, and 200 micromolar dNTP each.

The electrophoresis process aims to evaluate the PCR results. The PCR results were visualized using a digital electrophoresis device using Qiagen’s QIAxcel DNA High Resolution Kit following the electrophoresis procedure from the manufacturer. If there is amplification, a band will appear. Based on the source of the primary sequence taken, namely the sequence with the access code DQ995342.1, SNP amplifying primer rs1828591, will produce a single band of DNA with a size range of 491 bp, primers for rs13118928 will produce single bands of DNA 381 bp, and primers rs10519717 will produce bands single DNA 545 bp. The amplicons was then sent for sequencing. Sequencing was carried out by a gene sequencing service provider, namely Genetika Indonesia Ltd, Jakarta, Indonesia. SNPs analysis was carried out on the SNPs rs1828591, rs13118928, and rs10519717 with bioedit and chromaspro software in both forward and reverse sequences. In electroferogram analysis using bioedit software, the nitrogen base sequence is represented by peaked waves with various colors. Base C is yellow, base G is white, base T is green, base A is pink. Generally, one position has only one nitrogen base, but at one SNP position there can be 2 electroferogram peaks, for example bases A and G. This shows that the site is a heterozygous AG genotype.

Statistical Analysis

The DNA samples of 110 male Javanese smokers with and without COPD were genotyped, and three SNPs (rs13147759, rs1828591, and rs13118928) were evaluated. Genotype distributions were compared between patients and controls. The statistical analysis was carried out with the SPSS program with a chi-square test. Data analysis used IBM SPSS Statistics software version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Participant characteristics

The characteristics of the participants can be seen in Table 1 which is described in detail regarding the differences between the COPD and control groups. There was no significant difference between the COPD group and the control group in each variable related to participant socio-demographic characteristics. There were a significant difference in the lung fuctions (\(p=0.000\)), history of respiratory tract infections at childhood (\(p=0.004\)) and body mass index (\(p=0.030\)).

The Association between HHIP gene polymorphisms in smokers with COPD and Control

The Genotype distributions were compared between patients and controls can be seen in table 2. There were 3 genotype of the HHIP gene sequences in the SNP rs1828591 position, namely AA, GG and AG. In COPD who were smokers, there were 29 cases of GG (52.72%), GG 2 cases (3.63%) and AG 24 cases (43.63%), while in control AA 21 (38.18%), GG 5 (9.09%) and GG 29 (52.72 %). Statistically, it was found that there was no \(p\) value < 0.05 in the genotype comparison of the HHIP gene at position rs1828591. This means that there were
no differences in the genotype frequency of the SNP rs1828591 of HHIP gene between COPD smokers and healthy smokers in Javanese in Lampung Province, Indonesia.

In table 2 it can be seen that in the control group there were only 2 genotype of the HHIP gene sequence in the SNP position rs13118928, namely AA and AG. In the control group obtained AA 26 (47.27%), and AG 29 (53.72%), while in COPD group, AA 29 (52.72%), GG 1 (1.81%) and AG 25 (45.45%) were obtained. Statistically, there was no p value <0.05 on the AA, GG and AG genotypes, meaning that there was no difference in the frequency of the SNP rs13118928 HHIP gene polymorphism between COPD smokers and healthy smokers in Javanese in Lampung Province, Indonesia.

In table 2 it can be seen that there are 3 genotype of the HHIP gene sequences in the SNP position rs10519717, namely CC, TT and CT. In COPD who were smokers, there were 19 cases (34.54%), CC 8 cases (14.56%) and CT 28 cases (50.90%), whereas in controls TT 13 (23.63%), CC 9 (16.37%) and CT 33 (60.00) %. Statistically, there was no p value <0.05 in the genotype comparison of the HHIP genotype at position rs1828591. This means that there is no difference in the frequency of the SNP rs10519717 genotype of the HHIP gene between COPD smokers and healthy smokers in the SNP rs10519717 genotype in Javanese in Lampung Province, Indonesia.

**Table 1. Characteristic of Participants**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Participant</th>
<th>COPD</th>
<th>Control</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td>64.46</td>
<td>61.62</td>
<td>0.790</td>
</tr>
<tr>
<td>Indeks Brinkman (packyears)</td>
<td></td>
<td>378.41</td>
<td>325.90</td>
<td>0.632</td>
</tr>
<tr>
<td>FEV1 % (Mean±SD)</td>
<td></td>
<td>45.40 ± 20.47</td>
<td>103.81 ± 20.07</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>FCV % (Mean±SD)</td>
<td></td>
<td>74.50 ± 19.49</td>
<td>94.64 ± 16.18</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>FEV1/FVC % (Mean±SD)</td>
<td></td>
<td>60.89 ± 14.51</td>
<td>110.64 ± 18.64</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Work place</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room (n/total)</td>
<td></td>
<td>7/55</td>
<td>13/55</td>
<td>0.056</td>
</tr>
<tr>
<td>Outdoor (n/total)</td>
<td></td>
<td>48/55</td>
<td>42/55</td>
<td></td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (n/total)</td>
<td></td>
<td>46/55</td>
<td>41/55</td>
<td>0.273</td>
</tr>
<tr>
<td>Medium (n/total)</td>
<td></td>
<td>6/55</td>
<td>11/55</td>
<td></td>
</tr>
<tr>
<td>High (n/total)</td>
<td></td>
<td>3/55</td>
<td>3/55</td>
<td></td>
</tr>
<tr>
<td>Income level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (n/total)</td>
<td></td>
<td>48/55</td>
<td>47/55</td>
<td>0.485</td>
</tr>
<tr>
<td>Medium (n/total)</td>
<td></td>
<td>5/55</td>
<td>7/55</td>
<td></td>
</tr>
<tr>
<td>High (n/total)</td>
<td></td>
<td>2/55</td>
<td>1/55</td>
<td></td>
</tr>
</tbody>
</table>
## Table 1. Characteristic of Participants

<table>
<thead>
<tr>
<th></th>
<th>Participant</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COPD. n=55 (%)</td>
<td>Control. n=55 (%)</td>
</tr>
<tr>
<td><strong>IMT (Kg/m2)</strong></td>
<td>21.10</td>
<td>23.20</td>
</tr>
<tr>
<td><strong>Albumin (g/dl)</strong></td>
<td>3.92</td>
<td>3.91</td>
</tr>
</tbody>
</table>

Note: *significant if \( p < 0.05 \)

## Table 2. Genotype frequencies among study groups.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Participant</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COPD. n=55 (%)</td>
<td>Control. n=55 (%)</td>
</tr>
<tr>
<td>rs1828591</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>29 (52.72)</td>
<td>21 (38.18)</td>
</tr>
<tr>
<td>GG</td>
<td>2 (3.63)</td>
<td>5 (9.09)</td>
</tr>
<tr>
<td>AG</td>
<td>24 (43.63)</td>
<td>29 (52.72)</td>
</tr>
<tr>
<td>rs13118928</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>29 (52.72)</td>
<td>26 (47.27)</td>
</tr>
<tr>
<td>GG</td>
<td>1 (1.81)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>AG</td>
<td>25 (45.45)</td>
<td>29 (52.72)</td>
</tr>
<tr>
<td>rs10519717</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>19 (34.54)</td>
<td>13 (23.63)</td>
</tr>
<tr>
<td>CC</td>
<td>8 (14.56)</td>
<td>9 (16.37)</td>
</tr>
<tr>
<td>CT</td>
<td>28 (50.90)</td>
<td>33 (60.00)</td>
</tr>
</tbody>
</table>
Discussion

COPD is defined as a disease state characterized by poor progressive and reversible airflow limitation that is usually associated with an abnormal pulmonary inflammatory response. Nearly 90% of COPD is caused by long-term smoking, however, only 25% of smokers end up being COPD patients. In addition, COPD tends to occur more frequently in smokers with a family history of obstructive airway disorders including asthma and COPD. All of this suggests that, apart from smoking, there are other genetic factors underlying the development of COPD(1).

Many factors can contribute to COPD. COPD can be influenced by racial, ethnic, gender and environmental factors as well as genetic factors. The variability found in lung function and the risk of COPD in people with a similar smoking history, together with family aggregation studies, supports the important role of genetics in the pathophysiology of COPD(14).

Genome association studies have identified several genomic regions that are clearly associated with COPD susceptibility. However, despite recent advances in the genetics of COPD, much of the heritability of COPD remains unexplained. To date AAT deficiency is the only well-identified genetic risk factor for COPD. It is strongly suspected that several other genes are involved in this disease process. Variants of several genes have been investigated and identified to have a close association with COPD, such as TLR-9, HHIP, IREB2, CHRNA3/5, and HHIP(7).

Pathophysiology COPD is a multifactorial process with a complex profile of inflammatory cells including eosinophils, macrophages, neutrophils, and lymphocytes. Levels of several cytokines, such as interleukin (IL) 8, interleukin (IL) -6, TNF-α, and VEGF are elevated in stable COPD patients, suggesting their key role in the pathogenesis of COPD. Therefore, COPD develops as a result of multiple steps involving inflammatory cells and mediators, of which local inflammation in the lungs is essential as it affects airway remodeling and parenchymal destruction(1).

The results of this study were the HHIP gene polymorphism had no effect on the incidence of COPD in Javanese. The HHIP protein is an inhibitor of the HH signaling pathway. HH protein is an important morphogen for various developmental processes, including the anteroposterior pattern of the limb and the regulation of left-right asymmetry in embryonic development(15). HH protein is also an important mediator for diseases caused by cigarette smoke such as lung cancer and COPD. HH protein activity is regulated by HHIP. This HHIP protein is an inhibitor of HH signaling, and it has been shown that HHIP expression protects mice from cigarette smoke-induced emphysema(5).

The HHIP gene is found on chromosome 4 arm of the p locus 13. This gene is highly expressed in the lungs and brain. Several genomic regions that code for HHIP are associated with COPD susceptibility. Polymorphisms can cause changes in gene expression leading to functional changes and ultimately COPD. A significant reduction in HHIP gene expression on mRNA and protein was found in COPD patients compared to smokers with normal lung function(6). Haploinsufficiency of the HHIP gene in genetically engineered mice caused an increase in emphysema due to exposure to cigarette smoke. Mice with haploinsufficiency (HHIP+/-) are more susceptible to developing severe emphysema due to exposure to cigarette smoke than wild type mice (HHIP+/-)(5). In this study, it was found that the presence of polymorphisms in the HHIP gene at positions rs13118928, rs1828591 and rs10519717 did not make Javanese smokers susceptible to COPD(16).

The results of this study differed from several studies in Europe and Asia. Study in Europe has shown that two single nucleotide polymorphisms (SNPs) of the HHIP gene, namely SNP rs1828591 and rs13118928, are associated with the risk of COPD. Study in Asia has concluded that the HHIP gene is involved in COPD susceptibility in Chinese Han populations(8, 9, 17) and in Mongolians(10).

This study is a preliminary finding in pioneering a road map for genetic susceptibility study against
COPD in Indonesia. There is only one publication of the relationship between gene polymorphisms and COPD in Indonesia, namely the study of Tarigan et al\textsuperscript{18}. Study on genetic susceptibility to a disease requires a lot of study with a homogeneous sample and needs to be carried out in various tribes. This is because Indonesia is a country with ethnic and ethnic diversity, which of course has a large genetic variation. This study will contribute to the successful prevention of COPD. This study could contribute to the initial screening of individuals who are susceptible to COPD, as well as play a role in early screening that will prevent smokers from developing COPD.

Groups at high risk of COPD could be screened using genetic screening in the future, although much effort must be made in this clinical area. We focus on pulmonary function and airway inflammation in patients with COPD. More comprehensive data should be obtained to reveal the mechanisms of COPD in further studies\textsuperscript{(11)}.

However, this study has drawbacks because it does not directly examine the levels of the HHIP protein to determine the genetic expression of the HHIP gene and this study also does not examine other downstream HHIP proteins, such as T lymphocyte activation and degradation of the pulmonary extracellular matrix (ECM) which increases the risk of COPD. This study is a cross-sectional study, so it is not possible to know how often there is a history of infection in COPD patients and how the development of nutritional status in COPD patients.

**Conclusion**

HHIP SNP gene polymorphisms including rs13118928, rs1828591 and rs10519717 were found in Javanese smokers in Lampung. There is no relationship between the HHIP SNP gene polymorphisms rs13118928, rs1828591 and rs10519717 and the incidence of COPD in Javanese in Lampung. Future studies can examine genes for other genes that may be associated with COPD in Javanese and it is necessary to carry out research on other ethnic groups in Indonesia.

**Ethical Approval:** Participants have expressed their consent to participate in this study and have signed informed consent. This research has been approved by the Health Research Ethics Committee of the Faculty of Medicine, University of Lampung, Bandar Lampung, Indonesia (2739/UN26/PP.05.02.00/2019).

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**Reference**


Effect of Nano-Coating on Microleakage of Different Capsulated Glass Ionomer Restoration in Primary Teeth: An In Vitro Study

Sohaib Qais Alwan¹, Athraa Mustafa Al-Waheb²

¹B.D.S, College of Dentistry, Tikrit University, ²Professor, Department of Pedodontics and Preventive Dentistry, College of Dentistry, University of Baghdad, Iraq

Abstract

Aim: The aim of this in vitro study was to assess and compare the effect of the nano-coating on microleakage of different capsulated conventional glass ionomer cement (CGIC), resin-modified glass ionomer cement (RMGIC) and hybrid glass ionomer cement in primary molars.

Materials and Methods: A total of 36 primary molars samples were divided into three groups. Group 1 - teeth restored with capsulated RMGIC (Fuji II LC) without nano-coating. Group 2 - teeth restored with capsulated CGIC (Fuji IX) with nano-coating. Group 3 - teeth restored with capsulated hybrid glass ionomer (Equia Forte) with nano-coating. Microleakage was tested using immersed the teeth in 2% methylene blue dye penetration for 24 hours after the thermocycler and measured at x40 magnification under the stereomicroscope.

Results: The results of this in vitro study showed that coating with nano-coating showed a reduction in microleakage in hybrid glass ionomer (Equia Forte) and more microleakage in non-coating RMGIC (Fuji II LC) but also more microleakage in coating CGIC (Fuji IX), which was statistically significant. CGIC with nano-coating and RMGIC without coating showed highest microleakage followed by hybrid glass ionomer (Equia Forte) with coating.

Conclusion: Significant reduction in microleakage was seen in Equia Forte (hybrid glass ionomer) with nano-coating and the coating should use with all types glass ionomer.

Key word: Conventional glass ionomer cement, G-Coat Plus, microleakage, primary molars, resin-modified glass ionomer cement, thermocycling.

Introduction

Dental caries is the foremost common chronic disease in childhood. Around the world, the commitment of dental caries to the burden of oral infections is around ten times higher than that of periodontal infection and the other common oral condition. In dentistry, dental caries is the major reason for loss of the teeth, representing a major challenge for oral health. (¹). The work of a pediatric dental practitioner treating children is twofold, we must put quality work for our patients and we must be experts at managing our patient’s involvement. We must be able to work with speed and precision. This may only be possible in case we have materials that can hand easily and provide reliable results. One such restoration is glass ionomer cement capsule (GIC) (²). Glass ionomer has been utilized as a dental restoration since its presentation in 1972. With its presentation as a restorative material, there were a few restrictions to the material (³). Since their presentation, glass ionomers have advanced and have been progressed colossally. Today’s glass ionomers are simpler to handle by capsule, have superior wear resistance, and have superior esthetics than the first GIC (⁴). Glass ionomers have numerous
preferences as a restorative material. These incorporate, but are not constrained to the capacity to bond chemically to dentin and enamel, biocompatibility, favorable thermal extension, diminished dampness affectability, and the capacity to discharge fluoride, and after that act as a fluoride store. Resin-modified GIC (RMGIC) has resin monomers, like HEMA or Bis-GMA consolidated in its composition, making it a dual-cured cement having higher flexural, compressive, and pliable qualities than ordinary GICs (5).

EQUIA (GC, America) may be a new glass ionomer restorative framework. It may be a combination of a self-adhesive, chemically cured, exceedingly filled GIC (Fuji IX GP Additional, GC) and a self-adhesive, light cured, filled resin surface sealant (G Coat Furthermore, GC). The producers of EQUIA claim that the fabric has expanded break durability, flexural quality, and flexural weariness resistance which are required in Class V restorations (6).

As a result of these qualities, GIC is perfect for the uncooperative child as well as the “high caries risk” children (7). One of the most disadvantages of the filling materials is the microleakage around a tooth, which can be seen as recoloring around the edges of the filling, postoperative sensitivity, secondary caries, pulpal pathology or pulpal rot and the moment being decreased bond quality of the restorative cement driving to fractional or add up to disappointment of the filling itself. According to Nakabayashi and Pashley, in 1998 microleakage is characterized as the section of liquids and substances through minimal holes on the interface of the filling and teeth (8). Within the glass ionomer cements (GICs), water plays an vital part within the setting. Water is capable for the transport of calcium and aluminum cations, which can respond with the polyacid to make a polyacrylate framework. Joining of water with glass ionomers is related with increment within the translucency of the GIC. The freely bound water can be misplaced from the surface by drying up. This causes an unattractive chalky appearance as infinitesimal breaks create within the drying surface and leads to microleakage. Moreover, the nearness of moisture can lead to retention of water and hygroscopic setting extension. To avoid this, it is critical to secure the cement by covering it with an filling varnish or petroleum jelly (9). Another advantage of utilizing such surface defenders is that they fill little surface voids and absconds and may offer assistance to protect the first color of the restorations by diminishing the take-up of stains (10). The 21st century is the period of nanotechnology. Nanofillers make strides the wear resistance of coating operator, in this way giving more defensive coating over CGIGs. As of late, G-Coat Additionally and Equia coat a nanofilled, self-adhesive, light-cured defensive coating has been presented for ordinary GIC, RMGIC, composite resin, and compomer. Past studies have affirmed that when connected, the consistently scattered film thickness of G-Coat Additionally gives higher wear resistance, reinforces the rebuilding, and gives exceedingly gloss appearance (11). Therefore, the present study was undertaken to investigate the effect of G-Coat plus (nanofilled, self-adhesive, lightcured protective coating) on microleakage of conventional GIC and RMGIC in primary molars.

Materials and Methods

Sample’s selection & mounting:

Thirty-six extracted primary molar teeth for orthodontic purposes or because of still over time will select for the study collected for no more than 3 months inspected beneath magnifying lens to avoid any samples with (cracks, Hypoplastic or hypocalcified teeth), as well as the teeth with caries including more than one-fourth of the occlusal surface, will removed from the study. All the teeth were collected from dental specialized centers in Baghdad or from dental private clinics. The teeth belong to child patients.

The teeth were cleaned with hand-scaler then polished by pumice and rubber cups in handpiece low speed and stored at room temperature in distilled water (12).

Covered the roots of teeth by a layer of sheet wax short of the cervical line to work as the soft tissue and
act as a separating medium between the tooth and silicon. A base of silicon is mounted so that it covers the roots of the teeth short of the cemento-enamel junction and the mounting was in plastic molds.

**Samples distribution:**

The teeth were randomly distributed and numbered according to the filling materials used into three groups each contain twelfth samples as follows:

- **Group 1:** restored with non-coating Fuji II LC capsulated glass ionomer cement.
- **Group 2:** restored with coating Fuji IX capsulated glass ionomer cement.
- **Group 3:** restored with coating Equia forte capsulated glass ionomer cement.

**Cavity preparation and restoration:**

In order to standardize cavity preparations for all the teeth, used a modified dental surveyor. Turbine (high speed handpiece) attached to the movable arm of the surveyor in a way put the long axis of the bur in perpendicular to the long axis of the tooth during the preparation. Used diamonds depth cutter 2 mm-Crown Prepration bur was used and the bur was replaced after four teeth preparations.

The teeth received class V cavity width 4mm, length 2mm and depth 2mm prepared on the buccal surface of each tooth with no mechanical retention used diamonds depth cutter 2 mm-Crown Preparion bur under air-Water cooling perparation.

The depth and width of the cavity was checked by digital caliper and periodontal probe (12).

After cavity prepared the teeth were rinsed and dried, and moist cotton pellet was placed in the cavity to prevent complete dehydration of the tooth, cavity conditioning was carried out using 20% acrylic acid (GC, Tokyo-Japan) for 10 s by micro brushes and rubbing movements. Then, cavity was rinsed using water spray for 15 s, after that, a piece of cotton was placed in the cavity and gently dried for 5 s using air spray; then, the cotton was removed to prevent complete dehydration (13, 14).

After cavity preparation and cavity conditioner applied to the 36-primary molar.

...was restored with glass ionomer cement capsule powder and liquid in capsule according to manufacture, mixed in amalgamator 10 second and restore the cavity to full depth 2mm to cavosurface line angle of cavity after chemical setting take 6 min to group 2 (Fuji IX-GC, Tokyo -Japan) and group 3 (Equia forte-GC, Tokyo - Japan) but group 1 (Fuji II-GC, Tokyo -Japan) according to manufacture used curing with light cure 20 seconds, then finish and polish the filling with enhance polish bur during finish use internal water hand piece then applied layer of G-Goat plus (GC, Tokyo -Japan) it is Nano-fill protecting layer above the filling to group 2 (Fuji IX), and use Equia coat (GC, Tokyo -Japan) it is Nano-fill also to group 3 (Equia forte) then curing with light cure 20 seconds.

**Sample’s storage and thermocycling of the samples:**

All the specimens were then stored for 24 hours in normal saline, then use thermocycler device for thermocycling.

Thermocycling was carried out by soaking the teeth alternatively into (5-55 ±1~2°C) water bath chambers with 30 seconds immersion time in each bath and 10 seconds transition time (ISO/TS (E) 11405:2003; (15, 16, 17, 18). The thermocycling 500 cycle all the 12 specimens were thermocycled in the same time.

**Sample’s dye and microleakage measurement:**

All the teeth were dried and two layers of nail varnish were applied to the teeth to cover the complete tooth surface except for 1mm around the restoration edges. The root apices of the teeth were sealed with adhere wax and after that submerged in 2% methylene blue color for 24 hours. The teeth had been washed beneath running...
water and dried.

The samples were blocked with clear epoxy resin; this was done by fabrication of a mold using a specially fabricated plastic molds with dimensions (3-2-1) cm.

Cut longitudinally in the center of the teeth into two pieces using microtome by disk thickness of 0.01mm cutting at high speed and water coolant. The cut halved the tooth bucco-lingually. The cut was made parallel to the long axis of the samples.

The presence of microleakage was affirmed by the visualization of a blue line of the color at the tooth-restoration interface for the occlusal and the cervical dye entrance by visualization by two spectators utilizing stereomicroscope at magnification 40X (19).

The extent of microleakage was evaluated and recorded according to the depth of dye penetration scores given by two way:-

1-Each prepare section will investigation using a sterio microscope (Kruss-Germany) with video output device (20).

Use software (Optika Vision Lite) to measure dye penetration between Glass ionomer filling and tooth in millimeter.

2-The scoring was done as described by Khera and Chan (21).

0 = (No micro-leakage).

1 = Dye penetrating is to the lesser than and up to one half of the depth of the prepared cavity.

2 = Dye penetrating is to more than one-half of the depth of the prepared cavity but not up to the angle of the axial and occlusal or gingival wall.

3 = Dye penetrating up to the angle of the axial and occlusal or gingival wall but not have the axial wall.

4 = Dye penetration have the axial wall.

Pilot study:

After doing pilot study for 5 samples, intercalibration absolute agreement for microleakage (mmm) and its score using Intraclass correlation coefficient (ICC) and weighted Kappa is 0.8 and 0.85 respectively.

Statistical Analysis

Using Statistical Package for social Science (SPSS version 21, Chicago, IL, USA), Descriptive statistics is Frequencies and percentage for nominal variables, minimum, maximum, mean, and standard deviation (SD) for quantitative variable, while median and mean rank for qualitative variable and cluster chart bars. Inferential statistics are Weighted kappa, Intra Class Correlation Coefficient (ICC) Levene test, One Way Analysis of Variance (One Way ANOVA) with Dunnett’s T3 posthoc test and finally Kruskal-Wallis test with Multiple Wilcoxon Sum rank and Mann-Whitney U tests adjusted by Dunn-Bonferroni method.

Results

Test of Normality:

The table 1 indicates that micro leakage variable is normally distributed among groups by using Shapiro-Wilk test (p >0.05) so the parametric tests (One way Analysis of Variance “ANOVA”.

Measure of microleakage in millimeter penetration of dye:

Results in table 2 demonstrate that microleakage is higher in Capsulated FUJI IX followed by Capsulated FUJI II while the lowest is in the EQUIA FORTE with significant results among them further multiple pair wise comparisons using Dunnetts’s T3 post hoc test indicates that there is no significant finding between both capsulated of FUJI IX and FUJI II while each one of them is statistically significant with EQUIA FORTE (Figure 1).

Measure of microleakage in score for dye penetration:
Findings in table 3 show that microleakage score is higher in Capsulated FUJI IX followed by Capsulated FUJI II while the lowest is in the EQUIA FORTE with significant results among them further multiple pair wise comparisons using multiple wilcoxon sum rank test indicates that there is no significant finding between both capsulated of FUJI IX and FUJI II while each one of them is statistically significant with EQUIA FORTE (Figure 2).

### Distribution of scores among groups:

Results in table 4 shows that score 0 occurs in EQUIA FORTE only, score one mostly occurs in Caps. Fuji II followed by Caps. Fuji IX while lowest in EQUIA FORTE, score 2 finds in caps. FUJI IX followed by Caps.Fuji II, lastly score 3 finds only in Caps. Fuji IX (Figure 3).

### Table 1: Tests of Normality of micro leakage area among groups

<table>
<thead>
<tr>
<th>Types</th>
<th>Statistic</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caps. Fuji II</td>
<td>0.867</td>
<td>12</td>
<td>0.0599</td>
</tr>
<tr>
<td>Caps. Fuji IX</td>
<td>0.870</td>
<td>12</td>
<td>0.0654</td>
</tr>
<tr>
<td>EQUIA FORTE</td>
<td>0.872</td>
<td>12</td>
<td>0.0693</td>
</tr>
</tbody>
</table>

### Table 2: Descriptive and statistical test of Micro leakage among groups using One Way ANOVA and Dunnett T3 post hoc test.

<table>
<thead>
<tr>
<th>Types</th>
<th>N</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>±SD</th>
<th>F</th>
<th>P value</th>
<th>Groups</th>
<th>MD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caps. Fuji II</td>
<td>12</td>
<td>0.476</td>
<td>1.900</td>
<td>0.9487</td>
<td>0.5393</td>
<td></td>
<td></td>
<td>Caps. Fuji II</td>
<td>-0.1231</td>
<td>0.9040</td>
</tr>
<tr>
<td>Caps. Fuji IX</td>
<td>12</td>
<td>0.534</td>
<td>1.943</td>
<td>1.0718</td>
<td>0.4502</td>
<td>13.297</td>
<td>0.000*</td>
<td>Caps. Fuji II</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EQUIA FORTE</td>
<td>0.7169</td>
<td>0.0022*</td>
</tr>
<tr>
<td>EQUIA FORTE</td>
<td>12</td>
<td>0.000</td>
<td>0.567</td>
<td>0.2318</td>
<td>0.2522</td>
<td></td>
<td></td>
<td>Caps. Fuji IX</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EQUIA FORTE</td>
<td>0.8400</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

Levene statistics=3.962, p value=0.029 (*), df=2, *.=significant at p<0.05, MD=mean difference.
Table 3: Descriptive and statistical test of Micro leakage score among groups using Kruskal-Wallis and Multiple Wilcoxon sum rank test adjusted by Dunn-Bonferonni method.

<table>
<thead>
<tr>
<th>Types</th>
<th>N</th>
<th>Min.</th>
<th>Max.</th>
<th>Median</th>
<th>Mean rank</th>
<th>Chi square</th>
<th>P value</th>
<th>Groups</th>
<th>MRD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cap. Fuji II</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>1.00</td>
<td>21.125</td>
<td></td>
<td></td>
<td>Fuji II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cap. Fuji IX</td>
<td>12</td>
<td>1</td>
<td>3</td>
<td>1.00</td>
<td>23.875</td>
<td>14.187</td>
<td>0.001*</td>
<td>Fuji II</td>
<td>-2.750</td>
<td>1.00</td>
</tr>
<tr>
<td>Cap. Fuji IX</td>
<td>12</td>
<td>0</td>
<td>1</td>
<td>0.50</td>
<td>10.500</td>
<td></td>
<td></td>
<td>Fuji IX</td>
<td>-13.375</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Df=2, MRD=mean rank difference.

Table 4: Distribution of scores among groups.

<table>
<thead>
<tr>
<th>Types</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caps. Fuji II</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Caps. Fuji IX</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>EQUIA FORTE</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 1: Micro leakage among group.
Discussion

Enamel of deciduous teeth contains less calcium and phosphorus than permanent teeth; additionally, Deciduous teeth have more sensitive of enamel rods with more density. The Dentina tubule numbers in deciduous teeth are more than permanent teeth. All of these variables can cause higher microleakage score in deciduous compared with permanent teeth. (22).

Thermocycling has been utilized in this study to mimic oral conditions. This prepare may highlight the mismatch in thermal extension between the restoration and tooth structure, coming about in numerous volumetric changes amid temperature changes and causing weakness of the adhesive joint with consequent microleakage. This is often in understanding with other investigate, which expressed that, thermo-cycling as intra-oral temperature varieties and subjecting the restorations on the tooth to temperature extremes consistent with oral cavity (11).

Glass ionomers (GIC) have been used as a restorative material since of their capacity to chemically bond to tooth structure and discharge fluoride. They are broadly utilized in dentistry for restoration, as a luting and liner or base. GICs are most sensitive restorative materials to the moisture during the early stages to placement. (25).

Water plays a key part within the development of GIC. Drying out and water defilement amid the starting setting stages can compromise the physical properties of restoration (11).

According to Gemalmaz et al. (1998), when GIC restorations were contaminated with moisture, their mechanical quality diminished and the surface of the material disintegrated or wore quickly. The ability of (GIC) to minimize the degree of microleakage at the tooth or restoration interface is an important factor in clinical success. In the studies microleakage can happen due to disintegration of the tooth-restoration interface, contrasts between thermal expansion coefficients of the restorative material – tooth tissue or polymerization shrinkage, causing recoloring, recurrent caries, and restoration failure. (26).

To overcome the downsides of GIC from moisture defilement and drying up amid starting setting arrange, it has continuously been suggested that GICs must be covered quickly after put (GIC) with a water-proof
surface coating \(^{(9)}\).

Studies have suggested protection of the surface amid the starting setting of GIC with different surface coating agents such as cocoa butter, waterproof varnish, and also nail varnish \(^{(11)}\).

Earl \textit{et al}. (1989) have appeared that quick covering of immature (GIC) with a light-cured resin bonding is the foremost viable strategy of restricting movement of the water across to the surface \(^{(11)}\).

According to Tyagi., \textit{et al}, (2020)\(^{(26)}\) the amount of soluble framework is greatest amid the early stages of GIC formation and the foremost sensitive period is the primary six minutes after mixing. Any contamination by moisture amid this stage can cause the loss of soluble network and diminish its physical properties, thus the GIC ought to be ensured from extra water contamination amid the starting stages to avoid disintegration of particles though once it sets.

In this study found Fuji IX (conventional GIC) with coating and Fuji II (RMGIC) without coating have high microleakage and not significant different between them but equia fort with coating have not and less microleakage.

In previous studies founds Fuji II LC (resin modified glass ionomer) without coating less microleakage than Fuji IX (conventional glass ionomer) without coating because resin-modified GIC (RMGIC) has resin monomers, like HEMA or Bis-GMA incorporated in its composition, making it higher flexural, compressive, and tensile strengths than conventional GICs \(^{(27, 28, 29)}\) thus in this study the coating give improve to the Fuji IX become the same degree of microleakage to the Fuji II LC without coating.

According to Chuang, \textit{et al}. (2001)\(^{(30)}\), resin-modified glass ionomer cements (RMGIC) were developed to replace conventional GICs. RMGICs are materials that set concurrently via a dominant acid-base reaction and auxiliary photopolymerization. With the addition of resin monomer (2-hydroxyethyl methacrylate [HEMA]), and light-polymerized initiators, RMGICs are polymerized immediately after visible light irradiation. Compared with conventional analogs, RMGICs have been characterized as having a longer working time, a rapid set, improved appearance and translucency, and higher early strength. However, RMGICs retain some properties of their conventional counterparts. Additional resin monomer and supplementary photopolymerization have not significantly reduced the susceptibility of RMGICs to dehydration problems. Thus, the maintenance of water balance in the modified cements is important, resin-modified glass ionomers can be finished immediately, they remain moisture sensitive, the results suggest that resin adhesive should be used as a surface protection to reduce margin microleakage of resin-modified glass ionomer restorations. Their results indicated that RMGICs should be protected from water for at least 1 hour after cement mixing. In contrast, most manufacturers’ instructions indicate that RMGICs can be used with or without surface protection.

Whereas the most elevated scores were in Fuji II LC. This may well be come about from polymerization shrinkage that happens in light cured tar adjusted glass ionomer cements. Polymerization shrinkage creates inside 5 minutes after curing and proceeds for another 24 hours. This shrinkage brought about in withdrawal stress which can break the adhesive interface and make the gaps in the margin \(^{(12, 31)}\).

The display study shown that there was significant contrast in microleakage between three groups i.e. EQUIA specialty appeared lesser leakage. The advantage of GC EQUIA Strong point is an inventive restorative framework based on a new glass hybrid innovation, which has more voluminous glass fillers of EQUIA Specialty Fil were supplemented by smaller, exceedingly receptive fillers that reinforce the restoration. In combination with the EQUIA Strong point coat, a composite coating the flexural strength increments by 17\% and flexural energy by nearly 30\%. EQUIA Specialty Coat enters the surface porosities, hence increasing the quality of the generally EQUIA filling and diminishes the microleakage around the restoration \(^{(32)}\).
Conclusion

According to this study an in vitro study conclusion into:

1- Conventional glass ionomer (GC-Fuji ix) with nano-coating (GC G-Coat plus) have high microleakage equal to resin modified glass ionomer (GC-Fuji II LC) without coating.

2- Nano-Coating (GC-Equia coat) with resin modified glass ionomer (GC-EQUIA FORTE) have very low microleakage.

3- Maximum score in Fuji IX with coating then in Fuji II LC without coating but minimum score in Equia Forte with coating.

4- Thus, prefer use Nano-Coating with RMGIC to minimum microleakage.

5- Nano-Coating nanofilled resin-coating provides a high hydrophilicity combined with an extremely low viscosity, thus accounts for a perfect seal to GIC also provide color stability to cement and glossy appearance and bond chemically to tooth structure.

Declarations

Conflict of Interest the authors declare that there are no potential conflicts of interest related to the study.

Source of Funding: Nil

Ethical Clearance: This research has exemption as it a routine treatment (no new materials were used).

References


The Application Program of Smoking Free University Policy Campaign in Sisaket Rajabhat University

Tanaprat Thuksin¹, Chuthamat Nopparat², Tanapat Sriwarom³

¹Lecturer of Community Health Program, Faculty of Liberal Art and Science, Sisaket Rajabhat University, Sisaket, 33000, Thailand, ²Assistant Professor of Public Health. School of Public Health, Walailuk university, Nakhon Si Thammarat, 80160, Thailand, ³Lecturer of Local Politics and Governments Chalermkarnchana University, Sisaket, 33000, Thailand

Abstract

Background: These policy campaign were developed in law enforcement protection a non-smoking for smoking free university by students union president. Method: This quasi-experimental research aimed to evaluate the application program of smoking free university policy campaign. To study the effect of the student leadership development program in the management of the smoking free university, Sisaket Rajabhat University and study the level of participation of the student leaders in the management of the smoking free university towards the smoke-free zone in Sisaket Rajabhat University. by selecting the students union president population of 20 people, the samples were selected according to the inclusion criteria and the exclusion criteria, which the research pattern was used to conduct a comparative study within One Group Pretest - Posttest Design in 12 weeks with 4 activities. The data were collected by using questionnaires and using descriptive statistics presented in the form of a frequency distribution. The data distribution is normal. They were presented with the statistical values of frequency, percentage, mean and standard deviation. And translate the results into score values comparison of the mean, differences before and after the experiment. Within the experimental group using statistics : Paired Samples t-Test. Results: The results of the research were found that after the development of the experimental development program, the students’ leadership in the management of the non-smoking university, knowledge, skills, social roles, imagination or concept about oneself, motivation / mindset Participation in the development of student leadership potential in the management of the non-smoking university. Significantly better than before development (p <0.05). Conclusion: In conclusion of the study : Results of the Student Leadership Development Program in Management of Smoking Free Universities, Sisaket Rajabhat University. It can cause success factors in managing the non-smoking university. In organizing smoking free zones in Sisaket Rajabhat University. To bring about effective action and efficiency and developed as the non-smoking university model including campaigning activities establishing a banner that announces the non-smoking area that covers the entire university. To create participation of university students and the student leaders, The students union president have a clear understanding of roles and duties in their work operations and have an action plan. They realized the importance of organizing the non-smoking zone in the university. There are regulations / measures to prevent and control smoking in non-smoking zones jointly agreed by the leaders. Established a mechanism to support the construction of the non-smoking area in Sisaket Rajabhat University. The measures are followed up and monitored, and implement a participatory and sustainable non-smoking university.

Keywords: smoking free university, policy campaign, non-smoking area

Introduction

The tobacco control policy is an effective way to help people quit smoking and to assist and support smokers, which is an effective way to reduce the number of deaths. Smoking in Thailand has decreased, but there
are still 10.7 million smokers as reported by the Thai National Statistical Office in 2017.\\(^1\) For the purpose of reaching a prevalence of 15.75 percent or less, this goal is what Thailand agreed to achieve in the WHO’s Global Action Plan for the Prevention and Control of NCDs, 2013–2020, and the five-year National NCD Prevention and Control Strategy and Action Plan (2017–2021).\\(^2,3\)

The 2006 U.S. Surgeon General’s Report, The Health Consequences of Involuntary Exposure to Tobacco Smoke, has concluded that (1) second-hand smoke exposure causes disease and premature death in children and adults who do not smoke; (2) children exposed to secondhand smoke are at an increased risk for sudden infant death syndrome (SIDS), acute respiratory problems, ear infections, and asthma attacks, and that smoking by parents causes respiratory symptoms and slows lung growth in their children; (3) exposure of adults to second-hand smoke has immediate adverse effects on the cardiovascular system and causes coronary heart disease and lung cancer; (4) there is no risk-free level of exposure to second-hand smoke; (5) establishing smokefree workplaces is the only effective way to ensure that second-hand smoke exposure does not occur in the workplace, because ventilation and other air cleaning technologies cannot completely control for exposure of non-smokers to second-hand smoke; and (6) evidence from peer-reviewed studies shows that smokefree policies and laws do not have an adverse economic impact on the hospitality industry.\\(^4\)

Therefore, the researcher aimed to evaluate the application program of smoking free university policy campaign in Sisaket Rajabhat University. To compare before and after implementation of the program in subjects practicing smoking free university policy campaign by participation. This research also improved policy campaign were developed in law enforcement protection a non-smoking with a positivestudent leadership development program. In addition, there is also an increase in terms of knowledge, skills, social roles, imagination or concept about oneself, motivation / mindset Participation in the development of student leadership potential in the management of the smoking free university.

**Objective**

The objectives of the research were to evaluate the application program of smoking free university policy campaign in Sisaket Rajabhat University and to compare in terms of knowledge, skills, social roles, imagination or concept about oneself, motivation / mindset, management of the non-smoking university, Participation in the development of student leadership potential in the management of the smoking free university before and after implementation of the program within group.

**Material and Methods**

**Research Design**

This research was a total quasi-experimental research which aimed to evaluate the application program of smoking free university policy campaign in Sisaket Rajabhat University. The samples were total 20 student by selecting the students union president population, the samples were selected according to the inclusion criteria and the exclusion criteria.

The period of this research was 12 weeks and included 4 interventions. The research instruments of non-smoking university policy campaign in Sisaket Rajabhat University Program for the experimental groups.

- **Intervention 1** A competency and participation improving in smoking free university policy campaign.
- **Intervention 2** Set a smoking free area and notice for non-smoking area at all building in University. Let’s stage a campaign to stress the importance of the law enforcement protection a non-smoking Acts.
- **Intervention 3** The students union president MOU in managing the smoking free university. In organizing smoking free zones. To bring about effective action and efficiency and developed as the non-smoking university model Including campaigning activities establishing a banner that announces the non-smoking area that covers the entire university.
Intervention 4 The regulations/measures to prevent and control smoking in non-smoking zones jointly agreed by the leaders. Established a mechanism to support the construction of the non-smoking area in university. And assessment process and outcomes.

Population and Sample Characteristics Inclusion Criteria

The Population consisted of 20 students union president who had to students leader worker at them faculty are included 5 faculty in Sisaket Rajabhat University is Faculty of Liberal Arts and Sciences, Faculty of Education, Faculty of Humanities and Social Sciences, Faculty of Business Administration and Accountancy and College of Law and Government.

Research Period

The research has been undertaken in 3 phases; pre-research, research, and post-research. Data were collected from April 1, 2020 to August 30, 2020, obtained over a 12 week period.

Research Instruments

The evaluation form was provided for participants to answer questions by themselves, and the test of evaluation form consisted of 2 parts:

Instruments for collecting data

Instruments for collecting data consisted of Demographic data

Instruments of experimental

The instruments of quasi-experimental program had a guidebook of the experimental program for research assistants process for smoking free university campaign. The experimental program encouraged smoking free university policy campaign by using 4 interventions for the law enforcement protection a non-smoking Acts.

Data Collection

Before collecting data, ten registered public health students in who trained in the standard protocols and received a research assistance guidebook of the research program were assembled. The data were collected by the principal investigator and the other ten trained research assistants. The researcher and research assistants collected the data from 20 participants who were informed about this research and instructed in how to complete the evaluation form, including requesting consent by signing the research form. The data were corrected before and after the experimental phases. Afterward, collected assessment forms and questionnaires were checked for data accuracy then the results were put into a data entry in SPSS file format.

Data Analysis

Statistical method used to analyze the data was Statistical Package for the Social Sciences (SPSS) in the following aspects:

1. Descriptive statistics included the number, percentage, mean and standard deviation, to indicate the general information and smoking situation of the participants.

2. Statistical use paired samples T-test to compare within the group before and after the experiment to indicate in terms of knowledge, skills, social roles, imagination or concept about oneself, motivation / mindset and Participation.

Results

The application program of smoking free university policy campaign in Sisaket Rajabhat University, The results revealed that the program in terms of demographic data in this study showed that 20 student. They showed most were female, there were 11 (55.0%) and male, there were 9 (45.0%) and the most age range was 19 years, there were 9 people (45.0%), 21 years, there were 5 people (25.0%), 18 years, there were 2 people (10.0%) 20 years, there were 2 people (10.0%) and 22 years, there were 2 people (10.0%) respectively. Faculty studyingshowed that is Faculty of Liberal Arts and Sciences, there were 4 people (20.0%), Faculty of Humanities and Social Sciences, there were 4 people (20.0%), Faculty of Business Administration and
Accountancy, there were 4 people (20.0%), Faculty of Education, there were 4 people (20.0%), College of Law and Government, there were 4 people (20.0%). For College Years, They showed most were third-year, there were 7 people (35.0%), first-year, there were 6 people (30.0%), second-year, there were 6 people (30.0%) and fifth-year, there were 1 people (5.0%) respectively.

Table 1 described the results of the research were found that after the development of the experimental development program, the students’ leadership in the management of the smoking free university, knowledge, skills, social roles, imagination or concept about oneself, motivation / mindset and participation in the development of student leadership potential in the management of the smoking free university. Significantly better than before development (P-value < 0.05).

Table-1: The comparative within group in term of knowledge, skills, social roles, imagination or concept about oneself, motivation / mindset and participation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before experimental</th>
<th>After experimental</th>
<th>t</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td>In terms of knowledge</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before experimental</td>
<td>15.45</td>
<td>19.61</td>
<td>11.297</td>
<td>&lt;0.001*</td>
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<tr>
<td>After experimental</td>
<td>2.694</td>
<td>1.706</td>
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<td></td>
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<tr>
<td>In terms of skills</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before experimental</td>
<td>27.48</td>
<td>46.55</td>
<td>29.752</td>
<td>&lt;0.001*</td>
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<tr>
<td>After experimental</td>
<td>2.755</td>
<td>1.947</td>
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<tr>
<td>In terms of social roles</td>
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<tr>
<td>Before experimental</td>
<td>26.94</td>
<td>57.45</td>
<td>43.785</td>
<td>&lt;0.001*</td>
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<tr>
<td>After experimental</td>
<td>3.356</td>
<td>1.457</td>
<td></td>
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<td>In terms of imagination or concept about oneself</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Before experimental</td>
<td>26.35</td>
<td>46.39</td>
<td>41.666</td>
<td>&lt;0.001*</td>
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<td>After experimental</td>
<td>2.138</td>
<td>1.801</td>
<td></td>
<td></td>
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<tr>
<td>In terms of motivation / mindset</td>
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<td>26.81</td>
<td>44.94</td>
<td>45.524</td>
<td>&lt;0.001*</td>
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<td>2.227</td>
<td>2.337</td>
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<tr>
<td>In terms of Participation</td>
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<td>Before experimental</td>
<td>98.23</td>
<td>168.03</td>
<td>63.872</td>
<td>&lt;0.001*</td>
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<tr>
<td>After experimental</td>
<td>5.162</td>
<td>3.860</td>
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</table>

**Conclusion and Discussion**

The application program of smoking free university policy campaign in Sisaket Rajabhat University. The development of the experimental development program, the students’ leadership in the management of the smoking free university, knowledge, skills, social roles, imagination or concept about oneself, motivation / mindset and participation in the development of student leadership potential in the management of the smoking free university, the results were statistically significant at (p < 0.05). In conclusion of the study: Results of
the Student Leadership Development Program in Management of Smoking Free Universities, Sisaket Rajabhat University. It can cause success factors in managing the smoking free university. In organizing the non-smoking zone in Sisaket Rajabhat University. By using program included 4 interventions in 12 weeks. To bring about effective action and efficiency and developed as the smoking free university by organizing for the non-smoking zone. Including campaigning activities establishing a banner that announces the non-smoking area that covers the entire university and set a smoking free area and notice for non-smoking area. In a self-report survey, more than 75% and 50% of students reported being exposed to posters and public service announcements, respectively. Recognition of campaign theme was more than 80%. Almost half of respondents reported that the posters were interesting, one-third reported that the posters prevented them from smoking, and 10% reported that the posters encouraged them to cease smoking. Stock media posters had a significantly higher affective reaction than the customized media posters. To create participation of university students and the student leaders, The students union president have a clear understanding of roles and duties in their work operations and have an action plan. They realized the importance of organizing the non-smoking zone in the university. There are regulations / measures to prevent and control smoking in non-smoking zones jointly agreed by the leaders. Established a mechanism to support the construction of the non-smoking area in Sisaket Rajabhat University. The measures are followed up and monitored, and implement a participatory and sustainable non-smoking university. They were accorded to the research who found that the sample size had Perception in hazards of tobacco overall Ranking is good and perception is related behavior were statistically significant at p <0.05. with the finding of the research found that before the attitude test which related to cigarettes, there was no difference and after the attitude test related to cigarettes, there was statistically significant difference at p < 0.05. Overall, participants views about smoking on campus during the implementation of a smoke free policy suggest broad agreement but reflect concerns about enforcement, boundaries, non-smokers and designated areas. Consistent and systematic processes for implementation, maintenance and enforcement of policy goals, and cessation support, is needed to create a non-smoking culture on university campuses. They were accorded to the research this study adds to the existing literature by highlighting how clueless most college student smokers are about quitting, despite the availability of cessation programs and the fact that tobacco use is considered the single largest preventable cause of death and disease in the United States. Students experiment with various quit strategies in a random fashion with limited chances of success, given the addictiveness of the product.

However, it was found that the process of smoking free university policy campaign, the development of student leadership potential of the students union president in the management of the smoking free university. In addition, there is also an increased in terms of enforcement of policy goals.

**Ethical Clearance:** This research was approved for ethical certification by the Institutional Review Board of Faculty of Liberal Arts and Science. Sisaket Rajabhat University. (reference number: AF02-09 / COE 002/2563 Date.06 Feb 2020). Participants could refuse and/or leave this research at any time. The data in the evaluation forms was kept confidentially without specifying the participants’ names in the document. When the participants had completed their evaluation forms, they were sealed in the envelopes by themselves before returning to the researcher.

**Conflict of Interests:** This study has no conflicts of interest.

**Source of Funding:** This study was supported by Faculty of Liberal Arts and Science. Sisaket Rajabhat University. Sisaket Province, Thailand.

**Acknowledgments:** The authors are grateful for the Senior students of Community Health, Siriwan Yotha et
al., Faculty of Liberal Arts and Science. Sisaket Rajabhat University.

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The Diagnostic Dilemma of the Most Common Yet the Most Complicated Malignancy Involving the Retromolar Region: A Case Report

Tanha Khan¹, M. K. Sunil², Megha Srivastava³, Arpan Manna¹, Chhavi Srivastava¹, Lakshay Vishnoi¹
¹PG Student, ²Professor and Head, Dept of Oral Medicine and Radiology, ³Senior Lecturer, Dept of Oral Medicine and Radiology, Teerthanker Mahaveer Dental College and Research Centre

Abstract

The most common type of oral malignant neoplasm is reported to be Oral Squamous Cell Carcinoma (OSCC), accounting for more than 90% of all malignant lesions in the oral cavity. The commonest site of OSCC are the tongue, oropharynx and the floor of the mouth. OSCC is more common in men as compared to women as men are often more associated with habits such as smoking and tobacco chewing. It is important to have proper knowledge of this disease so that early diagnosis can be done and mortality rate can be reduced.

Keywords: Oral Squamous Cell Carcinoma (OSCC), TNM Staging, Targeted therapy

Introduction

Oral Squamous Cell Carcinoma is the most commonly seen oral carcinoma with various types of clinical presentations¹. OSCC account for more than 90% of all malignant lesions usually occurring in the oral cavity². OSCC of mandibular region is reported to have lowest survival rate as compared to other carcinomas affecting the oral cavity³. Oscc can be seen clinically as ranging from a white plaque to an ulcerated lesion⁴. There can be delay in the proper treatment of oral carcinomas as they can often be misdiagnosed as other inflammatory lesions involving the oral cavity⁵. Thus, it is necessary for the dental practitioners to diagnose this disease as early as possible so as to deliver prompt treatment².

Case Report

A 35 year old male patient came to the department of Oral Medicine and Radiology with the chief complaint of swelling on right side of the face since 2 months. The patient mentioned that he noticed the swelling for the first time, 2 months back. But he didn’t consult any oral physician at that time. Suddenly 1 week before he reported, he started feeling pain in his right lower back tooth region. He also mentioned that he had a mobile tooth in his right lower back tooth region. So having thought of that tooth to be the root cause of the pain, he went to a local practitioner and got his mobile tooth extracted. After extraction, within 1 day, a small growth appeared on right lower back tooth region and the swelling of the right side of the face also started increasing. According to the patient, the size of the growth showed gradual increase from the day of extraction of the mobile tooth till the day he reported and has attained the present size.

The patient also gave history of analgesic medication for 4 days after extraction. The patient’s medical history and family history was not relevant. As per his statement, he had a habit of chewing smokeless tobacco (gutkha) since 15 years with a frequency of 10 packets/day. He used to...
keep the quid in his right lower buccal vestibular region.

General clinical examination revealed normal mouth opening. On extra-oral examination, a diffused swelling was present on right side of the face at the lower border of the mandible measuring approximately 3*4 cm. On palpation, the swelling was non-tender, non-fluctuant and hard in consistency. Multiple tender lymph nodes were noted on right side of the face and neck region. On palpation, submandibular and cervical lymph nodes were tender and palpable (approximately 4 cm in diameter) and were bony hard in consistency and fixed to the skin.

The intraoral examination revealed a large ulceroproliferative lesion measuring approximately 3*4 cm and was erythematous along with white keratotic appearance. Anteroposteriorly, the lesion was extending from the buccal vestibule of 46 region to the posterior aspect of the retromolar region and mediolaterally, it was extending from buccal vestibule to the lingual vestibular region of 45, 46, 47 region. The border of the lesion was everted (Fig.1).

For primary radiological investigation OPG was advised and it revealed ill-defined radiolucent area with ragged margin measuring 2*3 cm extending from distal aspect of 46 to the middle third of the ramus in right side (Fig.2).

After co-relating all the clinical findings, habit history and radiological findings, the provisional diagnosis was made as “Carcinoma of the Mandible”. Later exfoliative cytology was carried out followed by Incisional biopsy.

The final diagnosis of Oral Squamous Cell Carcinoma was confirmed based on the histopathological report. The TNM cancer staging of this case is Stage IVA and stage grouping T2 N2a M0 as the size of the tumor was approximately 3 cm and metastasis was seen in single ipsilateral lymph node approximately 4 cm in size, without any distant metastasis.

Later, the patient was referred to the department of Oral and Maxillofacial Surgery for the needful treatment.

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Fig: 1: Intraorallylarge ulceroproliferative lesion measuring approximately 3*4 cm and was erythematous along with white keratotic appearance is present
Discussion

Squamous Cell Carcinoma is considered to be the most malignant neoplasm of the oral cavity in India. It is estimated that OSCC constitutes of about 90% of all oral neoplasms. The most common sites for OSCC are tongue, oropharynx and floor of the mouth and Squamous Cell Carcinoma of lips and gingiva are rarely seen. Men are more commonly affected by OSCC as compared to women as men are more exposed to high risk habits such as smoking and tobacco chewing. Additionally, OSCC is increasing nowadays in white people with age group of 18-44 years, particularly among white women.

Some OSCC tends to occur on apparently normal mucosa while others may preceded by some premalignant lesions, such as erythroplakia and leukoplakia. Usually, OSCC presents as an ulcer with raised irregular margins. It tends to show various clinical appearances such as a lump, as a red lesion, as a white lesion or mixed white or red lesion, a non-healing extraction socket or a cervical lymph node enlargement, characterized by hardness or fixation. It is important to note that OSCC should be considered when any of these above mentioned features exists for more than 2 weeks. In our present case, intraoral findings revealed similar features of OSCC which have been mentioned in literature, most commonly ulcero-proliferative growth of the lesion.

As mentioned above, the highest risk factor for OSCC in Western countries is the use of alcohol as well as tobacco. Although the risk factors were independent, it is seen that their actions are seen combinedly. The risk of OSCC increases fifteen folds in users as compared to non-users when combination of tobacco and alcohol is used. It is important to know that there are other risk factors such as betel quid chewing and is associated with increased risk of OSCC. As in our present case patient was chronic gutkah consumer and he used to place the quid in the affected side.

Regional lymph node metastasis is the other common feature of OSCC. In the literature, it is said that cervical lymph nodes of the submandibular triangle and upper jugular regions have more strong predilection of regional lymph node metastasis in most of the cases of OSCC involving lower alveolus. In this present case also, tender and palpable submandibular and cervical lymph nodes of about 4 cm were detected.
Prognosis of OSCC is mainly based on metastasis. Prognosis is better when it is well-differentiated and without metastasis. But most commonly, cases of OSCC are not diagnosed in early stage which ultimately leads to poor prognosis of the disease. Prognosis also varies on numerous other factors related to the tumor or treatment or to the patient.

Radiographically, the bony defects seen in OSCC can be classified as follows: (a) erosive-well defined margins of the absorbed bone and (b) moth eaten-irregular, ill-defined margins of absorbed bone. Further, the extent of the radiographically bone defects are described as follows: (a) grade 1- bone defects limited to the alveolar bone (b) grade 2- bone defect exceeded the alveolar bone but do not extend beyond mandibular canal and (c) grade 3- bone defects extending beyond the mandibular canal. This present case shows, ill defined radiolucency with ragged margin measuring 2*3 cm extending from distal aspect of 46 to the middle third of the ramus in right side extending up to the superior border of the mandibular canal indicative of grade 3-bone defect.

OSCC, being the most dreaded form of carcinoma is responsible for the loss of numerous lives. So, it must be diagnosed at an early stage and treated accordingly. OSCC is being treated traditionally by surgery, chemotherapy, radiotherapy and combination therapy depending on size, site, age, TNM staging.

Treatment modalities of OSCC includes primarily a surgical excision followed by radiation therapy and chemotherapy as an adjunct treatment modalities. In case where lymph node metastasis is present, Radical neck dissection is often required. When the bone defects do not show extension beyond the mandibular canal, marginal resection can be considered as a treatment option, whereas in cases when the bone defects extend beyond the mandibular canal, segmental resection is considered as a treatment option. Newer therapy technique called Targeted therapy have begun to emerge. This method tends to identify the mutated gene responsible for carcinoma and corrects the pathway through which these mutated genes act.

Early diagnosis can be done by other methods such as incisional biopsy, brush biopsy, the ViziLite (highlighting of keratin), oral autofluorescence, photodynamic detection, Optical Coherence Tomography (OCT).

**Conclusion**

As OSCC has various clinical presentations, dentists must be aware of all the characteristics of this disease. Various diagnostic investigations should be considered immediately after the provisional diagnosis in order to reduce the morbidity and mortality rate. Correct and timely diagnosis of the disease is of utmost importance so as to deliver proper treatment modalities to the patients.

**Conflict of Interest:** There is no conflict of interest.

**Donation:** Nil

**Ethical Clearance:** Taken from ethical committee of the college.

**References**

2005.
Suicidal Deaths Amid COVID-19 Pandemic: A Cross-Sectional Autopsy-Based Study

Tanmay Sardar1, Ranjan Biswas2, Achintya Biswas3, Saptarshi Chatterjee1

1Associate Professor, Department of Forensic Medicine and Toxicology, 2Assistant Professor, Department of Psychiatry, Bankura Sammilani Medical College, Bankura, West Bengal, 3Associate Professor, Department of Forensic Medicine and Toxicology, Calcutta National Medical College, Kolkata, West Bengal

Abstract

Background: Suicide is a global issue, with an estimated 75.5% of the cases occurring in developing countries, and India alone accounting for 26.6% of all global suicidal deaths. With an advent of COVID-19 in the early months of 2020, India observed a rapid rise in suicidal deaths. Though, various media reports predicted loneliness, mental illness and economical instabilities as the major triggering factors, there is a lack of analytical or descriptive studies confirming this hypothesis. In this context, the present cross-sectional study was planned to determine the socio-demographic profiles of the victims and the triggering factors of the suicidal deaths during the COVID-19 phase, in context to the victims of suicide from 2017 to the Pre-COVID phase.

Methods: The present cross-sectional study was conducted by analyzing the suicidal deaths from 2017 to 30th June, 2020, interviewing the deceased family members during the COVID-19 phase and studying the Inquest reports, with the documents from the Institutional Medical Record Section.

Conclusion: The authors feel that suicide is an act of moment in mind, so any decision made under excitement or incitement is the real culprit. To curb the menace of suicide, state and society should ensure education, employment and socioeconomic well-being, along with strict law enforcement.

Keywords: Coronavirus, Mental illness, Pandemic, Suicide, Domestic violence

Introduction

Suicide, ranked among the leading causes of death in the world,1 is a global phenomenon, with an annual global suicide prevalence of 10.4 per 100,000 population.2 Suicide is the second leading cause of death in people aged 15-29 years and an estimated 75.5% of all global suicides occur in developing countries, with India alone accounting for 26.6% of all global suicide deaths. India’s contribution to the global suicidal deaths increased from 25.3% in 1990 to 36.6% in 2016 among women, and from 18.7% to 24.3% among men.3 Because of the large population size (18% of the global population), suicides and suicidality in India have global implications towards achieving a sustainable development goals (SDGs) by 2030, with suicide mortality rate as one of the key indicators.

The coronavirus disease (COVID-19) struck India in the early months of 2020. Concerning disease models led to historic and unprecedented public health actions to curb the spread of the virus. Remarkable social distancing interventions have been implemented to fundamentally reduce human contact. While these steps are expected to reduce the rate of new infections,
the potentiality for adverse outcomes on suicide risk is high. And in concordance to this, suicide became the leading cause for over 300 ‘non-coronavirus deaths’ reported in India due to distress triggered by nationwide lockdown in the months of April and May, 2020. There have been a staggering number of suicides, caused by fear of infection or being tested positive for the virus, loneliness, lack of freedom of movement, and alcohol withdrawal during the lockdown.

Suicide is a multi-faceted and complex event. So, the approach to understanding suicide must be multidisciplinary, involving psychiatrists, forensic experts and physicians. Hence, this study was planned with a purpose to know the magnitude and socio-cultural factors of the problem of suicides, the trend during the nation-wide lockdown and to assess the physical and psycho-social comorbidities associated with these suicidal deaths, so that a sound prevention program could be suggested, planned and implemented for flattening the ascending curve of suicidal deaths.

**Aims and Objectives**

1. To compare the proportion of suicidal deaths from January, 2017 to June, 2020

2. To study the socio-demographic profiles of the victims of suicidal death

3. To analyze the events that triggered the suicidal deaths

**Material and Methods**

a. Place of study: BS Medical College Police Mortuary, Bankura, West Bengal, India

b. Period of study: 1st March, 2020 to 30th June, 2020

c. Study population: All the patients sent for autopsy examination at BS Medical College Police Mortuary between 1st January, 2017 to 30th June, 2020

ii. Inclusion criteria: All the victims of suicidal deaths between 1st January, 2017 to 30th June, 2020, who attended to BS Medical College Police Mortuary for autopsy examination.

i. Exclusion criteria:

ii. Cases with incomplete or inadequate history

iii. Unknown cases

d. Sample size: All the victims of suicidal deaths, attended to BS Medical College Police Mortuary for autopsy examination between 1st January, 2017 to 30th June, 2020.

e. Study design: Cross-sectional descriptive study

f. Source of data:

i. Police or Magistrate inquests

ii. Statements of the family members of the deceased

iii. Postmortem register, Bankura Sammilani Medical College

g. Statistical analysis: All the data were manually checked and edited for completeness in a pre-determined format and were then coded for computer entry. Collected data was recorded and analyzed in Microsoft Excel worksheet and SPSS IBM 19.

**Results**

In the present study, the study population have been divided into five groups, namely subjects from the years 2017, 2018, 2019 and Pre-COVID Phase (1st January, 2020 – 29th February, 2020) with the COVID-19 Phase (1st March, 2020 – 30th June, 2020).

1. Proportion of suicidal deaths

**Table 1: Proportion of suicidal deaths**
2. Age-distribution

3. Gender distribution

The Male: Female ratios for the years 2017, 2018, 2019, Pre-COVID phase (January, 2020 - February, 2020) and COVID-19 phase (March, 2020 – June, 2020) were 1.6:1, 1.2:1, 1.4:1, 1.5:1 and 0.9:1 respectively.

4. Monthly variation

Methods of suicide
Table 2 Distribution according to methods of suicide (in percentage)

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hanging</td>
<td>53</td>
<td>56.3</td>
<td>53.2</td>
<td>60.6</td>
<td>58.4</td>
</tr>
<tr>
<td>2</td>
<td>Poisoning</td>
<td>39.2</td>
<td>38.3</td>
<td>39.4</td>
<td>37.9</td>
<td>33.3</td>
</tr>
<tr>
<td>3</td>
<td>Burn</td>
<td>4</td>
<td>3</td>
<td>5.2</td>
<td>1.5</td>
<td>8.2</td>
</tr>
<tr>
<td>4</td>
<td>Drowning</td>
<td>1.7</td>
<td>1</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Railway runover</td>
<td>1.4</td>
<td>1.3</td>
<td>1.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Cut throat</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Wrist slash</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

6. Victims of COVID-19 Phase

6a. Occupation

42.5% victims of suicide were farmers, followed by the unemployed persons (23.7%) and homemakers (21.9%), and the rest of the population were students, retired person or salaried Government servants.

6b. Marital status

Majority of our subjects (55.5%) were married, followed by persons yet to marry (32.9%) and 10.1% of the subjects were either widow or widowers. 3 male subjects in the present study were divorced.

6c. Per Capita Income

![Figure 3 Distribution according to Per Capita Income (B.G. Prasad Scale)](image)

6d. Mental illness
13.04% of the victims were either suffering from mental illness or had a past history of mental illness, of which 37.03% of the subjects had a family history of mental illness. Considering all the victims of COVID-19 Phase, 23.67% of the victims had at least one member in their families with mental ill-health.

6c. Educational status

Table 3 Distribution according to Education status

<table>
<thead>
<tr>
<th>Sl.</th>
<th>Status</th>
<th>Number (n)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Illiterate</td>
<td>103</td>
<td>49.8</td>
</tr>
<tr>
<td>2.</td>
<td>Non-formal literate</td>
<td>39</td>
<td>18.8</td>
</tr>
<tr>
<td>3.</td>
<td>Primary</td>
<td>33</td>
<td>15.9</td>
</tr>
<tr>
<td>4.</td>
<td>Secondary</td>
<td>15</td>
<td>7.2</td>
</tr>
<tr>
<td>5.</td>
<td>Higher secondary</td>
<td>07</td>
<td>3.4</td>
</tr>
<tr>
<td>6.</td>
<td>Graduation and above</td>
<td>10</td>
<td>4.8</td>
</tr>
</tbody>
</table>

6f. Previous suicidal attempts

27 victims (13.04%) had a history of previous suicidal attempts. There was one woman in our study, who tried to commit suicide by slashing her wrists and received medical attention. That very night, she committed suicide by partial hanging.

6g. Events leading to the suicidal deaths

Table 4: Distribution of events leading to suicidal deaths

<table>
<thead>
<tr>
<th>Sl.</th>
<th>Events</th>
<th>Number (n)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Domestic violence</td>
<td>60</td>
<td>28.9</td>
</tr>
<tr>
<td>2.</td>
<td>Dowry deaths*</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>3.</td>
<td>Economic crisis</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>4.</td>
<td>Depression</td>
<td>24</td>
<td>11.6</td>
</tr>
<tr>
<td>5.</td>
<td>Chronic debilitating diseases</td>
<td>23</td>
<td>11.1</td>
</tr>
<tr>
<td>6.</td>
<td>Love and breakups</td>
<td>15</td>
<td>7.2</td>
</tr>
<tr>
<td>7.</td>
<td>Unfulfilled demands**</td>
<td>13</td>
<td>6.3</td>
</tr>
<tr>
<td>8.</td>
<td>Withdrawal***</td>
<td>9</td>
<td>4.3</td>
</tr>
<tr>
<td>9.</td>
<td>Corona phobia</td>
<td>5</td>
<td>2.4</td>
</tr>
<tr>
<td>10.</td>
<td>Sudden demise of Bollywood actor</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
**All the suicidal deaths in married women within 7 years of marriage, irrespective of the events leading to death.**

**Included the victims who committed suicide due to unfulfilled demands for smartphones, musical instruments, dining out and outdoor sports**

***Withdrawal from lack of availability of alcohol and other substances of abuse***

**Discussion**

The COVID-19 pandemic began in December 2019 at Wuhan, China, and has quickly spread globally affecting more than 16.5 million people. India reported her first confirmed case on 30th January 2020 in Kerala. However, with an affection of more than 1.4 million people and a death toll of 33,000, India is ranked third in the world in terms of infection, immediately next to the United States of America and Brazil. Understandably, medical professionals and public health specialists are focused on the treatment and containing the spread of the disease in the general population, with less attention to the psychological and social consequences of the Covid-19 crisis. New research warns that the rapid rise in unemployment, coupled to the prevailing mental health problems amid this pandemic can raise the suicide rates.

Though there is a paucity of literature on the trends of suicidal deaths amid COVID-19 crisis, multiple lines of evidence suggest that, this crisis may increase the prevalence of psychiatric disorders and suicide rates during and after the pandemic. This speculation is further amplified by the reports of 54 cases of suicidal deaths in the state of Punjab during the lockdown months of April and May in India. Reports from Nagpur claimed a decline in the suicidal deaths in the months of March and April, with an increase in incidence of suicidal deaths in May and a sudden spike in the month of June. The present study reflects a significant rise in the incidence of suicidal rates by more than 20%, as compared to the previous three years. Suicide, with an average prevalence rate of 25-30% among all the medicolegal autopsies, is uniformly distributed throughout the year in Bankura, West Bengal. However, a rise in the suicide rates is noticed from the early days of January 2020, with rapid developments from the months of March, reaching its peak by June. This explains the growing instabilities and uncertainties among the general people in a region, where more than one-third of the population is living below poverty lines.

Globally, suicide is a leading cause of death among teenagers and adults under 35 years of age, with a Male: Female ratio of 1.8 in age-standardized suicide rates, as per Global Health Observatory (GHO) data. Studies report that the age group of 20-63 years is most vulnerable, followed by the age group of 30-64 years. In our study, people of age group of 20-30 years were mostly affected, followed by the individuals of 30-40 years, with a gender distribution in continuity with the Global Health Observatory data from 2017 to 2019. But during March to June 2020, an altered ratio of 0.9:1 was seen, indicating more incidence of suicides in females, as compared to males. Women’s greater vulnerability to suicidal behaviour is likely to be due to gender related psychopathological and psychosocial stressors, coupled to the lower number of reasons for living during the COVID-19 days. The availability of specific means for suicide in one particular region depends upon various factors like age, sex, availability and access of methods, the socio-economic condition, as well as prevailing culture and religious customs. As the United States of America reports firearms as the most common method of suicide, China and South Korea, catering large rural populations, exhibits self-poisoning with pesticides as the usual method. However, in contrast, the National Crime Records Bureau in India reported that suicide by poisoning has gone down, with hanging becoming the preferred method. In concordance, our study reflects hanging as the most preferred method, followed by poisoning, with a fewer number of suicidal deaths attributable to self-immolation, drowning and railway runover. A systemic review on suicide in India found hanging as the most frequently reported method of suicide (10-72%), followed by self-poisoning (16-
49%), drowning (3-39%) and self-immolation (6-57%). Though poisoning and hanging are the two most preferred means of suicide over the decade in India, the gap between the two decreasing over time. 

Virtually all mental disorders carry an increased risk of suicidal ideation, suicidal attempt, and suicide. About 90% of individuals who attempt or commit suicide suffer from mood disorders, psychoses, and personality disorders. However, the prevalence of depression or other diagnostic mental disorders recorded by psychological autopsy for the suicidal victims in Asian countries is lower than non-Asian high-income countries. Depression is a public health issue in the South-East Asia region, with a prevalence ranging from 6.9% to 51.7%, among people who committed suicide. Identical results are reflected in the present study, which is also in concordance to a study in Indonesia, where major depressive episode was diagnosed in more than half of the people who died by suicide. And according to another report from the United States of America, suicide rates are largely unrelated to antecedent physical activity and alcohol consumption, and substantially higher among men reporting personality traits as insomnia, exhaustion, cyclothymia and self-consciousness.

The transition from suicidal ideation to actual suicide occurs impulsively as a reaction to acute psychosocial stressors. Numerous studies have examined the precipitating factors influencing suicidal behaviours, that include suicidal ideation, plan, attempts and completed suicides. Being unemployed, unmarried, low education and income were reported to be associated with suicidal behaviours. Additionally, family history of mental disorders, chronic debilitating diseases, depression, loneliness, and illicit drug use have been documented to predict suicidal behaviours. Contrastively, this study portrayed that the farmers and unemployed persons constituted majority of the victims, with married persons being more vulnerable to suicidal behaviours. This deviation in findings can be attributed to the COVID-19 pandemic, which struck India in the month of March 2020. The realization of the non-availability of vaccine and/or effective antiviral drug against SARS-CoV-2 virus, and understanding that social distancing and quarantine or self-isolation is the only available remedy, forced governments of most of the countries to declare the nationwide lockdown which had substantial effects on the global economy and the socio-cultural environment. Social isolation, anxiety, fear of contagion, uncertainty, chronic stress and economic difficulties may lead to the development or exacerbation of stress-related disorders and suicidality in vulnerable populations including individuals with pre-existing psychiatric disorders, low-resilient persons, individuals who reside in high COVID-19 prevalent areas and people who have a family member or friend who has died of COVID-19. Domestic violence alone accounted for almost 30% of all the cases in our study, followed by economic unrest. Fuelled by mandatory stay-at-home, physical distancing, economic uncertainties, and anxieties caused by the pandemic, domestic violence has increased globally. The National Commission of Women (NCW) noted a rise in the number domestic violence complaints in India. However, the number of cases reported are most likely not proportional to the actual rise in domestic violence, as most avenues to seek help or to physically remove themselves from their situations are impaired.

Conclusion

Suicide is a vast varied and intricate topic, yet our study is a tangible attempt of exploring the socio-demographic profiles of victims and the triggering events leading to suicides. Our study reported a suicidal incidence of 45.39% in the COVID-19 phase, as compared to a mean value of just over 26% from 2017 to Pre-COVID era. Majority of the victims belong to 20-30 years of age, with an overall male predominance, except during the COVID-19 phase, reflecting an unusual female dominance. Though the proportion of suicides is fairly distributed across the year, the months of pandemic reported a spike, with hanging and poisoning as the most preferred methods. Analysis during the pandemic revealed that farmers, unemployed and homemakers constituted the burden, with married persons from low-middle and low income groups. Victims with
low education and with a history of previous suicidal attempts were found to be most vulnerable. 13.04% of the victims were either suffering from mental illness or had a past history of mental illness, of which 37.03% of the subjects had a family history of mental illness. Considering all the victims of COVID-19 Phase, 23.67% of the victims had at least one member in their families with mental ill-health. However, domestic violence was depicted as the single most culprit behind these suicidal deaths.

To curb the menace of suicide, state and society should ensure education, employment and socioeconomic well-being, along with strict law enforcement. But authors feel that suicide is an act of moment in mind, so any decision made under excitement or incitement is the real culprit. Therefore, we would like to wrap up this by suggesting to improve the ability to think over any problem, with a balanced and reasonable tolerance.

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Ethical Clearance: Taken

Source of Funding: None declared

References
Uncertainty-Based Critical Nursing Model on Family of Family Needs in Bangil General Hospital, Pasuruan, Indonesia

Tanty Wulan Dari1, Moch Bahrudin1

1Lecturer, Department of Nursing, Health Polytechnic Ministry of Health, Surabaya, Indonesia

Abstract

Holistic care in the intensive unit should be provided for patients and their families. The patient’s family waiting in the intensive care room may experience uncertainty. Not only because of the unfamiliar treatment rooms, but also because of the health workers who are strangers to them and speak with medical terms. The objective of this research is to develop the CCFN (Critical Care Family Need) Nursing Model towards the adaptation of the patient’s family in the Intensive Care Unit (ICU). The research was conducted with an explanatory design. The population in this study was patients’ most influential family member in making decisions (related to patients’ care and medication) at Bangil General Hospital in 2020. The sample size was 105 with consecutive sampling technique. Research variables included: factors related to the patient, family psychology, health services, family needs and family adaptation. PLS testing was performed, then FGD was done to strengthen the statistical model. Results showed a different finding compared to the initial concept, where there the families’ thinking capacity p showed no significant effect on the families’ coping factor. The families’ coping factor showed no significant effect on the critical care family need (CCFN). However, family coping factor showed a significant effect on family adaptation factor. In conclusion, there are two indicators that can explain the fulfilling of family needs factors, namely indicators of mental support from health workers and their closeness to patients. Calmness of family during discussion with health workers, as well as enthusiasm felt by the patient’s family, are things that must be noted, so that the family adaptation process occurs optimally.

Keywords: Patient’s family, CCFN, Uncertainty, Adaptation.

Introduction

Separation between patients and their family may cause stressful situations, especially for nuclear (main) families in Indonesia. People in Indonesia generally adhere to very close kinship, where if a family member is sick, all members would also feel the pain. When a patient undergoes treatment in the intensive care unit (ICU), the patient must be isolated and separated from their family. In Indonesia, a family can be seen as a system, where change in health or separation of a family member will impact other family members. Conditions like this are likely to create a situation where the family experiences stress or feelings of uncertainty1,2.

Other causes of the feeling of uncertainty in patients’ family is the lack or absence of supporting facilities to fulfill biological needs of the family in the waiting room, such as the waiting room itself, lighting, and bathroom/toilet facilities1,2.

A research revealed that the feeling of anxiety was experienced by 27 parents for 11 months whose child was treated in the ICU. Through this study, 5 (five) nursing diagnoses arose, including, uncertainty, conflict in the role of parents, high risk of ineffective nutrition for children, high risk of damage of relationship with children, high risk of lack of home care and high risk of tension in the role of service providers. Based on these
problems, nurses need to intervene with parents and other family members regarding to child care in the ICU to overcome problems that arise\textsuperscript{3,4}.

According to a preliminary study conducted by several researchers in February 2019 at Bangil Hospital, an interview was done to 10 families who were waiting outside of the ICU. Families stated that they almost never receive an explanation of the patient’s illness, prognosis, development, treatment and action. The family would only be called if needed to buy medicine or when the patient’s condition is in a nearly dying state. Other than that, every family that is called into the ICU always come out crying, which causes feelings of anxiety and uncertainty for the other families\textsuperscript{5,6}.

Families of patients in the ICU hope that nurses provide extra attention and care to patients. Around 90\% of the families want nurses to explain the patients’ situation, development and treatments using a language that is easily understood. Families also want to be able to be beside the patient. Efforts or research to solve the problems mentioned above has never been carried out before\textsuperscript{5,6}. The services provided so far are routines and researches previously carried out at Bangil Hospital.

Based on the results of several research journals, the effort of medical staffs increasing the intensity of communication with the patients’ family and showing affection towards them shows positive results. Often, patients’ family members feel uncertain, in need of help, guilty, fatigued, sad, scared and anxious. Effective use of communication by health workers, improving communication strategies, helping families relieve emotions and increasing family independence are goals that have been set\textsuperscript{7}.

Families who receive attentive care will be able to provide greater comfort for their illness, encourage improvements in care, and improve the skills assessment of health care providers. Leske categorizes five family principles that can be used to guide nursing interventions, which should be initiated at initial contact with family members\textsuperscript{4}. This would provide assurance and a calm and relaxed atmosphere that would support trust and empathetic relationships. Increased closeness means allowing family members to become closer to the patient by visiting the patient. The five family categories according to Leske: Assurance, Proximity, Information, Comfort, Support\textsuperscript{8}.

The need for information about the patients in the ICU is proven to be the top need of the patients’ family members. A study was initiated to determine the level of satisfaction of family members, and it was found that providing complete information of the patients in the ICU was associated with family members’ overall satisfaction\textsuperscript{5}. There are several ways to deliver information to the family: educational orientation programs, classes providing social support and information on disease management and recovery, and information packages. Learning the balance between too little and too much information and how to convey this information is an important skill for nurses in the ICU to learn\textsuperscript{6}.

The nurse must consider the nature of the information delivered to the family after deciding the best manner to deliver the information. In a study of 390 families of patients who passed away in the ICU, researchers found that the majority of respondents (82.6 percent) criticized the hospital, 17 percent felt the information received about the diagnosis was insufficient or unclear, and 30 percent expressed dissatisfaction regarding to the information received on the cause of death (particularly among family members who were notified of death by telephone and not in person)\textsuperscript{7}. Programs for family members to determine whether this program would increase family member’s satisfaction with treatments, meeting their need for better information, and reducing disruption to ICU care. The intervention consists of three components, namely: 1) discussion with the nurse approximately 24 hours after admission of the patient 2) an informational pamphlet given during the discussion 3) daily phone calls from nurses who treat the patients that day\textsuperscript{7,8}.

The number of incoming calls from family members was significantly lower in the experimental group than in
the control group. In the experimental group, satisfaction with patient treatment increased significantly compared to before. Family members’ perception is based on how well their information needs are met. The family goes through three stages of adaptation in response to news on a family member suffering from a fatal disease. Various emotional responses may arise during this phase, including disorganization/division, anxiety, and emotional instability. The first phase is the preparation phase. This phase begins when the first symptoms appear and continues through the initial diagnosis.

Nurses can help families by informing them that it is natural to go through these phases and that they can expect direction from complex problems in each phase of a fatal illness. Helping families accept their feelings and directing them to appropriate service resources, such as hospitals, family support groups, social workers, and family conferences, can be very useful.

**Method**

In this research, correlation analysis was performed and carried out in two stages. An explanatory design was used in the first stage and FGD was done in the second stage to reinforce the findings. Explanatory design was used to develop a Critical Care Family Need (CCFN) nursing model on family members of patients in the ICU. The approach used is cross sectional. This study examined the effect of patient, family psychology, service and family needs: CCFN factors on family adaptation. The research was carried out at Bangil General Hospital, Pasuruan, Indonesia in an integrated intensive care installation room which involved 265 respondents. Sampling was done in October-November 2020.

**Results and Discussion**

The majority of respondents’ answers are summarized as following: Disease indicator, the majority stated that the family knew about the patient’s illness, with 60% of respondent answers. Prognosis indicator, the majority said the family knew about the prognosis of the patient, with 55.1% of respondent answers. Disease history indicator, the majority of the patient’s families knew about the patient’s history, with 89.4% of respondent answers. Action indicator, the majority of the patient’s families knew enough about patient treatment that cause pain, with 70.2% of respondent answers.

The following is a summary of the majority of respondents’ answers related to family psychological factors: Communication indicator, the majority of the patient’s families do not have the communication skills to handle the uncertainties they experience, with 75.1% of respondent answers. Appreciation indicator, the majority said that there was no ability to appreciate things to overcome the uncertainty they experienced, with 86.4% of respondent answers. Togetherness indicator, the majority said that there was sufficient togetherness among family members to handle the uncertainty they experienced, with 72.1% of respondent answers. Health indicator, the majority of the patients’ family stated that they had sufficient health to handle the uncertainty they experienced, with 60.4% of respondent answers.

The following is a summary of the majority of respondents’ answers related to indicators on family coping factors: Regulatory indicator, the majority of the patient’s family had no opinion on regulatory regarding to a family member being treated in intensive care, with 54% of responses. Empathy indicator, the majority stated that there was no empathy service provided by intensive care officers to the patient’s family, with 85.3% of responses. Assurance indicator, the majority stated that there was no service assurance provided by the intensive care staff to the patient’s family, with 90.2% of responses. Tangible indicator, the majority stated that there was sufficient tangibility (real evidence) of the services provided by intensive care workers to the patient’s family, with 70.6% of responses.
Congenator indicator, the majority of the patient’s family had no opinions on congenator regarding to a family member being treated in the intensive care unit, with 70.9% of responses.

The following is a summary of respondents’ answers related indicators on family coping factors: Therapeutic communication indicator, the majority of the patient’s families were not given therapeutic communication between them and health workers, with 75.8% of responses. Involvement on family in treatment indicator, the majority of patients’ families were not involved in patient treatment, with 88.3% of responses. Mental support by health workers indicator, the majority of the patient’s family felt that they were not given mental support by health workers, with 88.7% of responses. Comfortableness with health facilities indicator, the majority of the patient’s family did not feel comfortable with existing health facilities, with 84.9% of responses. Closeness to patient indicator, the majority of the patient’s family was given the opportunity to be close to the patient, with 75.1% of responses.

The following is a summary of respondents’ answers related to indicators on family adaptation factors: Enthusiasm indicator, the majority of the patient’s family felt that they did not feel enthusiastic, with 91.3% of responses. Discussion indicator, the majority of the patient’s family felt uneasy discussing their experience, with 54.3% of responses. Decision making indicator, the majority of the patient’s family felt uneasy in making decisions related to their experience, with 95.8% of responses. Participation indicator, the majority of the patient’s family felt no sense of calmness in terms of participation in their experience, with 87.9% of responses.

The Family Needs on Family Adaptation factor has the highest value, namely 0.227. This indicates that when the needs of a family are increased by 1 unit, family adaptation value would increase by 0.227 times. Patient factor on family adaptation factor has a value of 0.042. This indicates that if the patient factor is increased by 1 unit, family adaptation value would increase by 0.042 times. The Service Provider Structure Factor on the Family Adaptation factor is -0.032 which indicates that these factors are inversely related. If the value of the service provider structure is increased by 1 unit, the value of family adaptation would decrease by 0.032 times.

**Conclusion**

Family coping factors affect family adaptation factors. The value of the effect is -0.126, meaning that if the family coping factor is given a value of 1 unit, the patient’s family adaptation value would decrease by 0.126 times. There is no effect of the Family Coping Factor on the Fulfillment of Family Needs Factor/CCFN. The family coping factor has no effect on the Fulfillment of family needs factor. Fulfillment of family needs factor affects the family adaptation factor. The effect has value of 0.227, meaning that if the family needs fulfillment factor is given a value of 1 unit, the patient’s family adaptation factor would increase by 0.227 times.

Findings in this study reveal that the factors required to fulfill the needs of families’ whose family member is treated in the ICU include: therapeutic communication, family involvement in treatment, mental support, comfort and closeness to patients. These factors directly affect the adaptation of the patient’s family. Meanwhile, indirect factors include the patient, psychology of family, services and family coping. In conclusion, the most effective factors that help patient’s family adapt to the situation are closeness to patient and mental support. Not all factors affected family adaptation. This may be related to the location of study, which was at Bangil General Hospital.

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**References**


Comparison of Lower Organ Disorders between Wrestlers with Meniscal Injury, Anterior Cruciate Ligament (ACL) and Healthy People in Kermanshah

Tayebeh Mahvar¹, Somayeh Mahdavikian², Afsaneh Mohammadi³, Ferdos Pelarak⁴, Masoud Fallahi⁵, Hamideh Mashalchi⁶

¹Assistant Professor, Nursing Department, Nursing and Midwifery School, Kermanshah University of Medical Sciences, Kermanshah, Iran, ²Tutor, Nursing Department, Nursing and Midwifery School, Kermanshah University of Medical Sciences, Kermanshah, Iran, ³Tutor, Physical education and Sport Science Department, Islamic Azad University, Islamabad-E-Gharb, Kermanshah, Iran, ⁴Tutor, Department of Nursing, Dezful Branch, Islamic Azad University, Dezful, Iran, ⁵Tutor, Nursing department, Nursing and Midwifery School, Kermanshah University of Medical Sciences, Kermanshah, Iran, ⁶Tutor, Nursing department, Nursing and Midwifery School, Dezful University of Medical Sciences, Dezful, Iran

Abstract

Introduction: The present paper studied three lower organ linear disorders, including tibial rotation (rotating tibia bone around linear axis) (femoral anteversion), femoral normal rotation, and varus complications as possible risk factors of common sports injuries of knee.

Methods: In this comparative descriptive study, 60 men were divided into three meniscal injury, anterior cross ligament and healthy groups (n=20). All of the participants were current wrestlers at different clubs of Kermanshah province and towns who had at least four years of experience in various sports events. The samples of the injured groups were selected through questionnaire and referral to sports clubs and the healthy group sample was randomly selected. The healthy individuals had no record of knee injury.

Results: The results of the present study revealed that anteversion (internal rotation of femur) affected both common anterior cruciate ligament and meniscal impairments, and tibial rotation only significantly affected meniscal injury not anterior cruciate ligament. Also, increase of knee varus angle did not have any effect on meniscal and anterior cruciate ligament injuries.

Conclusion: The results of this study can be helpful to recognize the risk factors of sports injuries.

Key words: lower organ linear disorders, wrestlers, anteversion, knee varus angle

Introduction

Movement is an inseparable part of all living organisms. This vital factor is provided by the muscular-skeletal system of the living organism¹-⁴. Proper physical condition with no threatening motor diseases is one of the requirements of the living organism. Lower organ is the supporting surface, balance factor and motor element for the organism²,⁵-⁷. Thus, the disorders associated with this organ, in addition to changing the standing position, affect movements, too. As the largest joint of the body, knee is where most threatening factors and lower organ disorders occur³,⁴,⁷-⁹. Anatomical disorders and various distortions are seen in knee joints and rotation of lower organ as valgus, varus, hyperextension, anteversion
femur and tibia, which can be a reason for ligaments’ injury and stabilizing elements of knee joint\cite{10}. Increase in varus and valgus angles are associated with diseases like osteoarthritis and other types of arthritis. Nowadays, various studies are being conducted to investigate the effect of disorders such as knee joint disorders, rotation of lower organ, and incidence rate of injuries in this region. A lot of these studies have been conducted to prevent common sports injuries and to identify the possible risk factors of knee injury\cite{11-13}. Age, gender, body type, flexibility, joint laxity, muscular power, previous injuries, rehabilitation programs and anatomical disorders are the risk factors of lower organ injury\cite{14}, among which anatomical disorders are the most important causes. Based on the previous studies, knee joint includes most of the lower organ injuries so that 4.35% of the injuries, followed by the highest frequency, are associated with knee. According to the report by the orthopedics faculty of the United States, meniscal injury is the second most common knee injury and is often followed by 22- 86% anterior cruciate ligament injury. The annual cost of the injuries resulting from the sports around the world is estimated one billion dollars, a large sum of which is on knee injury treatment. Researchers have predicted remarkably lower costs for injury than treatment. In line with this, many researchers have been trying to find the risk factors causing common and costly traumas\cite{15-20}. Since dealing with injury is mainly focused on the preventive measures, identification of factors and predisposing risk factors of injuries are the most important issues to be taken into account to prevent injury\cite{21,22}. Previous research indicates that lower organ disorders are not known from the risk factors of common injuries in lower organ\cite{22-25}. The present study was aimed to study three lower organ linear disorders, including tibial rotation (rotating tibia bone around linear axis) (femoral anteversion), femoral normal rotation, and varus complications as possible risk factors of common sports injuries of knee. The results of this study can be helpful to recognize the risk factors of sports injuries.

**Material and Methods**

In this comparative descriptive study, 60 men were divided into three meniscal injury, anterior cross ligament and healthy groups (n=20). All of the participants were current wrestlers at different clubs of Kermanshah province and towns who had at least four years of experience in various sports events. The samples of the injured groups were selected through questionnaire and referral to sports clubs and the healthy group sample was randomly selected. The healthy individuals had no record of knee injury. Having signed the consent forms, the participants were informed about the study process and the orthopedics specialist explained the possible dangers of x-ray. Personal information, including height, weight, age, activity record, injury type, and time length after injury and time of inactivity due to injured foot, internal or external meniscal tear, time after surgery, superior leg and non-superior leg, body mass index (BMI) in both injured meniscus and anterior cruciate ligament was collected. Then, using 16-slide CT scan machine, the disorders of 100 people, including anteversion, rotation angle of tibia and varus angle were determined. Finally, the angles of disorders acquired for each person and each group were drawn and compared with the healthy group by the radiology specialist. In this study, 16-slide CT scan (Siemens, Germany) machine was used at Imam Hossein hospital in Kermanshah. To calculate the anteversion, intended cuts were taken from the pelvic region and distal part of femur. Then, in each region the best cut was chosen by the radiologist and by passing an imaginary transverse line through the same region, the tangent was drawn and the tangents on both distal and proximal parts of the femur were drawn to cross each other. The obtained angle from the crossed tangents was considered as anteversion angle. To calculate the rotation angle of tibia, taking the diagnosis of the radiologist into account, first the best cuts were selected from the proximal region of tibia and its distal region, respectively, then, the crossing lines and tangent on them were drawn and the obtained angle from the crossing lines was calculated as tibia rotation angle. Further, varus angle was calculated using the CT scan images and drawing the angle between the two imaginary transverse lines of the upper part and lower part of tibia. Finally, the angle of the acquired disorders in both groups of injured
Meniscus and anterior cruciate ligament were calculated by the radiologist and compared with the healthy group. Descriptive and inferential statistics were used to analyze the data. Also, Kolmogrov Smirnov was used to determine the normal distribution of the data. Then, independent t-test was used to determine the differences between healthy group and other groups. To analyze the data, SPSS (version 18) was used and Excel software was used to draw the graphs.

Findings

The findings indicated a significant difference between the tibial rotation of meniscal injury group (88.4±25.19) and healthy group (67.3±22.2) (p= 0.037), but there was no significant difference between rotation of tibia in anterior cruciate ligament injury group (58.3±85.21) and healthy group (88.4±25.19) (P=0.50).

In contrast, the anteversion level of the meniscal injury group and healthy group was statistically significant (p= 0.01), which was 73.1± 50.13 in the healthy group and 03.3± 65.15 in the meniscal injury group.

However, there was no significant difference between the anteversion level of anterior cruciate ligament injury group and healthy group (p=0.100), which was 73.1± 5.13 in the healthy group and 16.3± 85.16 in the anterior cruciate ligament injury group. Moreover, no significant differences were observed between the knee varus angles of the meniscal injury group (79.2±70.7), anterior cruciate ligament injury group (65.2± 75.7) and healthy group (59.3± 10.7) (p=0.50).

| Table 1 Personal data of the samples, including age, height, and weight |
|-------------------------------|----------------|---------------|
| Age (year) | Height (cm) | Weight (kg) |
| Healthy | 38.3± 88.28 | 62.7± 85.177 | 70.6± 9.76 |
| Injured meniscus | 38.5±65.26 | 69.4± 6.175 | 39.9± 3.77 |
| Injured ACL | 42.3± 57.26 | 35.5± 85.175 | 66.6± 55.77 |
| Total | 23.4± 37.27 | 20.6± 43.176 | 57.7± 25.77 |

| Table 2 Comparison of lower organ disorders between meniscal injury, ACL and healthy people |
|-------------------------------|---------------|----------------|
| Tibial rotation angle | Healthy | Meniscal injury | Anterior cruciate ligament injury |
| 88.4± 25.19 | 67.3± 2.22 | 58.3± 85.21 |
| Anteversion angle | 73.1± 50.13 | 03.3± 65.15 | 16.3± 85.16 |
| Knee drug angle | 59.3± 10.7 | 79.2± 70.7 | 65.2± 75.7 |
Discussion

The results of the present study revealed that anteversion (internal rotation of femur) affected both common anterior cruciate ligament and meniscal impairments, and tibial rotation only significantly affected meniscal injury not anterior cruciate ligament. Also, increase of knee varus angle did not have any effect on meniscal and anterior cruciate ligament injuries. However, increase of anteversion angle can be due to possible risk factors of common knee injuries. Therefore, based on the results of this study, it is necessary to identify the athletes with abovementioned disorders. Various studies have studied the effect of anatomical disorders on sports injuries and analyzed anatomical disorders as one of the predisposing risk factors.

Stanitski et al. in his study titled “anterior cruciate ligament tear in individuals with skeletal disorders” reported problems of skeletal disorders of lower organ as one of the most important causes of acute anterior cruciate ligament tear (about 6%). Scoville and Williams (2003) compared the injury rates and identified the risk factors associated with contact injury of anterior cruciate ligament between males and females on 859 people in a four-year period using a questionnaire. They reported that only 24 (16 male and 8 female) had noncontact injuries and other injuries were contact injuries. They also reported rotation of lower organ and dysfunctions of knee joint performance in them4. Alison Chang (2011) conducted a study on 90 people, divided into two groups, 46 with ACL injury and 44 healthy people. They analyzed the relationship between acute tear and inter condylar urethral stricture which is an anatomical disorder of the lower end of femur. Analysis of MRI for the knees of people with anterior cruciate ligament tear as well as the healthy people indicated a significant difference (p=0.001), and inter condylar urethral stricture was significantly associated with acute tear. chang (2011) investigated the rotation disorders of the lower organ on 50 people. The average time of anteversion for the right and left feet were 6.15° and 8.15°, respectively and the average time for tibial rotation in the right and left feet were 1.29° and 9.30°, respectively. Further, no significant difference was observed between rotation of different parts of right and left feet21.

Sung et al (2008) examined 53 people, 24 with tibial rotation and 29 healthy people. They showed more knee joint slipping and laxity in people with extra rotation of tibia than people with normal rotation. Thus, they suggested the extra rotation of tibia as an influential factor in knee injuries17. Braten et al. evaluated the anteversion angle in 50 male and female adults. They reported a significantly higher anteversion level in female than in male26.

Schmitz et al. (2008) investigated the internal and external rotations and increase of varus and valgus angles in men and women and reported more slipping and laxity of joints in people with higher varus and valgus angles. They also reported more possible injury for retaining and supporting ligaments of knee joint in people with internal and external rotation of knee than in other people. Further, they showed higher rotations and higher varus and valgus angles for females than in males7.

Conclusion

Based on the obtained results in this study regarding the significant effect of anteversion on common knee injuries as well as the effect of femur rotation on meniscal injury, it is recommended that incorrect sitting and sleeping habits of children be corrected. It is also suggested that exercises that cause an increase in disorders in athletes be avoided. Further studies are suggested to investigate the male and female athletes in other sports and compare them with non-athletes to analyze the effect of other disorders on common injuries in wrestlers and to examine the effect of disorders in terms of time duration after injury.

Acknowledgement: The authors would like to thank all co-workers of deputy of research and technology of Kermanshah University of medical sciences and Razi University of Sciences also the authors appreciate the participants who patiently participated in our study.

Conflicting Interest: The authors declare that they
have no competing interests

Consent for publication: We obtained informed written consent from all participants

Availability of data and material: The data used to support the findings of this study are available from the corresponding author upon request

Ethical Clearance: This trial was conducted in accordance with the Declaration of Helsinki and it was approved by the Razi University of Kermanshah, Vice-Chancellor for Technology Research with grant No. and the Ethics Committee of Razi University of Kermanshah endorsed the study protocol with reference number. Before the study, the objectives and methods were explained to all of the participants, and they were assured that their responses would remain confidential. Written informed consent was obtained from all participants before the study.

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Risk Factors of Pre-Eclampsia of Women Birth From History of Eating Behavior During Pregnancy

Teta Puji Rahayu¹, Suparji¹, Nana Usnawati¹

¹Lecture, Jurusan Kebidanan, Politeknik Kesehatan Kementerian Kesehatan Surabaya, Indonesia

Abstract

Introduction: Pre-eclampsia is the second leading cause of maternal death, affecting 3% to 8% of pregnant women worldwide. Pregnant women preeclampsia in Magetan Regency in 2017 were 189 people, while in 2018 there were 270 people. The purpose of this study was to analyze the risk factors for preeclampsia from eating behavior factors in pregnant women. Methods: A case control study design with a retrospective approach. The research was conducted in the working area of the Community Health Center, Panekan District, Magetan Regency. The population of this study was data of all mothers giving birth in 2018 of 210 mothers. The sample size of the control group was 27 mothers giving birth without preeclampsia. The ratio between the case group and the control group was 1: 1, so the number of samples was 54 people. The sampling technique was simple random sampling. The independent variable was eating behavior and the dependent variable was the incidence of preeclampsia. Data collection tools in the form of questionnaires and study of medical record data documentation. The data analysis used was descriptive and logistic regression analysis. The error rate is set at α <0.05. Results: The results of the study using logistic regression of eating behavior obtained p value = 0.00 (p <0.05) and OR (Exp B) 35.714. Conclusion: The conclusion of the research results is that eating behavior affects the incidence of preeclampsia. Pregnant women who have unhealthy eating habits have a 35x greater risk of developing preeclampsia than pregnant women with healthy eating habits.

Keywords: Pre-eclampsia, eating behavior, women birth, pregnancy

Introduction

Pre-eclampsia is a condition in which an increase in blood pressure is accompanied by signs of hypertension and an increase in urinary protein that usually occurs in TM III pregnancy. If you don’t get early treatment, preeclampsia can progress to eclampsia¹. Preeclampsia (PE) is a contributor to maternal and infant mortality and morbidity. Preeclampsia is a health problem that often occurs in pregnancy.

Corresponding Author:
Teta Puji Rahayu
Lecture, Jurusan Kebidanan, Politeknik Kesehatan Kementerian Kesehatan Surabaya, Indonesia, E-mail: tetapujirahayu@gmail.com, Telp.(031)5027058,Fax. (031) 5028141

Preeclampsia is the second largest cause of maternal death, affecting 3% to 8% of pregnant women worldwide. Nearly 18% of maternal deaths due to preeclampsia mostly occur in developing countries². In Indonesia, hypertension is the first cause of high maternal mortality. In 2015, the MMR was 305 / 100,000 KH. In 2024 it is targeted that the Maternal Mortality Rate (MMR) will decrease to 232 / 100,000 KH. The percentage of known causes of maternal death was 33.07% hypertension, 27.03% bleeding, 15.7% non-obstetric complications, 12.04% obstetric complications, 6.06% infections and 4.81% others SRS LITBANG³. The prevalence of preeclampsia in East Java in 2018 was 31% or as many as 163 people. This tends to increase compared to the incidence of preeclampsia in 2017 of 28.92% or as many as 153 people. Meanwhile, MMR in East Java in 2018 experienced a decline from the previous year, from 529
people to 522 people\textsuperscript{4,5}. Preeclampsia is the second contributor to maternal mortality. The percentage of causes of maternal death in East Java in 2018 is known to other causes, namely 32\% or 170 people. Pre-Eclampsia / Eclampsia, which is 31\% or as many as 163 people and bleeding, 23\% or as many as 119 people, heart 10\% or 51 people\textsuperscript{5}. While the smallest cause was infection by 4\% or as many as 19 people SRS LITBANG\textsuperscript{3}. Based on a preliminary study of LB3KIA data in Magetan Regency in 2017, the prevalence of preeclampsia in pregnant women was 189 people. Meanwhile, in 2018 there were 270 people. This shows that there has been an increase in the incidence of preeclampsia from 2017 to 2018, namely as many as 81 people. The highest incidence was in the area of the Panekan Community Health Center as many as 27 people. Preeclampsia is the second cause of death after bleeding, as many as 2 people died of preeclampsia or 15.38\% per 100,000 KH\textsuperscript{4,5,6}. AKI is an indicator of the success of maternal health efforts. This indicator is not only able to assess maternal health programs, but also able to assess the degree of public health, because of its sensitivity to improving health services, both in terms of accessibility and quality\textsuperscript{5}. The causes of preeclampsia are maternal health status, reproductive status, nutritional status, access to health services and health behavior\textsuperscript{7}. Health behavior is a response to stimuli. Health behavior consists of 3 aspects, one of which is nutritional behavior / eating behavior\textsuperscript{8}. According to Rahayu L.D and Suryandari A.E 2014 The habit of consuming foods high in fat and high in salt can cause pre-eclampsia to occur frequently in pregnant women so that adequate nutritional habits can prevent mothers from pre-eclampsia disorders\textsuperscript{8}. Research conducted by Rahayu L.D and Suryandari A.E 2014 with the title “Relationship between junk food consumption habits and the incidence of preeclampsia in pregnant women at Prof. Dr. Margono Soekarjo Hospital” concluded that the habit of consuming junk food can cause pre-eclampsia\textsuperscript{3,8}. There are 2 impacts that can be experienced by pregnant women with preeclampsia, namely the impact on the fetus and for the mother herself. For the fetus it can cause growth disorders (IUGR), fetal death in the womb (IUFD), LBW. Meanwhile, the impact on the mother is that the mother becomes unconscious (coma) until she dies\textsuperscript{1,2,3}.

The magnitude of the influence of preeclampsia on high maternal mortality and the risky impact of preeclampsia on maternal health, it is necessary to make efforts to prevent and handle preeclampsia cases. The government’s strategy in reducing the Maternal Mortality Rate in Indonesia based on RAKERNAS 2019 is through an intervention strategy consisting of four main things, namely increasing access to health services, improving the quality of health services, empowering communities and strengthening governance. The strategy includes quality ANC services according to the 10 T standard, pregnant women and giving birth in health facilities, quality ANC and PNC, early detection, an integrated referral system and a hospital for mothers and babies\textsuperscript{4,5,9}.

**Research purposes**

The purpose of this study was to analyze the risk factors for reaction of eating behavior factors in pregnant women.

**Materials and Methods**

The research design used was case control with a retrospective approach\textsuperscript{10}. The research was conducted in the area of the Community Health Center, Panekan District, Magetan Regency, East Java, Indonesia. The population of this study is data of all mothers giving birth at the Community Health Center, Panekan District, Magetan Regency, East Java, Indonesia. In 2018 there were 210 mothers. The sample of the case group was all women who gave birth with a history of preeclampsia and in the control group were some mothers who had no history of preeclampsia\textsuperscript{10,11}. The comparison between the case group and the control group was 1: 1, so the sample size was 54 people. The sampling technique used was simple random sampling technique\textsuperscript{12}. The independent variable in this study was eating behavior and the dependent variable was the incidence of preeclampsia. Data collection tools with questionnaires and study medical record data documentation. The data analysis was descriptive and analytical logistic regression.
error rate was set at $\alpha < 0.05$.\textsuperscript{11,12}

**Result**

The results of the study on a history of eating behavior during pregnancy with the incidence of pre-eclampsia in childbirth are

Data Characteristics Respondents

| Table 1. Case Group Data by Age, Parity, Education, and Occupation |
|------------------|------------------|------------------|
| Characteristics  | Frequency        | Percentage       |
| **Age**          |                  |                  |
| 20-35 years      | 20               | 74               |
| 35 years old     | 7                | 26               |
| **Total**        | 27               | 100              |
| **Parity**       |                  |                  |
| Primipara        | 7                | 26               |
| Multipara        | 20               | 74               |
| **Total**        | 27               | 100              |
| **Education**    |                  |                  |
| Primary school   | 10               | 37               |
| Junior High      | 11               | 40.8             |
| Highschool equivalent | 1           | 3.7               |
| **Total**        | 27               | 100              |
| **Profession**   |                  |                  |
| IRT              | 8                | 29.6             |
| Farmer           | 4                | 14.9             |
| Entrepreneur     | 3                | 11.1             |
| Private          | 1                | 3.7               |
| **Total**        | 27               | 100              |

Based on the research data which can be seen in table 1, an overview of the characteristics of mothers who experience preeclampsia based on age groups shows that most respondents who experience preeclampsia, based on the 20-35 year age group, are as many as 20 people (74%), from the level of parity Multipara more 20 people (74%), based on the education level of the senior high school group as many as 11 people (40.8%) and based on the type of work, the highest incidence of pre-eclampsia in the IRT group was 11 people (40.8%).
Table 2. Data for the Control Group by Age, Parity, Education, and Occupation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-35 years</td>
<td>26</td>
<td>96.3%</td>
</tr>
<tr>
<td>35 years old</td>
<td>1</td>
<td>3.7%</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primipara</td>
<td>8</td>
<td>29.6%</td>
</tr>
<tr>
<td>Multipara</td>
<td>19</td>
<td>70.4%</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>3</td>
<td>11.1%</td>
</tr>
<tr>
<td>Junior High</td>
<td>7</td>
<td>25.9%</td>
</tr>
<tr>
<td>Highschool equivalent</td>
<td>17</td>
<td>63%</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Profession</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRT</td>
<td>5</td>
<td>18.5%</td>
</tr>
<tr>
<td>Farmer</td>
<td>11</td>
<td>40.7%</td>
</tr>
<tr>
<td>entrepreneur</td>
<td>2</td>
<td>7.5%</td>
</tr>
<tr>
<td>Private</td>
<td>27</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>100%</td>
</tr>
</tbody>
</table>

The research data on the incidence of pre-eclampsia in pregnant women in the control group in table 2 shows that almost all of the mothers who did not experience preeclampsia in the 20-35 year group were 26 people (96.3%), the Mutlipara parity level was 19 people (70.4%), the level of education. 17 people (63%) and the type of work as self-employed as many as 11 people (40.7%).

Data History of Eating Behavior and Preeclampsia in Case and Control Groups

Table 3. Cross Distribution between Eating Behavior and Preeclampsia

<table>
<thead>
<tr>
<th>Eating Behavior</th>
<th>Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
</tr>
<tr>
<td>Not healthy</td>
<td>20(90.9%)</td>
<td>2(9.1%)</td>
</tr>
<tr>
<td>Healthy</td>
<td>7(21.8%)</td>
<td>25(78.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>27(100%)</td>
<td>27(100%)</td>
</tr>
</tbody>
</table>

The results in table 3 show that mothers who experience preeclampsia from exposure to unhealthy eating behavior are as much as 90.9%. And mothers giving birth are not preeclampsia but are exposed to unhealthy eating behavior as much as 9.1%.

Results of the analysis of the history of eating behavior and the incidence of pre-eclampsia in childbirth mothers
Table 4. Summary of Logistic Regression Test Results

<table>
<thead>
<tr>
<th>Variable</th>
<th>p value</th>
<th>Exp (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eating Behavior*Preeclampsia</td>
<td>0.000</td>
<td>35.714</td>
</tr>
</tbody>
</table>

The regression test results obtained p value = 0.00, (p < 0.05). These results indicate that eating behavior during pregnancy affects the incidence of preeclampsia in mothers during pregnancy and childbirth. The result of the odds ratio (Exp B) is 35.714, this shows that pregnant women who have unhealthy eating behavior have a 35x greater risk of experiencing preeclampsia compared to pregnant women who have healthy eating behavior. From the results of this study we can also conclude that 35.7% of preeclampsia is influenced by eating behavior, and as much as 64.3% is caused by other factors.

Discussion

The results showed that the incidence of preeclampsia in women who gave birth, the age group> 35 years showed a high number of cases compared to the age of mothers <35 years. The results of the research by Saifuddin2014, explained that the highest incidence of preeclampsia occurs in adolescence or early 20 years of age, but the prevalence increases in women over 35 years. Still according to Saifudin 2014 Age too young or too old is a factor in the occurrence of preeclampsia. Age less than 20 years is very risky because the reproductive organs are immature to get pregnant, and> 35 years of age the function of the reproductive organs also begins to decline so that they cannot work optimally. Mothers who are less than 20 years old and more than 35 years old have three times the potential to develop preeclampsia compared to pregnant women aged 20-35 years. But in fact, in the results of this study, the age of the mother who is not at risk also has a lot to contribute to the incidence of preeclampsia. Another study that explains the pre-eclampsia of pregnant and childbirth women is according to Transyah C.H 2018 in the Obstetrics room of Dr.M General Hospital. Djamil Padang 2016 shows that the age of pregnant women is mostly not at risk for preeclampsia and a small proportion of the age of pregnant women who are at risk for pre-eclampsia. The results of the study based on maternal parity showed that there were more mothers with a history of multipara who had pre-eclampsia during pregnancy and childbirth than primipara mothers. The results of research conducted by Pandiangan J.M and Kusnanto H (2017) stated that 25.23% of preeclampsia occurred in primipara, while the multipara group was 35.51%. Research shows that parity of mothers who experience preeclampsia occurs more in multipara than primipara. This is different from the opinion of Saifuddin 2014 which states that primipara is also a risk factor for preeclampsia both during pregnancy and during childbirth. Based on the research results, it can be concluded that preeclampsia can occur in Primipara and Multipara mothers. Primipara and Multipara mothers are both at risk of developing preeclampsia. Therefore, ANC services for mothers during pregnancy are very important so that they can detect any signs of preeclampsia.

The Effect of a History of Eating Behavior during Pregnancy on Preeclampsia

Based on the results of the regression test, it was found that pregnant women who have unhealthy eating behavior have a 35x greater risk of experiencing preeclampsia than pregnant women who have healthy eating behaviors. Research results that describe the same results as this study are the results of research by Retnawati and Suryanti 2017 that there is a relationship between the behavior of nutrient intake and the incidence of pre-eclampsia in pregnant women with a value of p = 0.000 (p <0.05). Rahayu and Suryandari’s research 2014 states that the habit of consuming foods high in fat
and high in salt can lead to pre-eclampsia. Food menu imbalances will form oxidative stress (Hyman Mark, 2006). Oxidative stress is said to be the result of an increase in free fatty acids and inflammation, this may be caused by low consumption of antioxidants or high consumption of foods rich in carbohydrates and fats. A diet like this is associated with an increase in free radicals in the body and then will cause pre-eclampsia. So that a pregnant woman will be better off if she consumes foods that are healthy and contain balanced nutrition. Another study conducted by Rahayu and Suryandari 2014 found that there was a relationship between junk food consumption habits and the incidence of pre-eclampsia in pregnant women (p-value = 0.012, 95% CI OR = 4.375). This shows that the habit of consuming junk food can lead to pre-eclampsia. According to Almadsier 2004 in Retnawati and Suryanti 2017 pregnant women need to eat foods that have complete nutrition, this is important, especially at 20 weeks of gestation, at this time pregnant women should not consume nutrients that are high in sodium and low in protein because they can cause pregnancy problems, such as pre-eclampsia. The impact of disruption of nursing patterns during pregnancy can result in an increasing number of pre-eclampsia which is characterized by symptoms of high blood pressure, excess protein levels in urine, leg edema, blurred vision, shortness of breath and decreased consciousness and seizures.

**Conclusion**

The conclusion of this study is the results of identification of the characteristics of pregnant women which show a description of the risk factors for eclampsia during childbirth, namely paretas, type of work and age group factors. The multipara group showed a greater incidence rate of preeclampsia than the primipara group. Based on the age group, the high incidence of preeclampsia during pregnancy and childbirth is the unhealthy age group, namely the age group <20 years and the age group> 35 years. The problem of eating behavior history during pregnancy in this study showed that a group of women with a history of unhealthy eating behavior during pregnancy contributed to the incidence of pre-eclampsia in childbirth. Based on the results of research on the history of eating behavior, that eating behavior during pregnancy affects the health status of the mother which will increase the morbidity rate of pregnant women, one of which is preeclampsia. Eating behavior during pregnancy affects the incidence of preeclampsia. Pregnant women with unhealthy eating habits have a 35x risk of experiencing preeclampsia compared to pregnant women with healthy eating behaviors.

**Conflict of Interest:** None

**Source of Support:** Self

Ethical Clearance: Ethical license is an approval from the Health Polytechnic Research Ethics Commission of the Ministry of Health Surabaya, this research does not use human and animal experiment objects, it only carries out surveys.

**References**


Lp-PLA2 Selective Inhibitors (Darapladib) Effect in Lowering Serum and Aortic Lysophosphatidylcholine (LysoPC), NF-κB and Lp-PLA2 Levels on Dyslipidemic Rats Model

Teuku Heriansyah1, Indah Nur Chomsy2, Anwar Santoso3, Muhammad Ridwan1, Fitria Nugraha Aini4, Titin Andri Wihastuti5

1Department of Cardiology and Vascular Medicine, Medical Faculty, Universitas Syiah Kuala, Banda Aceh, Indonesia, 2Doctoral Program of Medical Science, Faculty of Medicine, University of Brawijaya, Malang, 3Department of Cardiology and Vascular Medicine, Medical Faculty, Universitas Indonesia, Jakarta, Indonesia, 4Faculty of Medicine, University of Islam Malang, Malang, Indonesia, 5Department Basic Nursing Science, Faculty of Medicine University of Brawijaya; Malang, Indonesia.

Abstract

Background: Atherosclerosis is a chronic inflammatory disease, and is associated with upregulation of Lipoprotein-associated phospholipase A2 (Lp-PLA2), the enzyme that hydrolyzes phosphatidylcholine, producing lysophosphatidylcholine (LysoPC) and free fatty acids. LysoPC is a lipid mediator with known pro-inflammatory and pro-atherogenic properties, and is believed to be a critical factor in cardiovascular diseases.

Methods: Thirty male Rattus norvegicus divided into three groups, those are Normal group: fed with rats food contained 3.44 kcal/g total calorie energy, Dyslipidemia group: fed with high-fat diet (HFD) contained 5.30 kcal/g total calorie energy and Dyslipidemia + Darapladib Group: fed with HFD and darapladib orally 20 mg/kg BW once a day. Each group consists of two serials treatment time: 8 weeks and 16 weeks. Expression of NF-κB, Lp-PLA2, and level of LysoPC aorta and serum was the variable that measured.

Results: Darapladib has decreased the expression of Nuclear Factor Kappa B (NF-κB) (p = 0.003) and Lp-PLA2 (p = 0.022). Darapladib also decreased the level of LysoPC Serum on 8 weeks of serial treatment time (p = 0.001) but increased the level of LysoPC Serum on 16 weeks of serial treatment time (p=0.040). Pearson correlation test showed that serum and aortic tissue LysoPC level correlation was strong (r = -0.584) for 8 weeks of serial time and less strong (r = -0.284) for 16 weeks serial time.

Conclusion: Darapladib decreased expression of NF-κB, Lp-PLA2, and level of LysoPC serum on 8 weeks of serial treatment time but increased on 16 weeks of serial treatment time. Pearson correlation test showed that serum and aortic tissue LysoPC level correlation was an inverse correlation.

Keywords: NF-κB, Dyslipidemia, LysoPC, Lp-PLA2

Introduction

Enzyme Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a marker of arterial plaquedestabilization and can cause plaque rupture, myocardial ischemia, and the infarction(1-3). Enzyme Lp-PLA2 was also a marker of the lial dys function, which is the initial phase of atherosclerosis (4,5). Low-density lipoprotein (LDL) will oxidizing and become oxLDL(6,7). Receptor Lp-PLA2 was found in oxLDL. Lp-PLA2 will bind to its receptor and hydrolyze group of acyl short on position phospholipids sn-2 of oxLDL formed two mediators lipid bioactive, namely fatty acid oxidation (oxFA) lysophosphatidylcholine (LysoPC). LysoPC is a lipid mediator with known as proinflammatory and pro-atherogenic agent. It is believed to play an important role in atherosclerosis underlying cardiovascular diseases and several pathological conditions are associated with elevated LysoPC levels in the circulation(8).
The inhibitor enzyme Lp-PLA2 agent is considered capable of preventing atherosclerotic plaque formation\(^9\). There is more presence of active inflammatory cells and increased concentrations of Lp-PLA2 enzyme in unstable plaque, and that could be an important risk factor in the formation of atherosclerotic plaques and also in the process of the rupture\(^10\).

NF-κB has an important role in the early stages of atherogenesis. NF-κB modified the initial LDL lipids and the formation of inflammatory mediators, including secretory phospholipase A2, 5-lipoxygenase, 12-lipoxygenase, and COX-2. NF-κB also influences inflammation that occurs in atherosclerosis through the cytokines they produce, such as TNF, IL-1β, IL-6, IL-10, IL-12, and interferon-γ\(^11\).

Several studies prove that Lp-PLA2 is antiatherogenic and proatherogenic. In addition, it has developed preventive treatment of atherosclerosis through the Lp-PLA2 inhibitor. Based on the description above, it is very important to research to prove the provision of Darapladib as Lp-PLA2 inhibitor agents through the expression of Lp-PLA2, levels LysoPC tissue and serum, and the levels of the transcription factor NF-κB on dyslipidemia animal model divided in two serial times, that was 8 weeks and 16 weeks. The sample was sliced and stained in the Pathological Anatomy Laboratory, Faculty of Medicine, University of Brawijaya, Malang, Indonesia. Biochemical Parameters were measured at the Central Laboratory of Biological Sciences, Brawijaya University, Malang, Indonesia.

### Material and methods

#### Study Group

Thirty male Sprague-Dawley strain of *Rattus norvegicus*, two months of age, and weight around 150-200 g were obtained from Bogor Agricultural University, Bogor, Indonesia, and bred at Bioscience Central Laboratory of Brawijaya University, Malang, Indonesia. These rats were divided into three main groups. Those are Normal group: fed with rats food contained 3.44 kcal/g total calorie energy (67% carbohydrate, 21% protein, and 12% fat), Dyslipidemia group: fed with high-fat diet contained 5.30 kcal/g total calorie energy (58% fat, 17% carbohydrate, and 25% protein) and Dyslipidemia + Darapladib Group: fed with high-fat diet and given darapladib (purchased from GlaxoSmithKline) orally 20 mg/kg BW once a day. Each group consists of 2 serials treatment time: 8 weeks and 16 weeks. The sample was sliced and stained in the Pathological Anatomy Laboratory, Faculty of Medicine, University of Brawijaya, Malang, Indonesia. Biochemical Parameters were measured at the Central Laboratory of Biological Sciences, Brawijaya University, Malang, Indonesia.

#### Animal ethical approval

Ethical approval was obtained for the experimental animal processes in this study from the Animal Veterinary and had Committee Brawijaya University License Number 400/EC/KEPK/10/2016.

#### Lipid Profile Measurement

Measurement of total cholesterol concentration, HDL, and LDL / VLDL was done by the calorimetric method by using EnzyChrom AF HDL and LDL/VLDL Assay Kit (cat number: E2HL-100, Gentaur, Belgium)\(^12,13\).

#### Biochemical Test

The samples used were serum and aorta homogenates. Lp-PLA2 and NF-κB level were measured by immunofluorescence of aortic tissues that were previously fixated with PHEMO buffer and
were processed by immunofluorescence labeling with antirat antibody Lp-PLA2 using rhodamin secondary antibody and anti-rat antibody NF-κB using fluorescein isothiocyanate (FITC) secondary antibody (BIOS Inc., Boston, MA, USA). These parameters were observed with confocal laser scanning microscopy (Olympus Corporation, Tokyo, Japan) and were quantitatively analyzed using Olympus Fluov View software (version 1.7A; Olympus Corporation). LysoPC in serum level and aortic homogenate was measured with 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (16:0) (Avanti Polar Lipids, Inc., 700 Industrial Park Drive, Alabaster, Alabama, USA).

**Statistical Analysis**

Statistical methods used in this study were Kolmogorov-Smirnov for normality test and Levene for homogeneity test. Whereas, for the significance test, we used One-way ANOVA to determine the effects of Lp-PLA2, LysoPC, and NF-κB expression in Sprague Dawley rats with dyslipidemia condition and treated by Darapladib. This analysis was continued by the *post hoc* test using the LSD method to detect the differences of parameters in each group. This study also used a correlation test to define the correlation of LysoPC aorta and Serum. Statistical analysis was performed with SPSS software version 20 (IBM Corporation, Armonk, NY, USA).

**Results**

**Table 1. Measurement and analysis of Cholesterol Total, HDL, LDL/VLDL, LysoPC Aorta, LysoPC Serum, NF-κB Aorta and Lp-PLA2 in 8 weeks serial time**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol Total (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL / VLDL (mg/dl)</th>
<th>LysoPC Aorta (ng/g)</th>
<th>LysoPC Serum (ng/mL)</th>
<th>NF-κB Aorta (aU)</th>
<th>Lp-PLA2 aorta (aU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Group</td>
<td>72.80±6.050</td>
<td>34.74±3.310</td>
<td>19.83±3.070</td>
<td>488159±47.02</td>
<td>64445±247679</td>
<td>1140±1239.56</td>
<td>761±151</td>
</tr>
<tr>
<td>Dyslipidemia Group</td>
<td>115.57±6.50</td>
<td>8.36±2.070</td>
<td>32.42±3.08</td>
<td>102373±101</td>
<td>1316±75350</td>
<td>1389±151</td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia and Darapladib Group</td>
<td>81.37±3.980</td>
<td>20.02±0.360</td>
<td>551355±99824</td>
<td>1104.65±54.2</td>
<td>27.857</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data were presented as mean ± standard deviation (range) values. All the values of the parameters have been corrected into the International Standard of Mathematics (decimals).

*p <0.05 indicates a statistically significant difference.*

ANOVA = Analysis of Variance; HDL = High Density of Lipoprotein; LDL = Low Density of Lipoprotein; VLDL = Very Low Density of Lipoprotein; LysoPC = lysophosphatidylcholine; NF-κB = Nuclear Factor Kappa Beta; Lp-PLA2 = Lipoprotein-associated phospholipase A2; PAI-1 = Plasminogen Activating Inhibitor–1; aU = arbitrary Unit
Table 2. Measurement and analysis of Cholesterol Total, HDL, LDL/VLDL, LysoPC Aorta, LysoPC Serum, NF-κB Aorta and Lp-PLA2 in 16 weeks serial time

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol Total (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL/VLDL (mg/dl)</th>
<th>LysoPC Aorta (ng/g)</th>
<th>LysoPC Serum (ng/mL)</th>
<th>NF-κB Aorta (aU)</th>
<th>Lp-PLA2 Aorta (aU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Group</td>
<td>56.560 ±5.430</td>
<td>35.770 ±1.680</td>
<td>19.240 ±3.670</td>
<td>59838.6 ±3038</td>
<td>107737 ± 15452</td>
<td>850.4 ± 56</td>
<td>823.3394 ± 56</td>
</tr>
<tr>
<td>Dyslipidemia Group</td>
<td>117.770 ±117.110</td>
<td>18.150 ±0.890</td>
<td>102.140 ±15.650</td>
<td>351361 ± 41338</td>
<td>110104.2 ± 4867</td>
<td>1322.8 ± 68</td>
<td>879.363 ± 65</td>
</tr>
<tr>
<td>Dyslipidemia and</td>
<td>101.960 ±7.980</td>
<td>21.400 ±5.060</td>
<td>56.510 ±11.330</td>
<td>82042.4 ±6746</td>
<td>168838.2 ±10930</td>
<td>949.4 ± 52</td>
<td>1129.579 ± 1325</td>
</tr>
<tr>
<td>Darapladib Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>82.285</td>
</tr>
</tbody>
</table>

| P value | 0.043 | 0.000 | 0.001 | 0.010 | 0.040 | 0.000 | not significant |

Data were presented as mean ± standard deviation (range) values. All the values of the parameters have been corrected into the International Standard of Mathematics (decimals).

*p <0.05 indicates a statistically significant difference.

ANOVA = Analysis of Variance; HDL = High Density of Lipoprotein; LDL = Low Density of Lipoprotein; VLDL = Very Low Density of Lipoprotein; LysoPC = lysophosphatidylcholine; NF-κB = Nuclear Factor Kappa Beta; Lp-PLA2 = Lipoprotein-associated phospholipase A2; PAI-1 = Plasminogen Activating Inhibitor–1; aU = arbitrary Unit.

Table 1 and Table 2 showed that it is a significant difference in lipid profile (cholesterol total, HDL, and LDL) between normal, Dyslipidemia, and Dyslipidemia + Darapladib Group on both serial treatment time (p = 0.048). This table was also showed a significant increase in NF-κB and Lp-PLA2 expression in dyslipidemia group and a significant decrease in NF-κB and Lp-PLA2 expression in dyslipidemia + Darapladib group on both serial treatment time (p < 0.05).

Figure 1. Linear regression of LysoPC serum and LysoPC Aorta Level on both 8 weeks (A) and 16 weeks (B) serial treatment time.
Serum and aortic tissue LysoPC level correlation was an inverse correlation (Figure 1). The strength of serum and aortic tissue LysoPC level correlation was strong \((r = -0.651, r^2 = 0.424)\) for 8 weeks serial time and \((r = -0.83, r^2 = -0.689)\) for 16 weeks serial time.

There was a significant decreasing level of LysoPC Aorta \((p > 0.05)\) but an increasing level of LysoPC Serum \((p = 0.01)\) for 8 weeks of serial treatment time in Dyslipidemia Group. LysoPC Aorta and Serum levels were changed inversely at Dyslipidemia + Darapladib Group \((p < 0.05)\). However, for 16 weeks of serial treatment time, this study showed a different result than 8 weeks of serial treatment time. In the dyslipidemia group, there was a significantly increased level of both LysoPC Aorta and LysoPC Serum \((p < 0.05)\). On Dyslipidemia + Darapladib Group, LysoPC Aorta and Serum level were also changed inversely. Expression of NF-κB and Lp-PLA2, qualitatively observed using a confocal laser scanning microscope, can be seen in Figure 2.

![Figure 2](image-url)

**Figure 2.** Qualitative observation of NF-κB and Lp-PLA2 expression on aortic tissue with immunofluorescence staining (yellow arrows): (a) NF-κB expression; (b) Lp-PLA2 expression

Discussion

Lipid Fraction on High-Fat Diet Group

Lipid abnormalities fraction can be termed as dyslipidemia conditions. In the intestinal mucosal cells, free cholesterol is converted to cholesterol ester. Cholesterol ester joined to form VLDL triglycerides. VLDL then leave the heart and into the circulatory system. In the circulatory system, VLDL is converted to IDL by LPL and then into LDL. IDL and LDL are formed in the blood vessels can be entered into the endothelium of blood vessels or directly back to the liver. Ox-LDL phagocyted by macrophage cells. In macrophages, cholesterol esters hydrolyzed in to free cholesterol. Free cholesterol is brought to the surface of the cell membranes of macrophages and subsequently captured by HDL. Free cholesterol is converted into cholesterol esters and carried to the liver by HDL. This process will lead to the condition of Dyslipidemia.
There is a close relationship between the Lp-PLA2 enzyme and LDL, as well as a comparable and inverse relationship with HDL\(^{10}\). However, this study shows that the combination of Lp-PLA2 inhibitors and lowering hypercholesterolemia drugs gives better results to reduce dyslipidemia. This is because Lp-PLA2 inhibitors provide additional substances that can reduce LDL\(^{10}\).

Lp-PLA2 has a role in the pathogenesis of atherosclerosis and increases the risk of cardiovascular disease events\(^{16}\). Lp-PLA2 has been used as one of the early markers of atherosclerosis and as a therapeutic target\(^{17,18}\). Dyslipidemia conditions increase concentrations of LDL and VLDL in the plasma, thereby increasing the levels of Ox-LDL; fatty acids become oxidized fatty acids and will trigger an increase in free radicals. Increased Ox-LDL and oxidized fatty acids will stimulate an increase in Lp-PLA2 activity to convert it into LysoPC\(^{19,20}\). Receptor Lp-PLA2 was found in oxLDL, and Lp-PLA2 will bind to its receptor and hydrolyze the group of acyl short on position phospholipid sn-2 of oxLDL, formed two mediators lipid bioactive namely lysophosphatidylcholine (LysoPC) and fatty acid oxidation (Oxidized Fatty Acid/oxFA) which had an important role in the process of atherosclerosis\(^{21}\). Those products have proinflammatory effects that initiate and increase the progression of atheroma.

**Correlation Lysophosphatidylcholine (LysoPC) Level on aorta tissue and serum**

Lysophosphatidylcholine (LysoPC) is one of the Lp-PLA2 enzyme products which has the chemical formula \([(2R) -2-hydroxy-3 -[(9z,12z) -octadeca-9,12-dienoyl]oxypropyl]2- (trimethylazaniumyl) ethyl phosphate\ (National Center for Biotechnology Information. 2015). This study showed that in Dyslipidemia Group, there was a significant decreasing level of LysoPC Aorta (p > 0.05) but an increasing level of LysoPC Serum (p = 0.01) for 8 weeks of serial treatment time. And LysoPC Aorta and Serum level were changed inversely at Dyslipidemia + Darapladib Group (p < 0.05). However, for 16 weeks of serial treatment time, this study showed different results than 8 weeks of serial treatment time. In dyslipidemia group, there was a significant increasing level both of LysoPC Aorta and LysoPC Serum (p < 0.05). On Dyslipidemia + Darapladib Group, LysoPC Aorta and Serum level were also changed inversely.

**Serum and aortic tissue LysoPC level correlation** was an inverse correlation. The strength of Serum and aortic tissue LysoPC level correlation was strong (r = -0.651, r\(^2\) = 0.424) for 8 weeks serial time and (r = -0.83, r\(^2\) = -0.689) for 16 weeks serial time. That means the increase of the LysoPC serum level will decreasing the levels of LysoPC Aorta, and decreasing the LysoPC serum level will increase the levels of LysoPC Aorta. This correlation and linear regression pattern were shown in Figure 1A (for 8 weeks of serial treatment time) and 1B (for 16 weeks of serial treatment time). Lysophosphatidylcholine (LysoPC) is the main product of Lp-PLA2 activity, which can induce the synthesis of proteoglycans and play an important role in the thickening of the intimal layer of the endothelium\(^{22,23}\). In several studies, this LysoPC can stimulate proliferation apoptosis in endothelial and smooth muscle cells. LysoPC has also been instrumental in the recruitment of macrophages and differentiation of T cells into the arterial wall\(^{23,24}\). LysoPC is a chemotactic factor and led to the withdrawal of macrophages to the endothelium and induce the expression of adhesion molecules in endothelial cells mononuclear resulted in the initiation of lesions increased\(^{25}\). LysoPC performs its role in the pro-atherogenic process through this mechanism\(^{26,27}\).

**Nuclear Faktor Kappa Beta (NF-kB) expression and Lp-PLA2 on aorta tissue**

Table 1 and Table 2 were showed a significant increase (p < 0.05) in NF-kB expression in Dyslipidemia Group and a significant decrease in NF-kB expression in Dyslipidemia + Darapladib group on both 8 weeks and 16 weeks of serial treatment time. Excessive expression of NF-kB aorta is on the tissue will be very influential in the pathogenesis of atherosclerosis\(^{28}\). In the early
stages of atherogenesis, NF-kB plays a role in the modification of the initial LDL lipids and the formation of inflammatory mediators, including secretory phospholipase A2, 5-lipoxygenase, 12-lipoxygenase, and COX-2. NF-kB also plays a role in the expression of monocyte chemoattractant protein-1 (MCP-1), which plays an important role in the migration of macrophages into the extracellular matrix of the tunica intima.

Active NF-kB is also able to regulate some adhesion molecules, including P-selectin, E-selection, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1)\(^ {11,29}\). After arriving in the extracellular matrix of the tunica intima, monocytes will soon turn into macrophages. This change involves a variety of macrophage colony-stimulating factor (CSF, M-CSF, or CSF-1). These factors are known to be regulated by NF-kB. This transcription factor also has a function in the regulation of matrix metalloproteinase-9 (MMP-9) were able to degrade the extracellular matrix that allows the migration of macrophages into the tissue. NF-kB also influences inflammation that occurs in atherosclerosis through the cytokines they produce, such as TNF, IL-1\(\beta\), IL-6, IL-10, IL-12, and interferon-\(\gamma\)\(^ {28,30}\). NF-kB is a protein complex that works as a DNA transcription controller. The protein is involved in cellular responses to external stimuli such as free radicals, ultraviolet radiation, oxidized LDL, stress, cytokines, and bacterial or viral antigens\(^ {31}\). Therapy with LpPLA2 inhibitor has succeeded in reducing the expression of NF-kB. NF-kB canonical pathway stimulated by reactive oxygen species (reactive oxygen species / ROS), lipopolysaccharide, and the signal from cytokine receptors such as TNF, IL-1, and Toll-like receptors. NF-kB pathway activated by specific proteins of the TNF family, such as lymphotoxin \(\beta\), factor B cell activation, and CD40 ligand. Stimulation of these proteins causes the translocation of NF-kB complex / RelB: p52 into the nucleus\(^ {32}\).

Table 1 (for 8 weeks) and Table 2 (for 16 weeks) were showed a significant increase (\(p < 0.05\)) in Lp-PLA2 expression in Dyslipidemia Group and significantly decreasing Lp-PLA2 expression in Dyslipidemia + Darapladib group on both 8 weeks and 16 weeks of serial treatment time.

Lp-PLA2 is a single chain glycoprotein member of the serine protease inhibitors (or serpins) family. Endothelial cells, smooth muscle cells, fibroblasts, monocytes/macrophages, adipocytes, endometrium, peritoneum, liver cells, mesothelial cells and cardiac myocytes could produce Lp-PLA2. Lp-PLA2 is a major stress-induced gene. Once synthesized, Lp-PLA2 is mainly stored in platelets and secreted to blood flow or deposited on the subendothelial matrix. The increased expression of Lp-PLA2 \textit{in vivo} suppresses fibrinolysis, consequently leading to the pathological fibrin deposition and then could cause tissue damage\(^ {33}\). Inflammatory cytokines (IL-6, IL-1, TNF-\(\alpha\)), growth factors, and hormones (insulin, glucocorticoids) also influence Lp-PLA2. Chronic inflammation, insulin resistance, and obesity influenced the expression of Lp-PLA2. Several studies had been demonstrated that fibrinolytic dysfunction (defined by Lp-PLA2 levels) mediates the increased risk of CVD in individuals with metabolic syndrome, and increased Lp-PLA2 concentrations have been found both in blood and in coronary plaques of metabolic syndrome patients\(^ {34}\).

**Conclusion**

Lp-PLA2 inhibitor (darapladib) has affected a fraction of lipids (LDL / VLDL and increase HDL) and decreased NF-kB and Lp-PLA2 expression on both 8 weeks and 16 weeks of serial treatment time. Lp-PLA2 inhibitor (darapladib) also decreased the level of LysoPC Serum on 8 weeks of serial treatment time but increased the level of LysoPC Serum on 16 weeks of serial treatment time. Serum and aortic tissue LysoPC level correlation was an inverse correlation. That means the increase of the LysoPC serum level will decreasing the levels of LysoPC Aorta, and decreasing the LysoPC Serum level will increase the levels of LysoPC Aorta. The strength of Serum and Aortic tissue LysoPC level correlation was strong for 8 weeks of serial treatment time and less strong for 16 weeks of serial treatment time.
Conflict of Interest: The authors declare no conflict interest.

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14. Wihastuti TA, Sargowo D, Heriansyah T, Rahmawati


Isolation and Selective Drug of Chose for Pseudomonas Aeruginosa as a Causative Agent in Suppurative Otitis Media

Thamer J Ghali1, Husam Oudah ALjwaid1, Ali A. Al-Hisnawi2-3, Kasim Sakran Abass4

1 Assistant Lecturer, 2 Lecturer, Department of Medical Laboratory Technique, College of Medical Technology, National University of Science and Technology, Thi-Qar, Iraq, 3 Assistant Professor, Department of Biology, College of Science, University of Kerbala, Kerbala, Iraq, 4 Professor, Department of Pharmacology and Toxicology, College of Pharmacy, University of Kirkuk, Kirkuk, Iraq

Abstract

Background: Chronic Suppurative otitis media (CSOM), is a chronic inflammation of the middle ear and mastoid cavity, which presents with recurrent (otorrhea) ear discharge through a perforated tympanic membrane. The bacterial cause for CSOM can include both aerobic and anaerobic bacteria, the most common microorganisms found in CSOM are Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Klebseilla pneumonia, diphtheroid group, Citrobacter sp. and anaerobic bacteria and fungi like Candida sp as well as Aspergillus sp. P. aeruginosa was the most common iso (30500000) followed by S. aurease 27% (27 isolates), Proteus mirabilis 11% (11 isolates), E.Coli 11% (11 isolates), Klebseilla pneumonia 9% (9 isolates), respectively while other different species was 5% (5 isolates) and 2% (2 isolates) was fungi. Antimicrobial sensitivity for P. aeruginosa test by VITEK 2 System based on the main inhibitor concentration (MIC). The Piperacillin showed to be highly sensitive again P. aeruginosa 77.1% (27 isolation) followed by Meropenem, Imipenem, Levofloxacin and Amikacin 65.7% (23 isolation), 60% (21 isolation), 40% (14 isolation), 34.2% (12 isolation) respectively while Ciprofloxacin showed to be the less affected again P. aeruginosa 20% (7 isolation). Ceftazidime, Tobramycin and Ciprofloxacin were highly resistance 68.5% (24 isolation), 62.8% (22 isolation), 54.2% (19 isolation) respectively.

Keywords: Otitis media, P. aeruginosa, Antibiogram, Sensitive, Piperacillin

Introduction

Chronic Suppurative otitis media (CSOM), is a chronic inflammation of the middle ear and mastoid cavity, which presents with recurrent (otorrhea) ear discharge through a perforated tympanic membrane (1) depending on whether the disease process affects is classified into two types, tubotympanic the pars tensa and anticoanal or pars flaccida of the tympanic membrane (1,2) Incidence of the disease is higher in developing countries, especially among low socioeconomic societies because of malnutrition, overcrowding, poor hygiene, inadequate health care, and recurrent upper respiratory tract infections (2) The most important symptoms which make patients seek medical advice are hearing loss and suppurative drainage, reported in around 50% of the cases (3,4). CSOM is a persistent disease with high risk of irreversible complications which may range from persistent otorrhea, mastoiditis, labyrinthitis, and facial palsy to more serious intracranial
The bacterial cause for CSOM can include both aerobic and anaerobic bacteria, the most common microorganisms found in CSOM are *P. aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia*, diphtheroid group, Citrobacter sp., etc and anaerobic bacteria and fungi like Candida sp as well as Aspergillus sp. *P. aeruginosa* is one of the most predominant organisms to cause CSOM (10,11), with an incidence ranging from 21% to 52.94% (12).

The overall incidence of complications from otitis media is extremely low; infratemporal and intracranial complications occur in one in 100,000 children and in one in 300,000 adults per year (13,14). Patients with middle ear effusion may have persistent or fluctuating conductive hearing loss (14,15). Children with chronic middle ear effusion score lower on tests of speech, language, and cognitive abilities (15). Central perforation of the eardrum may cause chronic infection in the middle ear and mastoid cavity. Acute mastoiditis (i.e., pus in the mastoid air cells) may erode through the bone, forming a subcutaneous collection of pus (16). Contiguous spread or hematogenous seeding may infect the inner ear, petrous portion of the temporal bone, meninges, and the brain. Mastoiditis and intracranial complications of acute otitis media are more common in developing countries where persons have limited access to medical care (16,17).

**Material and Methods**

This study was carried out in the Department of Microbiology of Al-Haboby Hospital in Thi-Qar governorate. Total of 100 patients with bilateral and unilateral CSOM. All age and sex including in our study patient who had received antibiotics were excluded from the study.

**Sample collection**

Two sterile cotton swab was collated from patient ear discharge under aseptic precautions.

**Methods**

Case study and sample collected in Al-Haboby Hospital in Thi-Qar governorate. All age and sex including in our study patient who had received antibiotics were excluded from the study. Two sterile cotton swab was collated from patient ear discharge under aseptic precautions. One of swab was used for direct examination and gram staining and the second swab was used to culture onto Blood agar media, MacConkey agar media and nutrient agar media followed by incubation at 37 °C for 18-24 hrs Pseudomonas isolates and identified according to characteristic of colony morphology, pigment production and odor confirmed by VITEK 2 System for Identification and Antimicrobial Susceptibility Testing (bioMérieux).

**Procedure**

One of swab was used for direct examination and gram staining and the second swab was used to culture onto Blood agar media, MacConkey agar media and nutrient agar media followed by incubation at 37°C for 18-24 hrs Pseudomonas isolates and identified according to characteristic of colony morphology, pigment production and odor confirmed by VITEK 2 System for Identification and Antimicrobial Susceptibility Testing (bioMérieux) antibiotics tested were categorized into 6 classes.

**Diagnostic Card**

<table>
<thead>
<tr>
<th>CARD Name</th>
<th>Company</th>
<th>Country of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GN card</td>
<td>bioMérieux</td>
<td>France</td>
</tr>
<tr>
<td>AST card</td>
<td>bioMérieux</td>
<td>France</td>
</tr>
</tbody>
</table>
Results

A total of 100 cases of CSOM were included in the study

*P. aeruginosa* was the most common isolate accounting for 35% (35 isolates) depended on bionumber (000305130500000) followed by *S. aureus* 27% (27 isolates), *Proteus mirabilis* 11% (11 isolates), *E.Coli* 11%(11 isolates), *Klebseilla pneumonia* 9%(9 isolates), respectively while other different species was 5% (5 isolates) and 2% (2 isolates) was fungi as show in bellow Table 1.

<table>
<thead>
<tr>
<th>species</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeruginosa</td>
<td>35</td>
<td>35%</td>
</tr>
<tr>
<td>S. aurease</td>
<td>27</td>
<td>27%</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>11</td>
<td>11%</td>
</tr>
<tr>
<td>E.Coli</td>
<td>11</td>
<td>11%</td>
</tr>
<tr>
<td>Klebseilla pneumonia</td>
<td>9</td>
<td>9%</td>
</tr>
<tr>
<td>other species</td>
<td>5</td>
<td>5%</td>
</tr>
<tr>
<td>fungi</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Antimicrobial sensitivity

Antimicrobial sensitivity for *P. aeruginosa* test by VITEK 2 System based on the main inhibitor concentration (MIC).

The Piperacillin showed to be highly sensitive again *P. aeruginosa* 77.1% (27 isolation ) followed by Meropenem, Imipenem ,Levofloxacinc and Amikacin 65.7% (23 isolation) , 60%(21 isolation) , 40%(14 isolation) , 34.2%(12 isolation) respectively while Ciprofloxacin showed to be the les effected again *P. aeruginosa* 20% (7 isolation). Ceftazidine, Tobramycin and Ciprofloxacin were highly resistance 68.5% (24isolation). 62.8% (22 isolation) . 54.2%(19 isolation) respectively as showed below in table (2).
Table 2. Show the antimicrobial Pattern depended on MIC according to manufacture

<table>
<thead>
<tr>
<th>Family</th>
<th>Penicillins</th>
<th>Carbapenems</th>
<th>Aminoglycosides</th>
<th>Fluoroquinolones</th>
<th>Cephalosporins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>PRL</td>
<td>MEM</td>
<td>IM</td>
<td>AK</td>
<td>G</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Sensitive</th>
<th>Intermediates</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>27</td>
<td>5.7</td>
<td>6</td>
</tr>
<tr>
<td>Percent</td>
<td>77.1%</td>
<td>11.4%</td>
<td>17.1%</td>
</tr>
<tr>
<td>Frequency</td>
<td>23</td>
<td>11.4</td>
<td>10</td>
</tr>
<tr>
<td>Percent</td>
<td>65.7%</td>
<td>8.5%</td>
<td>28.5%</td>
</tr>
<tr>
<td>Frequency</td>
<td>21</td>
<td>28.5%</td>
<td>19</td>
</tr>
<tr>
<td>Percent</td>
<td>60%</td>
<td>22.8%</td>
<td>54.2%</td>
</tr>
<tr>
<td>Frequency</td>
<td>12</td>
<td>22.8%</td>
<td>11</td>
</tr>
<tr>
<td>Percent</td>
<td>34.2%</td>
<td>51.4%</td>
<td>31.4%</td>
</tr>
<tr>
<td>Frequency</td>
<td>9</td>
<td>28.5%</td>
<td>14</td>
</tr>
<tr>
<td>Percent</td>
<td>25.7%</td>
<td>62.8%</td>
<td>40%</td>
</tr>
<tr>
<td>Frequency</td>
<td>10</td>
<td>14.2%</td>
<td>16</td>
</tr>
<tr>
<td>Percent</td>
<td>28.5%</td>
<td>22.8%</td>
<td>45.7%</td>
</tr>
<tr>
<td>Frequency</td>
<td>7</td>
<td>28.5%</td>
<td>16</td>
</tr>
<tr>
<td>Percent</td>
<td>40%</td>
<td>8.5%</td>
<td>45.7%</td>
</tr>
<tr>
<td>Frequency</td>
<td>16</td>
<td>14%</td>
<td>16</td>
</tr>
<tr>
<td>Percent</td>
<td>20%</td>
<td>68.5%</td>
<td>68.5%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Piperacillin (PRL), Meropenem (MEM), Imipenem (IM), Amikacin (AK), Gentamicin (G) Tobramycin (TOB), Levofloxacin (LVO), Ciprofloxacin (CIP), Cefoxitin (CEF), Cefazoline (CFA), Ceftriaxone (CRO), Cefazidime (CZM).

Discussion

Chronic Suppurative Otitis Media may lead to fatal intra-cranial and extra-cranial complications like facial palsy, brain abscess and meningitis if not adequately treated with (18,19). *P. aeruginosa* is the most common bacteria isolated from chronic supportive otitis media. This finding is in tandem with the observations by other studies (20). Our study was disagreement with other studies done by (21,22), that reported staphylococcus aureus was predominant causative agent in CSOM followed by *P. aeruginosa*.

Among the β-lactamase inhibitor antibiotics, Piperacillin was found to be the most effective antibiotic against *P. aeruginosa* this funding was agreement with (23) which recommended the use of combinations Piperacillin + Tazobactem were more effected against *P. aeruginosa* infections followed by Carbapenems Meropenem and Imipenem similar to the findings seen in studies done by (24,25). Among the Aminoglycosides, Sensitivity was seen with Amikacin (34.2%). This finding is in agreement with studies. Cephalosporins are common used in treatment of *P. aeruginosa* Ceftazidime and Ciprofloxacin were very high resistance against Chronic Suppurative
Otitis Media\textsuperscript{(26,27)}, Although Cefoxitin, Cefazoline, Ceftriaxone, and Ceftazidime were more effected in this family again \textit{P.aeruginosa} which is in contrast with study done be \textsuperscript{(28,29)}, which showed cephalosporins were highly sensitivity in Chronic Suppurative Otitis Media. Fluroquilonones, Levoflaxacin was (40\%) sensitive while Ciprofloxacin (20\%) was again \textit{P. aeruginosa} which is similar to the study done by \textsuperscript{(30,31)}.

**Conclusions**

In conclusion, this is the first paper that provided original data concerning a \textit{P. Aeruginosa} as a causative agent in suppurative otitis media. \textit{P.aeruginosa} is the most common bacteria isolated from chronic supportive otitis media. Among the \(\beta\)-lactamase inhibitor antibiotics Piperacillin was found to be the most effective antibiotic again \textit{P. aeruginosa}. Selective the correct antimicrobial will help in decreasing the complications of CSOM. \textit{P. aeruginosa} were found to be the common cause of CSOM in the present study. Furthermore, \textit{P. aeruginosa} was resistant to commonly used antimicrobials. piperacillin is a drug of chose in our study. Antibiogram of isolates is recommended in decreasing the complications of CSOM. Finally, in spite of this paper, further studies are required under different laboratories and different complement components compounds in order to improve and to increase our knowledge about this very interesting infection with \textit{P. aeruginosa}.

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**Conflict of Interests:** The authors of this paper declare that he has no financial or personal relationships with individuals or organizations that would unacceptably bias the content of this paper and therefore declare that there is no conflict of interests.

**Source of Funding:** The authors have no sources of funding, so it is self-funding research.

**Ethical Approve:** We declare that the study does not need ethical approval.


A Study of Epidemiology of Poisoning Cases Brought For Autopsy at Tertiary Health Care Centre in South-Western Maharashtra: A Ten-Year Retrospective Study

Thippesh Kumar N1, Sandeep Bansal2, KV Radhakrishna3, Sarala M4, Shivakumar D Kalakabandi5, Gautam Dhokia6, Abhishek Rathee7

1Assistant Professor, Department of Forensic Medicine and Toxicology, Army College of Medical Sciences, New Delhi, India, 2Post-Graduate, 3Professor, 4Assistant Professor, 5Senior resident, 6Post-Graduate, 7Post-Graduate, Department of Forensic Medicine & Toxicology, Armed Forces Medical College, Pune, India

Abstract

Introduction: Present study is the study of epidemiology of poisoning cases brought for autopsy at tertiary health care centre in South-Western Maharashtra by finding out the incidence, pattern, sociodemographic factors, toxic agents, seasonal and other factors related to their deaths.

Material and Methods: Present study is a retrospective cross-sectional study done in mortuary of a tertiary health care centre in South-Western Maharashtra. Study included total 104 cases of death due to poisoning from Jan 2009 to Dec 2018 and the data collected from inquest papers, autopsy reports and regional forensic science laboratory reports was analysed using a structured proforma in Microsoft excel sheet.

Result: Out of 2570 medico legal autopsies conducted during the study period, deaths due to poisoning comprised 104 cases i.e. an incidence of 4.05%. Maximum deaths due to poisoning are found to be due to Intentional self-poisoning (suicides) and rural areas are more affected than the urban areas. Most commonly used toxic substance for poisoning is found to be pesticides and the maximum deaths occurred during the monsoon season. Gender-wise analysis shows that death due to poisoning is more common in males and majority of victims irrespective of their gender were married.

Conclusion: Study provides crucial information on various sociodemographic factors related to poisoning deaths in South Western Maharashtra which is relevant in understanding the causative factors behind the mortality due to poisoning and also helpful in devising preventive measure to reduce the loss of human life.

Keywords: Poisoning, Epidemiology, Autopsy, Suicide, Pesticides.

Introduction

Death by poisoning has been known since time immemorial. Some of the famous names and incidents such as Socrates, Meera Bai, Bhopal gas tragedy, Bradford sweet poisoning, Tokyo subway sarin attack and Punjab sweet poisoning, remind us of this dangerous threat to life. Death by poisoning whether intentional or unintentional is a significant global health concern as it is a major cause of worldwide mortality and morbidity. As per WHO estimates, in 2012 unintentional poisoning caused the loss of 193460 life’s and loss of 10.7 million years of healthy life all across the globe. Each year about 3.7 lac people lose their life by deliberate consumption

Corresponding author:
Dr. Sandeep Bansal,
Post-graduate, Department of Forensic Medicine and Toxicology, Armed Forces Medical College, Pune, Maharashtra. Mob. – 7620889356,
Email id – sandeep85w@gmail.com
of highly toxic pesticides and about 1 lac people become victim of fatal snake bite.\(^1\)

India is among the countries with high mortality due to poisoning and high incidence of suicide by farmers by pesticide consumption. About 50,000 people die in India every year due to poisoning as per the estimates.\(^2\) Mortality rate from poisoning in India varies from shocking 15 to 35%.\(^2\) The high incidence of farmer suicide has been plaguing the agricultural sector and is having a grave impact on our agriculture-based economy. Despite of being a highly debated issue and various measures like loan waivers for farmers, pesticide poisoning continues to be major contributor to the death by poisoning and much needs to be done to counter this menace.

Mortality due to poisoning varies from region to region and different regional studies have shown varying trends depending on multiple factors. Updated regional information on poisoning cases, availability of published data, regional poison information centers and robust surveillance system are must to significantly reduce the mortality and morbidity due to poisoning. Paucity of information on locally available toxic substances, population at risk, circumstances of poisoning is a hurdle in prevention of poisoning by targeted intervention programs and laws. Therefore, it is crucial to carry out region-based time to time review of poisoning patterns to help in reducing the mortality due to poisoning.

Present study aims at study of epidemiology of poisoning cases brought for autopsy at tertiary health care centre in South-Western Maharashtra by finding out the incidence, pattern, sociodemographic factors (i.e. age, gender, domicile and marital status), toxic agents, seasonal and other factors related to their deaths and to suggest preventive measures to avert loss of human lives due to poisoning.

**Material and methods**

This is a retrospective cross-sectional study done in mortuary of a tertiary health care centre in South-Western Maharashtra. All confirm cases of death due to poisoning from 1 Jan 2009 to 31 Dec 2018 were included in the study. Study included total 104 cases and data were collected from inquest papers, autopsy reports and regional forensic science laboratory reports. Ethical clearance was obtained from institutional ethical committee.

Data was entered in Microsoft excel sheet in a structured proforma consisting of age, gender, marital status, residence, type of substance causing poisoning, manner of poisoning, date, month and season of incidence and analysed. All the analysed data was presented in graphs, charts and tabular forms using the same software application.

**Results**

A total of 2570 medico legal autopsies were performed between Jan 2009 to Dec 2018 at the study centre. Deaths due to poisoning comprised 104 cases i.e. an incidence of 4.05% among the total autopsies conducted during the study period. Annual incidence rate of death due to poisoning was calculated for the study period and it showed a decreasing trend 2013 onwards. [Figure 1]
As per the manner of death, 93 (89.42%) cases were of suicidal poisoning, 8 (7.7%) cases were of accidental poisoning, 1 (0.96%) case of homicidal poisoning and in 2 (1.92%) cases, manner of poisoning was undetermined. [Figure 2]

Incidence of poisoning was found to be more common in rural areas (n=55, 52.88%) than in urban areas (n=49, 47.12%). [Figure 3]
The most common substances used for poisoning was found to be pesticides (n=79, 75.96%) and among them the commonest chemical used was organophosphates (n=54, 51.92%). [Figure 4]

The present study shows that maximum fatalities due to poisoning occurred during the Monsoon season (n= 49, 47.11%) and the month of March (n=14, 13.46%). [Figure 5]
FIGURE 5: DISTRIBUTION OF POISONING DEATHS ACCORDING TO SEASON AND MONTH

Study shows that death due to poisoning is more common among males (n=59, 56.73%) in comparison to females (n=45, 43.27%) with a male-female ratio of 1.3:1 and the most common age group affected is 21 to 30 years (n=34, 32.69%) irrespective of gender. [Table 1]

TABLE 1: DISTRIBUTION OF DEATHS DUE TO POISONING ACCORDING TO AGE AND GENDER

<table>
<thead>
<tr>
<th>Age Grp</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
<th>Male: Female Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10 yrs</td>
<td>2 (3.39%)</td>
<td>1 (2.22%)</td>
<td>3 (2.88%)</td>
<td>2:1</td>
</tr>
<tr>
<td>11 – 20 yrs</td>
<td>1 (1.69%)</td>
<td>6 (13.33%)</td>
<td>7 (6.73%)</td>
<td>0.17:1</td>
</tr>
<tr>
<td>21 – 30 yrs</td>
<td>17 (28.81%)</td>
<td>17 (37.77%)</td>
<td>34 (32.69%)</td>
<td>1:1</td>
</tr>
<tr>
<td>31 – 40 yrs</td>
<td>15 (25.42%)</td>
<td>10 (22.22%)</td>
<td>25 (24.03%)</td>
<td>1.5:1</td>
</tr>
<tr>
<td>41 – 50 yrs</td>
<td>08 (13.56%)</td>
<td>03 (6.67%)</td>
<td>11 (10.57%)</td>
<td>2.66:1</td>
</tr>
<tr>
<td>51 – 60 yrs</td>
<td>09 (15.25%)</td>
<td>04 (8.89%)</td>
<td>13 (12.5%)</td>
<td>2.25:1</td>
</tr>
<tr>
<td>&gt; 60 yrs</td>
<td>07 (11.86%)</td>
<td>04 (8.89%)</td>
<td>11 (10.57%)</td>
<td>1.75:1</td>
</tr>
<tr>
<td>Total</td>
<td>59 (56.73%)</td>
<td>45 (43.27%)</td>
<td>104 (100%)</td>
<td>1.3:1</td>
</tr>
</tbody>
</table>

Most of the victims were married, both among the males (n=47, 79.66%) and females (n=35, 77.78%) constituting total 82 (78.85%) married cases. [Figure 6]
Discussion

Analysis of the data in the present study gives an incidence rate of 4.05\% for death due to poisoning out of total 2570 autopsies conducted during the study period. Review of literature shows that the incidence rate in the present study is less in comparison to other studies.\(^3\)\(^-\)\(^11\)

The decrease in annual incidence rate of death due to poisoning after 2013 reflects an optimistic trend encouraging both government and society to boost the ongoing efforts in countering this menace. However, the emerging trend of relinquishing agriculture related occupations among the people as a factor behind the decline in incidence rate cannot be ignored.

In present study maximum poisoning cases according to the manner of death are found to be suicidal (n=93, 89.42\%) followed by accidental (n=8, 7.7\%). Only 1 (0.96\%) case of homicidal poisoning death was reported during the study period and in 2 (1.92\%) cases manner of poisoning was undetermined. The results are similar to other studies in respect of manner of death.\(^3\)\(^-\)\(^13\)

Present study shows that incidence of poisoning is more common in rural areas (n=55, 52.88\%) than in urban areas (n=49, 47.12\%). The results are similar to findings of other studies\(^3\)\(^-\)\(^7\)\(^,\)\(^11\) except the study conducted in Kanpur\(^{[10]}\) where more poisoning deaths were found to occur in urban area. Rural population forms about 54.77\% of the total population of Maharashtra which explains the more occurrence of poisoning deaths in rural areas.\(^{14}\)

The most common substances found to be used for poisoning in the present study is pesticides (n=79, 75.96\%) and the results are in concurrence with findings of other studies.\(^3\)\(^,\)\(^6\)\(^-\)\(^12\)\(^,\)\(^15\) Among the pesticides the commonest chemical found to be used is organophosphates (n=54, 51.92\%) followed by herbicides (n=10, 9.6\%). Maharashtra’s economy is predominantly agrarian with agriculture as the mainstay which explains easy availability of pesticides in most of agriculture related households.\(^{16}\) Participation of community in implementation of preventive measures like central storage facility for pesticides at village level, regulation of sale by registered person and purchase by certified person, will help in reducing the incidence of death by poisoning. Seemingly small precaution
of storage of pesticides in locked cabinets under the supervision of responsible elder member of the family can be of great help in preventing death due to pesticide poisoning.

The study shows the occurrence of fatalities due to consumption of easily available household items like mosquito repellent (n=1, 0.96%), Lysol (n=1, 0.96%), carbolic acid (n=4, 3.85%), and antiseptic savlon (n=1, 0.96%). Review of literature shows that results are similar to other studies.10,11,12,15 These items are effortlessly available in most of the urban homes and are usually handled casually. Safe storage of these harmless appearing toxic chemicals can prevent tragic loss to the family.

Consumption of overdose of antipsychotic medicines led to death of 2 (1.96%) people in the present study. Review of literature shows similar results in study conducted at Thane 11 but the study conducted at Manipal 4 shows high number of deaths due to medicinal overdose. The variation is probably because of different socio-economic profile of victims.

Snake bite was responsible for death of 4 (3.85%) people in the present study and the results are similar to study conducted in Bangalore 12 but deaths due to snake bite were far more in study conducted in Jamnagar 3 and Berhampur 15. The reason for the difference is geographical variation of the regions. Use of innovative measure like wearing of knee length rubber boots in fields and solar powered snake repellent sticks could prove beneficial in preventing snake bite.

Study found that 2 (1.92%) people lost their lives due to exposure to toxic gases while working in the sewages. Review of literature reveals that past studies do not mention such poisoning deaths.3-13,15 Despite of the existence of laws prohibiting employment as manual scavengers in sewages, such incidents are still happening and are taking toll on human lives. Mandatory wearing of protective suits by sewage workers and strict legal action against defaulters is the need of the hour to prevent such mortalities.

Present study shows that maximum poisoning fatalities occurred during the monsoon season (n=49, 47.11%) followed by summers (n=31, 29.80%) which is different from the results of other studies carried out in different regions of the country where maximum poisoning deaths have occurred during the summers.6,7,9,10,12,15 The study region in the South western Maharashtra faces long but uneven rainy season from June to October. Damage to the Kharif crops caused by the unexpected floods due to excessive rainfall and hailstorms lashing the regions is one of the major factors impelling farmers to take the extreme step. Plight of the farmers is further compounded by the health expenses, children education expenses and mounting debts. The proactive preparation by the hospitals for the expected increase in poisoning cases during the monsoons and establishment of regional poison information centre will prove advantageous in reducing the mortality.

Gender-wise analysis shows that males died more due to poisoning in comparison to females constituting 59 (56.73%) and 45 (43.27%) cases respectively with a male-female ratio of 1.3:1. These results of the present study are in concurrence with other studies.5-11 Present study shows that most of the victims were married, both among the males (n=47, 79.66%) and females (n=35, 77.78%) constituting total 82 (78.85%) married cases and the results are in concurrence with other studies conducted.3-13 Study shows that married status of individual plays a significant role as the sought-after bliss of marital life brings along many responsibilities and unexpected problems requiring maturity, emotional and psychological support. Inability to cope up with the marital responsibilities and to resolve marital discord, are some of the factors impelling the individual to take the extreme step of ending his or her life. Pre-marital and post-marital counselling should be encouraged for couples which will be helpful in resolving marriage related problems.

Maximum deaths due to poisoning occurred in the age group of 21 to 30 years (n=34, 32.69%) followed by age group of 31 to 40 years (n=25, 24.03%). These findings are in concurrence with the results of other
studies but different to findings of study conducted at Bankura where the maximum deaths were found to occurred in age group of 40 to 49 years owing probably to socio-demographic variations. 21 to 30 years is the age group when the youth starts shouldering the various social and family responsibilities. The stresses created by the vagaries of life may push the individual towards committing suicide. Establishment of a suicide helpline by the government may prove to be an effective method in curtailing the mortality due suicidal poisoning.

Study shows that 11 [10.57%] seniors citizens lost their lives due to poisoning. Death by poisoning among the senior citizens is less in comparison to younger people and the findings are similar to other studies. Depression due to chronic illness and a sense of alienation among the senior citizens are the major factors driving them to take the extreme step of committing suicide. There is need of sensitization of society towards the emotional and psychological needs of the senior citizens.

Study highlights the age group of 11 to 20 years where death of 6 female teenagers outnumbers the death of 1 male adolescent due to poisoning. Death of the teenagers by intentional self-poisoning warrants serious concern by all parents. There is need of sensitization of parents to understand the teenage behaviour in this era of social media technology and address the issues with patience, open mind and compassion especially involving the females.

The least number of cases (n=3, 2.88%) were found to be in the age group below 10 years. Death of 2 children below the age of 10 years had occurred by accidental poisoning which could have been prevented by secure storage of toxicological chemicals at home. 1 male child below the age of 10 years had become the victim of homicidal poisoning by a female relative out of jealousy of not being able to conceive a male child. This reflects another disturbing aspect of our society necessitating introspection and change in the patriarchal mindset of people.

Conclusion

Present study shows the incidence rate of death by poisoning and provides important information about the sociodemographic profile of victims in South-Western Maharashtra covering the period from Jan 2009 to Dec 2018. Study also provides crucial information about the common toxic substances used for poisoning and seasonal variation in poisoning deaths in South-Western Maharashtra. The information obtained is not only valuable in understanding the causative factors behind the mortality due to poisoning in the region but also of paramount significance for devising of preventive measures which can be implemented by family members, panchayat, local community, hospitals and the government authorities in combating this grave health concern and averting the loss of human lives.

Conflict of Interest: We declare that there is no conflict of interest.

Source of Funding: None

Ethical Clearance: Taken from IEC of AFMC, Pune

References


A Woman with Tuberculosis Multidrug Resistance and QTc Prolongation Repetitive Interval: A Case Report

Tutik Kusmiati, Ni Made Mertaniasih, Johannes Nugroho Eko Putranto, Budi Suprapti, Soedarsono, Abdul Rahman Bahmid

1Medical Staff, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, 2Professor, Department of Medical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, 3Associate Professor, Department of Vascular and Cardiology Medicine, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, 4Associate Professor, Faculty of Pharmacy, Universitas Airlangga – Universitas Airlangga Teaching Hospital, Surabaya, Indonesia, 5Associate Professor, Department of Pulmonology and Respiratory Medicine, 6 Resident, Pulmonology and Respiratory Medicine, Universitas Airlangga – Universitas Airlangga Teaching Hospital, Surabaya, Indonesia

Abstract

Background: Prolonged QTc interval is one of the side effects of the short-regimen MDR-TB. QTc prolongation is a risk factor for Torsades de pointes and is influenced by many factors.

Case: 47-year-old woman who was diagnosed with MDR-TB through GeneXpert examination and received short-regimen MDR-TB. This patient experienced repeated QTc prolongation, with peak QTc interval 600 msec occurring at 4th month with mild hypokalemia without clinical symptoms. The patient completed 9 months of short-regimen therapy with improve chest x ray followed by negative sputum culture.

Conclusion: Short-regimen MDR-TB contains several drugs that cause QTc prolongation. Clinical evaluation is required in patients with QTc prolongation before changing the regimen.

Keywords: TB-MDR, QTc prolongation, Moxifloxacin

Introduction

Multidrug-resistant tuberculosis (MDR-TB) is still a world health problem and a threat to TB control globally. WHO estimates that there are about 3.3% new MDR TB cases and 20% MDR TB re-treatment cases. Several factors that contribute to the spread of MDR-TB, especially in endemic areas, include limited treatment options, high costs, ineffective and prolonged therapy. In 2016 WHO announced a short-term regimen for MDR-TB therapy that is expected to reduce costs, reduce costs and be effective for MDR-TB patients worldwide. This regimen consists of kanamicin, moxifloxacin, etionamide, clofazimin, pyrazinamide and high doses of INH for 4-6 months of the intensive phase and is followed by a follow-up phase consisting of moxifloxacin, clofazimin, pyrazinamide and ethambutol for 5 months (1, 2).

Although the short-term MDR-TB regimen brings new hope, this cannot be separated from the side effects caused by some MDR-TB drugs, one of which is the lengthening of the corrected QT (QTc) interval. QTc prolongation is a risk factor for the incidence of Torsades de Pointes (TdP) as well as arrhythmias that have the
potential to cause death. According to a review study the prevalence of sudden death due to TdP due to QTc prolongation due to MDR-TB drugs may be less than 1%. Electrocardiogram (ECG) monitoring is required in MDR-TB patients receiving short-term therapy, but in resource-limited areas this is neither a limitation nor a condition of using short-term MDR-TB regimens (2, 3).

The QT interval is the portion of the EKG that is calculated from the start of the QRS complex to the end of the T wave, it describes the time it takes for the ventricular myocardium to depolarize and repolarize or the time it takes the myocardium to replenish between heartbeats measured in seconds (s) or milliseconds (ms). The QT interval is influenced by heart rate, which can shorten at a fast heart rate and lengthen at a slow heart rate so the QT interval needs to be corrected. The QT or QTc correction formula is needed to estimate the QT interval at a heart rate of 60 times a minute. The normal value of QTc in men <450 ms and in women <470 ms (4, 5).

Fluoroquinolones (FQ) are one of the most effective mycobactericidal classes. FQ has also been noted to cause prolongation of QTc and TdP especially in the elderly population. All FQ can be used for MDR-TB treatment including Levofloxacin (Lfx) and Moxifloxacin (Mfx). Mfx was noted to be the FQ that caused more QTc lengthening than the other FQs (5, 6). Based on the above description we are interested in reporting a recurrent case of MDR TB and QTc prolongation interval in a woman.

**Case Presentation**

A 47-years-old woman with a previous history of failure to treat category 1 OAT was diagnosed with rifampicin-resistant pulmonary TB by geneXpert examination. The results of the x-ray examination obtained the results according to figure 1. Patients received MDR TB therapy including Kanamicin (Km) 750 mg, moxifloxacin (Mfx) 600 mg, clofazimine (Cfz) 100 mg, Ethionamid (Eto) 500 mg, isoniazid (H) 600 mg, pyrazinamide (Z) 1500 mg and ethambutol (E) 800 mg. Before starting therapy, the patient was previously carried out a baseline examination, including a complete laboratory examination, electrocardiogram (EKG), audiometry and psychology.

After receiving therapy for 1 week the patient experienced prolonged QTc interval 545 msec, without laboratory abnormalities or clinical symptoms. Mfx was stopped for 3 days and an EKG was evaluated every day. After the QTc interval returned to normal (411 msec), Mfx was started at a dose of 400 mg. A week later the QTc interval of 468 msec and Mfx was again given at a dose of 600 mg while evaluating the ECG (Figure 2 & Table 1).

When entering the advanced stage of therapy, the patient again experienced a prolonged QTc interval of 600 msec so that Mfx was temporarily stopped and a laboratory evaluation was carried out. The laboratory results at that time showed that the patient had mild hypokalemia with a potassium value of 3.2 mmol/l. After correction of hypokalemia the QTc interval returns to normal and Mfx is started again at a dose of 200 mg for 7 days, and then continued at a dose of 400 mg until the end of therapy.

Entering the 7th month of therapy, the patient again experienced a prolonged QTc interval of 539 msec, without clinical symptoms and laboratory abnormalities (potassium 3.7 mmol/l). Currently Mfx is still given at a dose of 400 mg but the Cfz dose is reduced to 50 mg/day. After a week of evaluation the QTc returned to normal and Cfz was again given at a dose of 100 mg/day until the end of treatment. The patient had conversion of Acid-fast bacilli at 4th month followed by negative sputum culture until the end of therapy. The patient experienced clinical and radiological improvement, at the seventh month the treatment was completed and the patient was declared cured.
Table 1. QTC Serial

<table>
<thead>
<tr>
<th>Time</th>
<th>QTc interval</th>
<th>Action</th>
</tr>
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<tbody>
<tr>
<td>2nd week</td>
<td>545 msec</td>
<td>Mfx temporary stop</td>
</tr>
<tr>
<td>1st month</td>
<td>468 msec</td>
<td>Mfx 600 mg</td>
</tr>
<tr>
<td>2nd month</td>
<td>471 msec</td>
<td>Mfx 600 mg</td>
</tr>
<tr>
<td>4th month</td>
<td>600 msec</td>
<td>Mfx temporary stop</td>
</tr>
<tr>
<td>4th month</td>
<td>443 msec</td>
<td>Mfx 200 mg</td>
</tr>
<tr>
<td>5th month</td>
<td>491 msec</td>
<td>Mfx 400 mg</td>
</tr>
<tr>
<td>7th month</td>
<td>539 msec</td>
<td>Mfx 400 mg + Cfz 50 mg</td>
</tr>
<tr>
<td>7th month</td>
<td>433 msec</td>
<td>Mfx 400 mg + Cfz 100 mg</td>
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</table>

Table 2. Risk factors for QTc prolongation and torsades de pointes

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<tr>
<th>Baseline and unmodified predisposition</th>
<th>Acquired risk factors : clinical condition</th>
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<tr>
<td>Underlying conduction abnormalities</td>
<td>Electrolit imbalance : hypokalaemia, severe</td>
</tr>
<tr>
<td>(subclinical long QT syndrome)</td>
<td>hypomagnesaemia, hypocalcaemia</td>
</tr>
<tr>
<td>family history of sudden death</td>
<td>Structural and functional heart problems :</td>
</tr>
<tr>
<td></td>
<td>recent conversion from atrial fibrilation,</td>
</tr>
<tr>
<td></td>
<td>ischaemic and congestive heart disease,</td>
</tr>
<tr>
<td></td>
<td>ischaemic cardiomyopathy, dilated or</td>
</tr>
<tr>
<td></td>
<td>hypertrophic congestive heart disease,</td>
</tr>
<tr>
<td></td>
<td>congestive heart failure</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>Frequent conditions of TB patients : HIV</td>
</tr>
<tr>
<td></td>
<td>infection, low BMI (malnutrition, starvation, and wasting syndrome),</td>
</tr>
<tr>
<td>Female sex</td>
<td>severe vomiting and diarrhea creating low potassium levels</td>
</tr>
<tr>
<td>Advanced age (linearly increased risk after 60 years)</td>
<td>Impaired renal function</td>
</tr>
<tr>
<td></td>
<td>Impaired hepatic function</td>
</tr>
<tr>
<td></td>
<td>Hypothyroidism</td>
</tr>
</tbody>
</table>

Figure 1A. Chest X-Ray at baseline; B. Chest X-Ray after 6 month therapy; C. Chest X-Ray end of therapy
Discussion

Prolonged QTc interval greater than 500 msec is generally thought to increase the risk of developing TdP in the majority of the group with congenital long QT syndrome. The risk of TdP or sudden death is not always directly related to the length of the QT interval or the duration of QTc prolongation during therapy, but it can still be influenced by many factors. Drug-induced QTc prolongation has different properties from drug to drug. All FQs were noted to cause QTc prolongation, but according to case reports Mfx has the greatest risk of causing QTc prolongation\(^2,3\). The risk of QTc prolongation with FQ will be higher if there are electrolyte abnormalities and the use of other drugs that cause QTc prolongation\(^5,6\). In this case, when the patient experienced QTc lengthening of 600 msec, there was also mild hypokalemia (potassium 3.2 mmol/l). Apart from stopping Mfx we also provide potassium intake to the patient.

A 2018 Indian study involving 467 patients receiving the Mfx regimen after a 4-month evaluation did not show a significant increase in QTc. Although there were 5 patients who experienced prolonged QTc interval, Mfx had to be temporarily stopped and given again without experiencing a cardiac event. According to the study Mfx 400 mg daily given for 4 months did not cause prolonged QTc interval\(^7\).

In a South Korean study of 373 MDR-TB or NTM-TB patients receiving Mfx, Cfz and macrolide therapy, significant QTc prolongation occurred in 16.7% of patients with a maximum QTc value of 451 msec and a mean QTc lengthening of 33.6 msec from baseline.
This study concluded that drug combinations that cause QTc changes in MDR-TB or NTM therapy are generally safe to use\(^8\). Although Mfx significantly caused QTc prolongation it was of moderate duration with a mean increase of 4\% in the therapeutic dose of 400 mg. The effect of Mfx on ventricular repolarization occurs primarily at times of maximum plasma concentration, but QTc prolongation may be decreased with repeated administration, therefore the risk of TdP is small at 400 mg/day\(^9\). In this case, QTc prolongation still occurred in the advanced phase of therapy even though Mfx had been given at a dose of 400 mg/day, this might occur because the patient was still receiving other drugs that caused QTc interval lengthening, which is Cfx. After receiving low dose Cfx (50mg/day), QTc returned to normal. Although the evidence is limited, Cfx has been noted to increase QTc interval between 10-20 msec\(^10\).

Apart from FQ, there are several antibiotics used in MDR-TB therapy that are associated with prolongation of the QTc interval, such as bedaquiline, delamanid, and Cfx. The safety of using Mfx together with any of these drugs in MDR-TB therapy requires further clinical evaluation (Table 2). Until now, the incidence of TdP in clinical trials of TB patients is very rare, because the field conditions are difficult to evaluate for cardiotoxicity\(^10\). One study showed that the risk of Mfx-associated QTc prolongation was increased in individuals with certain genotypes\(^11\).

**Conclusion**

QTc prolongation is one of the side effects of the short-term MDR-TB regimen. This can be related to several factors, including the combination of several drugs that cause QTc prolongation, and certain clinical conditions, especially electrolyte disturbances. Although the incidence of TdP associated with QTc prolongation in MDR-TB therapy is rare, ECG evaluation in patients receiving short-term MDR-TB therapy is needed. Therefore a clinical evaluation should be carried out in patients experiencing QTc prolongation before stopping or changing their MDR-TB treatment regimen.

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**Funding:** None.

**Ethical Approval:** All procedures performed in studies involving human participants were in accordance declaration of helsinki the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

**References**

7. Nair D, Velayutham B, Marimuthu M, Navaneethapandian PGD, Chinnaiany P, Jawahar


Ummu Kamilah¹, Syamsiar S. Russeng¹, Masyitha Muis¹, Yahya Thamrin¹, Masni², Fridawati Rivai³, Rizky Maharja⁴

¹Student Master Program, Department of Occupational Health and Safety, ²Lecturer, Department of Biostatistics and Reproductive Health, ³Lecturer, Department of Hospital Management, Faculty of Public Health, Universitas Hasanuddin, Indonesia, ⁴Lecturer, Department of Industrial Hygiene, Occupational Health and Safety, Sekolah Tinggi Ilmu Kesehatan Makassar, Indonesia

Abstract

Accidents in a workplace are caused by many human errors in form of unsafe behavior. One of the efforts to prevent work accidents is to improve safety performance in form of participation and obedience. Improvement of safety performance in workplace is affected by management and individual factors. Management factor is mainly associated with safety management practices while individual factor is associated with individual characteristics and occupational health and safety knowledge and training. This study aims to analyze the effect of individual factors and safety management practices on safety performance among workers at PT. Masmindo Dwi area. This research is a quantitative research using analytic observational and cross sectional design which was conducted in November-December 2020. Interviews were conducted on 72 workers out of 289 workers. The methods used in assessing safety management practices, individual factors and safety behavior are questionnaires, observation and interviews. The results showed that there is a direct (p=0,024) and indirect effect (p=0,001) of safety management practices on safety performance with safety knowledge as a mediating variable. Writer highly advise that in improving safety performance, the management have to be consistent, show commitments to safety rules and procedures that have been made, improve safety training programs, and increase the quantity and quality of training programs.

Keywords: safety performance, safety knowledge, safety management practices

Introduction

Indonesia has various industrial sectors, one of them is mining which included the mineral and coal mining industry. The mineral and coal mining industry in developed and developing countries has implemented sophisticated technology but the dangers and risks faced by this field of work are still there, as evidenced by the many accidents of work occurred in the mining sector.

Statistical Data of Mine Safety and Health Administration noted that injuries which occurred in US coal mines in 2007 reached 3,203 cases with the number of cases in open cut-mining reaching 733 cases or 23% of the total injuries¹. In Indonesia, the incidence of work accidents in the mining sector is also still experiencing a significant increase every year. Based on data from the Ministry of Energy and Mineral Resources (ESDM) on all mining companies in Indonesia as per September 30, 2019, it showed that the total mining accidents that occurred from 2012 to 2019 were 1,318 cases, consisting of 194 accidents resulting in death, 674 serious work accidents and 450 minor work accidents².

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Corresponding Author:
Ummu Kamilah, S.KM
umumukamilah.kod@gmail.com
+6282243497159, Public Health Faculty, Universitas Hasanuddin, Makassar, South Sulawesi, 90245
Heinrich et al. argued that generally work accidents might occur due to two main factors, namely unsafe working conditions and unsafe behaviour\(^3\). Research by Kamilah shows that there was a significant corelation between unsafe behaviour and the incidence of work injuries on construction workers\(^4\). The Ministry of Energy and Mineral Resources also conducted an analysis upon the results of investigation of mining accidents which resulted in death during 2012 – 2019\(^2\). The analysis showed that 19% the causes of the accidents were from unsafe behaviour factor, specifically due to not following applicable work procedures (SOP). Then the analysis continued on personal factors which became the leading causes, with 52%. Most of it were due to lack of knowledge related to safety. In addition, another 19% came from the occupational factors due to job hazards that had not been well identified and 18% due to insufficient leadership and supervision. According to Hughes and Ferret, a person’s behaviour can be influenced by factors from the individual himself, both the conditions and their own characteristics\(^5\).

The government has shown its commitment to reduce the number of work accidents and protect the safety and health of mining workers by issuing several policies related to occupational safety and health in mining sector. Ministry of Energy and Mineral Resources has obliged all mining companies to implement SMKP (Safety Management System for Mining) which is a derivative of the Occupational Safety and Health Management System (SMK3). The success of implementing occupational health and safety regulations in a company will never be separated from the personal compliance attitude of both employees and managerial parties in implementing OHS-related regulations and policies. The role of employees is of course very much needed to support the success of occupational health and safety management efforts, by displaying safety performance\(^6\).

Safety performance is a performance that is measured based on a person’s safety related behaviour. According to Neal & Griffin, safety performance is formed from Safety Compliance and Safety Participation\(^7\). Safety compliance is safety performance carried out by employees in maintaining safety, while safety participation is employee behaviour to participate in safety activities or safety behaviour.

PT. Masmindo Dwi Area (PT.MDA) is the only company holding a 7th Generation of Work Contract which engaged in gold mining with its working area in Latimojong District, Luwu Regency, South Sulawesi Province with area of 14,390 hectares square. The majority of PT MDA employees work on fields where the risk of accidents is very high due to the use of many heavy equipment, high-risk work locations (mountains and adjacent to ravines), several drilling activities that requires precision in carrying out the work and unsafe environmental conditions.

The results of previous observations by the author, there were still several work locations with unsafe conditions which pose risk of work accidents and even fatality. Likewise, the safety performance of workers was still low which could be seen from the poor compliance of workers in implementing company OHS-related rules and SOPs and also the lack of safety participation in reminding or reprimanding working colleagues who did not comply to safety.

Safety management practices are practices, roles and management functions designed by companies to improve employee safety, which consists of 6 dimensions, namely management commitment related to safety, safety training, employee involvement in solving safety problems, safety communication, safety regulations and procedures and safety promotion policies. Vinodkumar and Bhasi found that safety management practices had an effect on safety performance, through safety knowledge\(^8\). Based on the explanations above, this research aimed to determine the effect of safety management practices through safety knowledge as an intervening variable towards safety performance on workers of PT. Masmindo Dwi Area.

**Materials and Method**

This research was conducted at the Awak Mas Site, the Contract of Work area of PT. Masmindo Dwi Area.
(MDA), Luwu Regency. The type of this research was observational using a cross-sectional study design.

The population was all workers at Awak Mas site of PT. Masmindo Dwi Area. 72 people from several departments were then selected as samples by using proportional stratified random sampling who already stated their willingness to participate in this study by signing the informed consent that had been issued by the Ethics Committee of the Hasanuddin University Faculty of Public Health.

Data collection was carried out by researchers. Safety management practice data including management commitment, safety training, worker involvement in safety, safety communication and feedback, safety rules and procedures, safety promotion policies, safety knowledge and safety performance were measured by interview using a questionnaire.

Individual factor data and frequency distribution were processed using SPSS 21 for windows. To assess the effect of safety management practices towards safety performance, a multivariate “path analysis” was conducted using the smartPLS application.

Result

Table 1. Characteristics of Workers at PT. Masmindo Dwi Area

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency (n)</th>
<th>Percent (%)</th>
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<tr>
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<tr>
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<td>Department of FM</td>
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<td>Department of GA/ Logistic</td>
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<tr>
<td>Young &lt;25 Years</td>
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### Table 1. Characteristics of Workers at PT. Masmindo Dwi Area

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<th>Old ≥25 Years</th>
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<td><strong>Safety Regulations and Procedures</strong></td>
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Cont... Table 1. Characteristics of Workers at PT. Masmindo Dwi Area

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Table 1 shows the distribution of respondents based on departments at PT. Masmindo Dwi Area. The most respondent came from Department of Geology with 12 people (16.7%). Then the most age group was in the old age (≥25 years), 62 people (86.1%), the most sex was male, 66 people (91.7%), the most work period was the oldone (≥ 1years), 43 people (59.7%). Then, based on the latest education, the most recent education was high school, 39 people (54.2%) and based on the health status category, the most respondents were in fit to work health status category, 39 people (54.2%).

Meanwhile, based on safety management practice indicators, management commitment variable was rated the most in good category by 65 respondents (90.3%), safety training was rated the most in good category by 64 respondents (88.9%), worker involvement was rated the most in good category by 70 respondents (97.2%), safety communication was rated the most in good category by 58 respondents (80.6%), safety rules and procedures were rated the most in good category by 49 respondents (68.1%), and safety promotion policies was rated the most in the good category by 38 respondents (52.8%).

The overall safety management practices were assessed by respondents to be in good category by 59 people (81.9%), safety knowledge was rated the most in good category by 58 people (80.6%) and safety performance was rated the most in good category by 56 people (77.8%).


<table>
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<td>10.3</td>
</tr>
</tbody>
</table>
Table 2 shows that out of 72 respondents, there were more workers who rated safety management practices in good category with good safety performance, 54 people (91.5%) compared to workers who rated safety management practices in poor category with poor safety performance, 11 people (84.6%). Furthermore, workers who have more safety knowledge tend to portrays good safety performance, 52 people (89.7%) compared to workers with poor safety knowledge who also tend to have poor safety performance, 10 people (71.4%).

![Figure 1 The Effect of Safety Management Practices Toward Safety Knowledge](image)

Figure 1 shows the research result model using path analysis. The analysis found that the effect of safety management practices towards safety knowledge has a value of $p = 0.011$ with an estimated contribution of 2.558, safety knowledge towards safety performance has a value of $p = 0.000$ with an estimated contribution of 4.397 and safety management practices towards safety performance has a value of $p = 0.024$ with an estimated contribution of 2.272. Then the results of simultaneous analysis of the safety management practices towards safety performance through safety knowledge have a value of $p = 0.001$ with an estimated contribution of 3.194.

**Discussion**

Work accidents generally can occur due to 2 main things, namely unsafe working conditions and unsafe behaviour. Safety Performance or safety behaviour can refer to safety related behaviour. In the occupational safety model at workplace by Christian et al. shows that safety performance antecedents are classified into individual factors and situational factors that are mediated by safety knowledge and safety motivation\(^9\). Situational factors are related to safety climate and safety leadership\(^10,11\).

The results explained before were in line with the statements of Vinodkumar and Bhasi who found that safety management practices had influence on safety performance\(^8\). Apart from being in line with the theory put forward by Vinodkumar & Bhasi, the results of this study were also in line with research by Froko et al. which found that there was a positive correlation between safety climate and safety performance that could predict compliance and safety participation\(^12\). Safety management practices had direct significant influence towards safety performance through its six indicators consisting of management commitment, safety training, worker involvement in solving safety problems, safety communication and feedback, safety rules and procedures, and safety promotion policies. Based on the results, most workers assessed that safety
management practices and safety performance were classified as good. This meant that management had maximized the implementation of safety management practices in order to improve worker safety performance so that work accidents could be avoided. However, even though it was dominated by workers with good safety performance, management also needs to pay attention to workers who had not been able to behave safely by assessing aspects of safety management practices which still could be improved.

Based on the observations result, the safety management practices at PT. Masmindo Dwi Area had been demonstrated properly based on safety management practice indicators. Management commitment indicators were shown through the application of the mining safety management system and the making of mining safety policies. Syamtinningrum suggests that OHS management in terms of commitment had a significant influence on unsafe behavior13. Safety training indicators were manifested through routine training for workers including refresher training. According to Saputra, workers who received training have a greater tendency to act and behave safely while working. Involvement of workers in safety at PT. MDA was demonstrated through the establishment of the Mining Safety Committee which was formed as a safety discussion forum for all workers through representatives of each department14. Safety communication and feedback at PT. MDA was demonstrated through the safety induction program, safety talk, pre shift meetings, and other safety programs. Geller states that one of the factors in behaviour that affects compliance in the safety triad theory is communication15. Based on this theory, it can be concluded that communication has an influence on the obedient behaviour of workers in the aspect of safety performance. Then the safety rules and procedures indicator were shown through making safety rules such as camp rules, standard operating procedures, working instructions, etc. However, even though management had maxed out the rules, there were workers who violate the rules and exhibit unsafe behaviour. The last indicator, namely the safety promotion policy. At PT.MDA, this indicator considered to be quite good but not optimal. This was because the safety reward and punishment program had not been implemented properly. Whereas Vredenburgh states that safety promotion policies which related to safety incentive programs could motivate employees to prioritize safety hence their safety performance might increase16. Maharja also said that reward system in the workplace affects the worker’s safety behaviour17.

Besides, Vinodkumar and Bhasi state that safety management practices affect safety performance through safety knowledge8. This study also shows results that were in line with Novi’s research and also research by Griffin and Neal which states that safety knowledge could mediate the correlation between safety climate and safety performance18,19. The significance in this study indicates that the good safety management practices at PT. MDA causes workers’ knowledge to improve where it led to a good safety performance. Safety knowledge was strongly related by employees’ knowledge of safety procedures that were applied in the company. With safety knowledge, workers would be more aware of unsafe environments. Having knowledge would tend came with the application of attitudes, and this plays an important role in reducing the rate of work accidents19.

The results showed that the majority of workers who rated safety management practices as good, also had good safety knowledge and had good safety performance. These results could conclude that if safety management practices were maximally implemented through the indicators such as management-made commitments then socialized to workers, safety trainings that were carried out routinely, involvement of workers in safety decisions making, good safety communication, rules and regulation and safety procedures were well made and enforced and also safety promotion policies through reward and punishment programs were also maximized, hence employees would possess and develop good safety knowledge and so in vice versa. In accordance with the results of the interview, worker’s knowledge about safety was in good category since PT. MDA had several OHS programs in improving safety knowledge which
was also closely related to safety management practices, namely safety training and safety communication.

**Conclusion**

As the result that had been presented previously, we could conclude that there was a direct and indirect effect of safety management practices towards safety performance through safety knowledge as an intervening variable. Researchers would like to suggest PT. Masmindo Dwi Area to increase and maintain workers to actively participate in the safety programs, management was also expected to increase the socialization of all programs related to safety management practices and to improve the quantity and quality of safety training for new workers as well as the refresher training.

**Conflict of Interest:** None

**Source of Funding:** Self

**Ethical Clearance:** Health Research Ethics Committee, Faculty of Public Health, Universitas Hasanuddin

**References**


16. Vredenburgh AG. Organizational safety: which management practices are most effective in


Decreased Antioxidant Capacity in Corn Farmers Occupationally Exposed to the Mixture of Herbicides

Unchisa Intayoung1, Kanyapak Kohsuwan1, Klintean Wunnapuk2, Ratana Sapbamrer3, Supakit Khacha-Ananda4

1Graduate Student, 2Assistant Professor, Department of Forensic Medicine, Faculty of Medicine, 3Associate Professor, Department of Community Medicine, 4Instructor, Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, 50200

Abstract

Background: The application of herbicides has been a common practice in worldwide agriculture, mainly with the goal to increase efficacy of weed control. So, most farmers faced a greater risk of herbicide exposure to develop adverse health effects from oxidative stress-induced herbicides. The aim of this study is to determine the urinary total antioxidant capacity (TAC) and 8-isoprostane levels in farmers using the mixture of herbicides in Long District, Phrae Province, Thailand.

Methods: Ninety-three participants were recruited. The spot urine samples (pre- and post-work) were collected. The urinary TAC was determined using ABTS radical scavenging assay. The urinary 8-isoprostane level was investigated by commercial ELISA kit.

Results: Most farmers worked on a farm during 1-5 h/day. Fifty-five percents of participants used the mixture of herbicide while working between glyphosate and paraquat as well as glyphosate and 2,4-D. The urinary TAC levels in pre-work urine sample of farmers applied combining herbicides were significantly higher than that level in their post-work urine sample. However, there was no significant difference between pre- and post-work urinary 8-isoprostane in farmers applied combining herbicides.

Conclusion: The results suggest that occupational exposure to mixture of glyphosate herbicides plus paraquat or 2,4-D could induce abnormal oxidative stress value especially antioxidant defense among agricultural workers.

Keywords: 8-isoprotane, Glyphosate, Paraquat, Total antioxidant capacity (TAC)

Introduction

Herbicides are being widely used for control the growing of weeds in agricultural activities1. Although herbicides are totally beneficial for agricultural activity, they are crucial sources of environmental pollution and exert irreversible adverse health outcomes both short- and long-term effects. The exposure of herbicides in workers generally caused from unintentional direct consumption, direct contact to skin due to improper application, inhalation of aerial sprays, or contaminated food consumption2. In the case of Thai farmers, there has been reported to have the high risk for herbicide exposure during occupation since most farmers practically apply high volume of the herbicide mixtures than recommended dosages for increase capability of weed control. As a result, these farmers faced greater risk of exposure to herbicides and development of adverse health effects3. The imbalance between oxidant and antioxidant defenses known as oxidative stress has been proposed as a key player in accounting for
herbicide toxic effect\(^4,5\). The overproduction of oxidants especially reactive oxygen species (ROS) caused adverse modifications to cell components such as lipids, proteins, and DNA damage. Lipid peroxidation is a one process of cell membrane-attacked ROS\(^6\). The by-products of this process such as malondialdehyde (MDA), propanal, hexanal, and 8-isoprostane were released and detected into various biological fluids\(^7\). In addition, the excess ROS was scavenged by effective protective mechanisms using antioxidant substances for preventing the attack of ROS on biomolecules and cells\(^8\).

Previous studies demonstrated that the increase of lipid peroxidation by-product (8-isoprostane) was induced in corn farmers exposed with mixtures of herbicide usage between atrazine and 2-(2,4-dichlorophenoxy) acetic acid (2,4-D)\(^9,10\). Available studies about antioxidant defense has been reported that superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) level was significantly decreased in herbicide-exposed workers\(^11,12\). Hence, the investigation of oxidative stress status in farmers who occupationally exposed with mixture of herbicides need to be focused for health status evaluation. In the present study, we hypothesized that the imbalance between reactive substance in forms of 8-isoprostane and antioxidant activity could be induced by mixture of herbicides (glyphosate or paraquat or 2,4-D). The main objective of this study was to determine the urinary 8-isoprostane and TAC levels in farmers using of the group of herbicides during agricultural activities in Long District, Phrae Province.

**Material and Methods**

**Ethical approval**

This study was approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University, Thailand (Study code: FOR-2562-06349). All subjects were informed about the protocol of this study and signed the consent form according to the guidelines of the Faculty of Medicine ethical committee.

**Location and population**

This study was conducted in Long district, Phrae province, Thailand. Ninety-three participants were eligible for this study. The inclusion criteria consisted of 1) farmers aged between 30 to 60 years 2) have been using combination of glyphosate or paraquat or 2,4-D during the study period 3) have never been diagnosed with kidney disease, diabetes and gout. All individuals were interviewed face-to-face using a questionnaire documenting their sex, working hours on the farm, year of work, and type of herbicide usage. All participants were divided into two groups based on the type of herbicide use 1) single application; farmers who applied only glyphosate 2) combined application; farmers who applied the mixture of herbicides in the group of glyphosate or paraquat or 2,4-D.

**Urine sample collections**

The spot morning urine before work (pre-work) and next morning urine after working (post-work) were collected from each participant. All samples were stored at -20°C prior the quantification of urinary creatinine, 8-isoprostane, and total antioxidant capacity (TAC)\(^13\).

**Quantification of urinary creatinine levels**

The urinary creatinine level was determined by colorimetric assay of Jaffé method\(^14\). In brief, 50 µL of urine samples (dilution 1:50) was mixed with 200 µL of working solution (1:1 of 4.365 mM picric acid (Sigma-Aldrich, USA) and 0.25 M sodium hydroxide (Sigma-Aldrich, USA)). After 45 min of reaction times, the absorbance of the orange-red creatinine picrate reaction product was measured at a wavelength of 492 nm using microplate reader (Synergy™ H4, BioTek Instruments, Inc., USA). The level of urinary creatinine was calculated by comparing with creatinine standard curve and expressed as mg/dL.

**Quantification of urinary 8-isoprostane levels**

The urinary 8-isoprostane were measured using commercial ELISA kit (Abcam, UK) according to the manufacturer’s instructions. The assay is based on the competitive ELISA between 8-isoprostane in sample and reference for binding to the primary antibody. The level
of urinary 8-isoprostane was calculated by comparing with 8-isoprostane standard curve and expressed as ng/mg creatinine.

**Quantification of urinary total antioxidant capacity (TAC) levels**

The urinary TAC was determined using ABTS radical scavenging assay\(^\text{15}\). Fresh ABTS\(^\bullet+\) solution was prepared by the mixture between 1.0 mM of AAPH and 2.5 mM of ABTS dissolved in 100 mL of phosphate buffer solution (PBS), pH 7.4. The mixture was incubated at 70°C for 30 min. Five microliters of urine sample was mixed with 245 µL of ABTS\(^\bullet+\) solution and incubated at 37 °C for 10 min. The color reaction was measured at a wavelength of 734 nm by microplate reader (Synergy™ H4, BioTek Instruments, Inc., USA). The urinary TAC level was calculated by comparing with ascorbic acid (Biochemica, Scotland) standard curve and expressed as mg vitamin C equivalent/L (mg VCE/L).

**Statistical Analysis**

The statistical analysis was conducted using the SPSS for Windows (Version 16.0. Chicago, SPSS Inc; 2007) and the GraphPad Prism (version 8.3.0 for windows, GraphPad Software, San Diego, California USA, www.graphpad.com). All demographic data (gender, working hours in farm, year of work, and type of herbicide usage) were analyzed by descriptive statistics. The levels of 8-isoprostane and TAC were calculated by comparing between pre-work and post-work urine samples using the Wilcoxon signed-rank test.

**Results**

Ninety-three participants were randomly recruited from five villages in Thung Lang subdistrict, Long District, Phrae province. The characteristics of these participants are shown in Table 1. The majority of participants were male (62.36%). Sixty-two percent of farmers worked on a farm during 1-5 h/day. Most of the participants worked for at least 20-40 years (60.44). Approximately 55.91% of farmers used the single type of herbicides (glyphosate) and 44.09% of farmers used the mixture of glyphosate plus paraquat or 2,4-D.

To determine oxidative stress in subjects, the finding of urinary TAC in all participants is shown in Figure 1. The urinary TAC level in the pre-work and post-work urine samples of farmers who used single herbicide was ranged between 0.08 – 1.04 and 0.15 – 1.30 VCE/L, respectively. The urinary TAC level in the pre-work and post-work urine samples of farmers who used the mixture of herbicides was ranged between 0.10 – 1.42 and 0.10 – 1.28 VCE/L, respectively. The urinary TAC levels in pre-work urine sample of farmers used combination of herbicide were significantly higher than that level in the post-work urine sample. However, there were no significant differences of the urinary TAC levels between the pre-work and post-work sample in farmer who used single herbicide.

To determine the lipid peroxidation, the urinary 8-isoprostane was measured using commercial ELISA kit. The urinary 8-isoprostane level is shown in Figure 2. The urinary 8-isoprostane level in the pre-work and post-work urine samples of farmers who used single herbicide was ranged between 119.6 – 242508 and 80.02 – 249859 pg/mg creatinine, respectively. The urinary 8-isoprostane level in the pre-work and post-work urine samples of farmers who used the mixture of herbicides was ranged between 230.0 – 11164 and 168.6 – 4601 pg/mg creatinine, respectively. There was no significant difference between the urinary 8-isoprostane in pre-work and post-work sample of both groups (single and combination of herbicides use).
Table 1 Demographic characteristics of participants from studied area in Thung Lang subdistrict, Long District, Phrae province.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>58</td>
<td>62.36%</td>
</tr>
<tr>
<td>female</td>
<td>35</td>
<td>37.64%</td>
</tr>
<tr>
<td>working hours in farms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5 hours/day</td>
<td>49</td>
<td>62.82%</td>
</tr>
<tr>
<td>6-12 hours/day</td>
<td>29</td>
<td>37.18%</td>
</tr>
<tr>
<td>year of work</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 19 years</td>
<td>13</td>
<td>14.29%</td>
</tr>
<tr>
<td>20 – 40 years</td>
<td>55</td>
<td>60.44%</td>
</tr>
<tr>
<td>&gt; 40 years</td>
<td>23</td>
<td>25.27%</td>
</tr>
<tr>
<td>Type of herbicides usage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>single</td>
<td>52</td>
<td>55.91%</td>
</tr>
<tr>
<td>combination</td>
<td>41</td>
<td>44.09%</td>
</tr>
<tr>
<td>Use of personal protective equipment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mask</td>
<td>90</td>
<td>96.77%</td>
</tr>
<tr>
<td>Glove</td>
<td>81</td>
<td>87.1%</td>
</tr>
<tr>
<td>Boots</td>
<td>92</td>
<td>99%</td>
</tr>
</tbody>
</table>

Figure 1 Box-plot demonstrating urinary TAC in herbicide-exposed farmers in the pre-work (open bar) and post-work (solid bar) urine sample. The line through the middle of the boxed represents the median of urinary TAC and the top and bottom of each box represents the first and third quartiles. The lower and upper error bars are computed as the lower and upper quartiles. *The TAC level was significantly different between the groups (P < 0.05).
Discussion

Occupational exposure of herbicides has been reported to induce several health outcomes. The mixing two or more herbicides together was practically applied in the farms rather than the single use of herbicide. Previous report revealed that glyphosate herbicide was mostly used in a mixture with other herbicides to enhance its potency for weed control\textsuperscript{16}. Therefore, it was probable that the farmers who applied the mixture of herbicides were at high risk of developing negative health effects resulting from oxidative stress-induced herbicides. The oxidative stress biomarker of lipid peroxidation, (8-isoprostane) and antioxidant defenses (TAC) indicating the imbalance of oxidative stress were determined this study. Our result found that the the urinary TAC levels in pre-work urine sample of farmers used combination of herbicides were significantly higher than that level in the post-work urine sample. The decrease of urinary TAC level could be indicative of oxidative stress or increased susceptibility to oxidative damage. Many classed of herbicides exerted the ability to generate reactive species, especially oxygen species molecules. Chronic oxidative stress could also reduce body antioxidant resources leading to lower antioxidant levels\textsuperscript{17}. Moreover, the level of two antioxidant substances; superoxide dismutase (SOD) and glutathione reductase (GR) was decreased in pesticide sprayers\textsuperscript{18}. Occupational exposure to pesticides among soybean farmers has been found to exert adverse effects at the biochemical level by observation of the alteration of antioxidant capacity in terms of ferric-reducing ability of plasma (FRAP) during exposure periods\textsuperscript{19}. According to the study of Ojha et al. 2011\textsuperscript{20} the level of antioxidant enzymes including catalase, superoxide dismutase, and glutathione peroxidase was decreased in dose-dependent in rats received the combined organophosphate pesticides.
Then, we aimed at assessing whether the decrease of antioxidant capacity from herbicide exposure may contribute to the elevation of oxidative stress marker. Previous studies reported that malondialdehyde (MDA) was the most popular marker for investigation of lipid peroxidation in vitro, however the level of MDA was a non-specific maker and depended on diet content\textsuperscript{21-23}. An alternative approach was to determine 8-isoprostanes since it was stable, unaffected by lipid contents in a diet, high specific, and sensitivity products of peroxidation\textsuperscript{7, 24}. The finding showed that there was no significant difference between the urinary 8-isoprostane in pre-work and post-work sample of both groups. There are two reasons for supporting our result. Firstly, the concentration of herbicides from occupational exposure may not be high sufficient to generate ROS, so the level of ROS may not be enough to disrupt lipid of cell membrane. Secondly, antioxidant defenses in the body can reduce ROS and maintain redox homeostasis resulting in unchange the level of 8-isoprostane\textsuperscript{25, 26}. Thirdly, working time of farmers was less than 5 h/day and they wore personal protective equipment (PPE) during their working, they could therefore be exposed to low level of herbicides\textsuperscript{27}. According to the study of Sapbamrer et al., 2019 the significant increase of SOD activity during the pesticide application season was associated with the number of working hours on the farm\textsuperscript{28}. Moreover, the oxidative stress markers including MDA and superoxide dismutase were reported to have the significant correlation with number of worked hours/day, hours of spraying pesticides/day in pesticide-exposed agricultural workers\textsuperscript{29}.

**Conclusion**

In the light of present findings, it can be concluded that occupational exposure to mixture of herbicides between glyphosate or paraquat or 2,4-D could induce abnormal oxidative stress index especially antioxidant defense among agricultural workers. Our findings could be applied for health surveillance in farmers who had a high risk to occupational herbicide exposure.

**Conflict of Interest:** The authors declare that they have no conflicts of interest.

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**References**


Mandibular Canine Index to Determine the Sex of the Individual- A Population based Study

Varsha A Shetty1, Vina Vaswani2, Suraj Shetty3, Mahabalesh Shetty4

1Assistant Professor, Department of Forensic Medicine and Toxicology, K S Hegde Medical Academy, Mangalore, 2Professor, Department of Forensic Medicine and Toxicology, Yenepoya Medical College, Mangalore, 3Associate Professor, 4Head of Department of Forensic Medicine and Toxicology, K S Hegde Medical Academy, Mangalore

Abstract

Introduction: The uniqueness of dental morphology plays an important role in Forensic Odontology, especially for human identifications. Mandibular canines may be considered vital for personal identification.

Objective: This study aims to establish the effectiveness of the Mandibular canine index in predicting the sex of an individual in a given population.

Materials and Methods: 400 participants (200Male, 200Female) were randomly selected from a given population between the age group 13 to 30 years. Using Digital Vernier calipers, the greatest mesio-distal crown width of both left and right Mandibular canines and Inter canine arch distance was measured.

Results: It show that canine dimensions and inter-canine distance were statistically larger in males compared to females and were statistically significant (p<0.001).

Conclusion: Canine dimensions can be considered as an adjunct for sex determination in our population

Keywords: Canine; Identification; Mandibular canines; Mandibular Canine Index; Odontology.

Introduction

Every human being has the right to protect their identity, whether living or dead. Forensic identification is the application of forensic science and technology to identify specific objects from the trace evidence often left at the scene. Establishment of the sex of an individual is a significant component of the identification process. Different odontometrical techniques are proven to be helpful in identification when the bodies are damaged beyond recognition.

Teeth are hard structures chemically stable and proven to be a good material for research and forensic identification. Role of teeth in the past dates back to centuries ago where teeth were used primarily for the identification or distinguishing a person from the rest based on their peculiarity. The identification of dental remains is of primary importance when the deceased person is skeletonized, decomposed, burned, or dismembered. Among all teeth, mandibular canines is known to exhibit good sexual dimorphism. Canine teeth are most likely to survive trauma. Hence, they can be considered as key teeth for personal identification.

Time and again, Forensic Odontology has proved that it has application with single identification or disasters with many people. The importance of forensic dentistry is increasing in the field of identification and solving crimes. The technique applied has started from the evolution beginning from the Garden of Eden to...
the present where all the identification of the accused of a rape trial in the state capital. It has an incredibly significant role, although the professional nature and its practices have evolved only in recent times and are still in progress.

This study aims to determine whether sex estimation is possible using mandibular canine tooth and investigate the reliability of canines in predicting sex in a given population.

**Materials and Methods**

This study group comprised of four hundred subjects (200 male, 200 female) from ages ranging from 13-30 years were randomly included from a given population. This age group was selected as all canine teeth would have erupted by this age, and intercanine distance would have been fixed. The attrition is expected to be minimal within this age group. All the participants included in the study had caries free teeth, normal overbite, absent spacing of teeth and no history of previous orthodontic treatment.

**Exclusion Criteria:**

- Absent canines or partially erupted or ectopically erupted teeth.
- Dental and occlusal abnormalities.
- Spacing in teeth.
- Physiological wear and tear (attrition, erosion, abrasion).
- Deleterious oral habits (bruxism).
- Bad/ poor oral hygiene.
- Previous orthodontic treatment.

**Ethical Clearance:** was taken from Yenepoya university ethics committee before starting the study. The participants who fit the criteria for this prospective study were randomly selected from a given population. The aims, objectives and procedure were clearly explained to each participant, and informed consent was obtained from the participant. All the measurements were taken intra-orally in a clean and well-illuminated room, keeping all the aseptic precautions in mind. A proforma filled with a detailed history including sex, age, address, date of birth, previous dental history, and the measurements of the teeth dimensions. Digital Vernier Calipers (Bruder Mannesmann, Germany), which is calibrated to 0.01mm, was used to take the teeth measurements.

**Mandibular canine width** was measured as the greatest mesiodistal dimension of mandibular canine on either side of the jaw using Digital Vernier calipers.

**The Intercanine distance** was measured as the linear distance between the cusp tips of the right and left mandibular canine using Digital Vernier calipers.

All these measurements were taken using the Hunter and Priest method. Two such measurements from both sides i.e., Right mandibular canine and Left mandibular canine are taken and then average of the two measurements is taken as the final value. This was done to rectify intra-observer error.

The measurement readings obtained were lay open to analysis to come to conclusions. The mandibular canine index was calculated using the formula:

\[
\text{MCI} = \frac{\text{Mesio-distal crown width of the mandibular canine}}{\text{Mandibular canine arch width}}
\]

Further the standard canine index was calculated for the mandibular canines using the formula derived by Rao et al.

\[
\text{Standard MCI} = (\text{Mean male MCI} - \text{SD}) + (\text{Mean female MCI} + \text{SD})
\]
According to Rao et al the calculated MCI was compared to standard MCI. The result obtained were both compared if the value was higher than the Standard MCI then it is considered a male canine tooth and lesser than standard MCI then it is considered a female canine tooth.11

All data were tabulated in the excel sheet and subjected to statistical analysis. SPSS software 16 was used and t test and multivariant discriminant analysis was applied and to develop a regression formula from this study.

**Results**

A total of 400 subjects participated in the study. The study included males and 200 females and 200 males. The parameters that were recorded were-

a) The Mesiodistal dimension of Left mandibular canine

b) The Mesiodistal dimension of Right mandibular canine

c) The Intercanine arch distance

Among the 400 participants, the mean value left mandibular canine was found to be 6.58 for males and 6.30 for females. The p value for this index is < 0.001 which is statistically highly significant as shown in Table No 1.

The mean value of the width of right mandibular canine was found to be 6.61 for males and 6.34 for females. The p value for this index is < 0.001 which is statistically as shown in Table No 1.

The intercanine distance of mandibular canines was measured for all subjects intra orally. The 400 participants the mean value of the intercanine distance of mandibular canines was found to be 27.51 for males and 26.31 for females. The p value for this index is < 0.001 which is statistically highly significant as depicted in Table No 1.

Multi variant Discriminant analysis was applied on the 400 samples from the participant to derive a formula for accurate of sex prediction.

The result is as follows: (Table 2)

1. In direct method, the percentage of accuracy using left mandibular canine, right mandibular canine and intercanine distance values the sex prediction is 63.60%.

Hence, the regression formula for direct method is,

\[ Y = 1.549 \times LMWidth + 0.704 \times RMwidth + 0.145 \times IC - 18.41 \]

2. In step wise method, it could be calculated only for left mandibular canine and intercanine distance because the right mandibular canine vales were insignificant. Hence using this method sex prediction for left mandibular canine and intercanine distance is 64.80%.

Hence, the regression formula for step wise method is,

\[ Y = 2.064 \times LMwidth + 0.177 \times IC - 18.05 \]

### Table no 1: RESULTS OF SEX WISE DISTRIBUTION OF MEAN OF CANINE MEASUREMENTS

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>SEX (200 FEMALES  200 MALES)</th>
<th>MEAN</th>
<th>SD</th>
<th>RANGE (95% confidence interval)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
<td>Upper bound</td>
</tr>
<tr>
<td>LEFT MANDIBULAR CANINE</td>
<td>FEMALES</td>
<td>6.30</td>
<td>0.42</td>
<td>6.24</td>
<td>6.36</td>
</tr>
<tr>
<td></td>
<td>MALES</td>
<td>6.58</td>
<td>0.31</td>
<td>6.53</td>
<td>6.62</td>
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</table>
### Table no 1: RESULTS OF SEX WISE DISTRIBUTION OF MEAN OF CANINE MEASUREMENTS

<table>
<thead>
<tr>
<th></th>
<th>FEMALES</th>
<th>MALES</th>
<th>FEMALES</th>
<th>MALES</th>
<th>P&lt;0.001</th>
<th>P&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIGHT MANDIBULAR CANINE</td>
<td>6.34 0.45 6.28 6.40</td>
<td>6.61 0.30 6.56 6.65</td>
<td>P&lt;0.001</td>
<td>HIGHLY SIGNIFICANT</td>
<td></td>
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</tr>
<tr>
<td>INTERCANINE DISTANCE</td>
<td>26.31 1.99 26.04 26.58</td>
<td>27.51 2.11 27.21 27.81</td>
<td>P&lt;0.001</td>
<td>HIGHLY SIGNIFICANT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table no 2: RESULTS OF DISCRIMINANT ANALYSIS

<table>
<thead>
<tr>
<th></th>
<th>Independent variables</th>
<th>Unstandardized coefficients</th>
<th>Standardized coefficients</th>
<th>Constant</th>
<th>Centroid</th>
<th>Section point</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIRECT METHOD</td>
<td>left mandibular mesio-distal canine width</td>
<td>1.549 0.574</td>
<td>-18.41</td>
<td>0.013</td>
<td>63.60%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STEPWISE METHOD</td>
<td>left mandibular mesio-distal canine width</td>
<td>2.064 0.765</td>
<td>-18.050</td>
<td>0.013</td>
<td>64.80%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

In forensic cases teeth can be an important deciding factor in the identification of an individual. Canines are different from other teeth with respect to survival and sex dichotomy. Although canine survival and sex dichotomy are not related to each other still they are useful in the process of identification. In the present study 400 participants were randomly selected from a community between the age group 13-30 years. The mean age for eruption of canine tooth is 10.87 years and
according to literature is fully erupted by the age of 11 to 12 years and also inter canine distance is usually fixed by 12 years of age. Hence the age minimum limit in this study was taken to be 13 years. The age group maximum limit to avoid teeth which has gone for attrition and other age-related teeth queries is 25 years. Hence the age maximum limit in this study was taken to be 30 years. However, participants who had visible attrition of teeth were excluded from the study irrespective of their age. In studies conducted by Rao et al, Muller et al, the study participants consisted of students from a particular college rather than a targeted population. In this study 400 participants who were selected were from a targeted population who had similar ethnic, cultural, and behavioral backgrounds.

In a preliminary study conducted by Rao et al in 1989 on 766 participants randomly selected between age group 15 to 21 years, Multi variant discriminate statistical analysis showed 85.9% accuracy in sex determination which was highly significant however, in our study with sample size of 400 randomly selected from a given community between the age groups 13-30 years showed multivariant discriminant analysis of 64.80%. This difference in result could be attributed to the fact that Rao et al study was not a population-based study and the measurements of the teeth taken were using dividers which could lead to errors.

In another study conducted by Muller et al in France 2001 among 424 students at university of Nice Sofia Antipolis with 210 females and 214 male participants in the age group 20.4 ± 2.9 years. The aim of the study was to calculate mandibular canine index for sex determination as done by Rao et al but by taking occlusion into consideration. There was effort made by the author to find out the reliability of the method and its influence when there is lower anterior dental crowding. In cases of correct alignment, the values were higher for males compared to females however the study showed lower sex predictability value of 59.57% which coincides with our study with sex predictability of on 64.80% which is not significant.

In a study conducted by Vishwakarma and Guha in 2011 in Gwalior medical college among 180 participants (90 females and 90 males) between the age group 17-23 years using vernier calipers study comments on left mandibular canine being a better parameter to identify sex. In their study the mesiodistal width of mandibular canine of males and females were found to be statistically significant for left mandibular canine compared to the right. But in our study mean value of right mandibular canine is higher compared to the mean of left mandibular canine. However, when using step wise selection in multivariant discriminant analysis for right mandibular canines the data was invalid, and no accuracy or result could be obtained.

In a study conducted in 2014 by Bakkanavar S et al among 500 students (250 females and 250 males) randomly selected of age group 15 – 25 years of South India using a digital calipers to take measurements of both maxillary and mandibular canine to see if this could be used as a tool for sex determination. The results showed that the mesio-distal crown width of both right and left mandibular canines in males were more than the females. The sex predictability according to the study was poor for mandibular canine index which was 74.8%. In comparison to our study where the value was 64.80% the value of Bakkanavar S et al seems higher. This can be attributed to the fact that unlike our study which is based on a particular population Bakkanavar S et al study participants were students from diverse and from different backgrounds which could have contributed to the higher values. However, Bakkanavar S et al study shows that results obtained show poor sex predictability which is similar to our study.

**Conclusion**

The developing field of forensic dentistry in India relies a lot on easy and inexpensive methods for identification. Forensic dentistry helps in investigation when other means fail to do so. A dentist can be asked upon to render on their expertise in the field of forensics, when in need during investigation. A data base could be created with morphometric measurements
with the purpose to determine if there is significance among larger population. This data may be beneficial for anthropological, genetic, legal, and forensic applications. It is recognized that canines among other teeth exhibit sexual dimorphism. This study measured linear dimensions of teeth. However more accuracy can be obtained by using sophisticated equipments and also the use of more standardized and complex mathematical equation. This might increase the accuracy of the prediction. However, one must not forget that only when more parameters are considered the more accurate the determination of sex will be. For this reason, clues from dentition must be correlated with other clues.

**Source of Funding:** Self

**Conflict of Interest:** Nil

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Application of Machine Learning and Deep Learning Technology in the Field of Dentistry among Top Five Gross Domestic Product (GDP) Countries - A Scientometric Analysis

Varshini Rajendran¹, V.V.Bharathwaj², P.Suganya³, D.Prabu⁴, Rajmohan⁵, M.R.Prashanthy³

¹Undergraduate Student, ²Lecturer, ³Postgraduate, ⁴Professor, ⁵Reader, Department of Public Health Dentistry, Chennai, Tamilnadu, India

Abstract

Background: The Scientometric analysis of the articles helps to provide more useful information about the field of research.

Aim: This study aims to evaluate the articles based on the application of deep learning and machine learning in the field of Dentistry among top five Gross domestic product (GDP) countries.

Materials and Method: The articles related to Deep learning (DL) and Machine learning (ML) in the field of Dentistry among top five GDP countries were retrieved from electronic databases such as Google Scholar, Scopus and PubMed using keywords and MeSH terms. The parameters such as author name, number of authorships, number of citations, year, place of the study, journal, type of article, field of dentistry, outcome and implementation of deep learning and machine learning in dentistry were evaluated. The collected data were analyzed and tabulated using descriptive analysis.

Results: Totally thirty seven number of articles regarding deep learning and machine learning in the field of dentistry among top five GDP Countries were obtained. The maximum number of studies was published in United States of America and minimum studies in Japan. The articles were published between the years 2001 and 2020 in different fields of Dentistry.

Conclusion: The Scientometric analysis of articles related to the use of deep learning and machine learning in the field of Dentistry helps the researchers and dentists to have an idea about the trends and improvement for better analysis and treatment in the future for greater benefit of patients.

Keywords: Deep learning, machine learning, research, Gross domestic product, dentistry.

Introduction

Artificial intelligence is a branch of computer science demonstrated by machine, contrary to natural intelligence laid out by living beings. Artificial intelligence plays a crucial role in the recent advancements of dentistry [1].

The ideas of machine learning have been applied in various fields including medicine and dentistry for decades. Machine learning is an application of artificial intelligence which provides the ability to automatically learn and improve from experience without being programmed. This mainly focuses the development of computer program to access data and learns automatically. It is used to assess the pattern in data and make better decision for the future. The main aim is to allow the computer to learn automatically without human intervention or assistance and use of instructions [2].
Deep learning is a subset of machine learning in artificial intelligence (AI) that has network capable of learning unsupervised from data. It is also known as deep neural learning or deep neural network. Deep learning methods have dramatically improved advances in speech recognition, vision, hearing, genomic and in the field of dentistry [3].

In the recent years, machine learning and deep learning have been implemented in dentistry and made a huge impact for better treatment decisions. This technology is very advantageous and beneficial for dental professionals as they play a convenient role in precise teeth detection and classification [4], dental diseases diagnosis [5], interpretation of dental images automatically [6], highly accurate automatic teeth type classification on CT image for forensic identification [7], assessment of Tooth morphology on radiograph [8], detection of Periodontal Bone Loss [9], detection of apical lesions [10], in orthodontic and Prosthodontic treatments [11], malocclusion assessment [12] dental Age Estimation, 3D prosthesis [13] etc.

Scientometric analysis is the field of study which concerns itself with analyzing and measuring the scientific literature. It helps to measure the impact of scientific articles and journals and use of measurements in management contexts and policy [14]. Gross domestic product (GDP) is the monetary value of all finished goods and service made within the country during a specific period [15] based on that the country with highest GDP are United States of America followed by China, Japan, Germany, India and so on [16].

This article helps to determine the scientific articles that were published about machine learning and deep learning in dentistry among top five GDP countries, i.e. United States of America, China, Japan, Germany, India, so that researchers can have knowledge about the expansion of technology.

Materials and Methods

This study was performed to evaluate the Scientometric analysis of articles related to machine learning and deep learning in the field of Dentistry among top five Gross domestic product (GDP) countries. The articles were obtained from electronic databases such as PubMed, Scopus, Google Scholar as ‘deep learning in dentistry’ and ‘machine learning in dentistry’. The data were collected using MeSH term and keywords such as deep learning, machine learning and dentistry.

The articles published among top five GDP countries were evaluated and the parameters like author name, number of authorships, number of citations, field of Dentistry, type of article, name of the journal, year of publication, outcome of the study and implementation in dentistry was included.

The inclusion criteria of this study includes only articles related to machine learning or deep learning in dentistry, only original and review articles were included. Only articles from the top five GDP countries are considered. The exclusion criteria of this study are the articles with no open access and articles published in other than English language were excluded.

Results and Discussion

A total number of articles related to the application of deep learning and machine learning in the field of dentistry among top five GDP countries were thirty seven. The articles were published from the year January 2001 to April 2020. Most of the studies are conducted in United States of America whereas the least number of articles are published in Japan with the lowest number of articles.
**Figure 1:** Type of articles related to deep learning and machine learning in dentistry among top five GDP countries.

<table>
<thead>
<tr>
<th>Type of articles</th>
<th>USA</th>
<th>China</th>
<th>Japan</th>
<th>Germany</th>
<th>India</th>
</tr>
</thead>
<tbody>
<tr>
<td>review article</td>
<td>3</td>
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<td>0</td>
<td>4</td>
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<tr>
<td>original article</td>
<td>8</td>
<td>9</td>
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<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

**Figure 2:** Implementation of machine learning and deep learning in different fields of dentistry among top five GDP countries

Figure 2 shows the implementation of articles related to deep learning and machine learning in various fields of dentistry among top five GDP countries. 1
Figure 3: Year wise distribution of articles related to application of deep learning and machine learning in dentistry

Figure 3 shows the number of articles published on application of machine learning and deep learning in dentistry among top five GDP countries. They were published between the years 2001 and 2020. The number of articles was found to be gradually increases from the year 2017. The maximum number of articles was published in the year 2019.

The study conducted by Suganya et al in the year 2020 had discussed about the bibliometric analysis of top cited artificial intelligence articles in the field of dentistry [44]. Gross domestic product (GDP) is the total monetary or market value of all the finished goods and service provided within the country’s border in a specific time period. The top five countries with highest GDP are United States of America, followed by China, Japan, Germany and India. There are articles published from these counties on the application of deep learning and machine learning in the field of dentistry. Information like author, number of authorships, journal, year, number of citations, field of dentistry, outcome of the study, were tabulated for each article.

Totally eleven articles were published from 2001 to 2020. One article had single authorship. Out of eleven articles, eight of them are original articles and three are review articles. The deep learning and machine learning were implemented in different fields of dentistry like orthodontics, Prosthodontic treatment, oncology, diagnosis and public health care. The articles with highest number of citations in United States of America were twenty four and there were four articles with nil citation.

Totally nine articles were published in China; all of them were original articles. Deep learning and machine learning methods were implemented in different fields of dentistry including radiology, orthodontics, forensics etc. They were published between the years 2013 and 2019. The articles with highest number of citations in an article published in China were nineteen and three articles had the lowest number with one citation.

Deep learning and machine learning methods were used for tooth identification and diagnosis. Implementing these methods gave better and accurate results. The
highest number of citations in an article published in Japan was eighty two and lowest number of citation was one.

Totally five articles were published in Germany; all of them were original articles. These articles were published between the years 2019 and 2020. Deep learning and machine learning techniques were implemented in endodontic periodontal diagnosis and in forensics. The highest number of citations in an article published in Germany was eight and two articles had nil citation.

Totally 8 articles were published in India, out of which 1 article had Single authorship. These articles were published from 2017 to 2020 and were applied in different fields. Table 10 shows the outcome and the implementation of the studies published in India based on the deep learning and machine learning in dentistry. Accordingly, machine learning and deep learning methods were used in dentistry for accurate and better diagnostic purpose. Highest number of citations in an article published in India was twenty and the six number articles had nil citation.

The highest number of citations in an article was eighty two which was published in Japan in 2016. There are twelve articles among all the five countries with no citation. According to figure 1, a total of thirty seven articles were published related to application of machine learning and deep learning in dentistry, among which eleven articles were published in the United states of America, nine articles in China, eight articles in India, five articles in Germany and four articles in Japan. All these articles have open access, and all were in English language.

Accordingly, China, Japan, and Germany had no review article, they were all original articles. In India, four articles were published on machine learning and deep learning was review article and four were original. In the United States of America, eight articles were original and three were review articles.

The number of articles with implementation of machine learning and deep learning in different fields of dentistry like namely Prosthodontics, Endodontics, Periodontics, Pedodontics, oral oncology, forensic dentistry and public health.

In the year 2020, till the month of April four number of articles was published regarding machine learning and deep learning in dentistry among top five GDP countries. From the figure it is evident that the number articles published in a year gradually increases than the previous year. Before the end of 2020 more articles might be conducted. Considering all the data, more number of researches may be conducted in the future, following a trend.

The limitation of this study includes only articles with full text and open assess. The articles published in other than English language were not taken into an account. Other databases should also be considered to get more relevant data. The data was obtained from only countries with highest GDP. Further studies should be conducted throughout the world to get more information and knowledge.

**Conclusion**

This article concludes that based on the previously published articles related to the application of machine learning and deep learning in dentistry, we can predict that more number of articles will be published in the future. This Scientometric analysis helps researchers to gain more knowledge about the advancements in dental treatment and diagnosis. Totally thirty seven number of articles were published among top five GDP countries, of which America has the highest number of articles. The application of deep learning and machine learning have progressively improved treatment plan by getting accurate results and less time consuming. It was useful to dental professionals as well as to public by providing self assessment of their oral health which prevents and reduce the further progression of diseases at an earlier stage.
Future Scope:

This article will be more useful for dental professionals and public regarding the recent advancements and treatment modalities of dental health. This paves a pathway for the scientists to be more updated on recent advancements in dentistry and also motivates them to conduct more researches to obtain maximum benefits for the people.

Acknowledgements: Nil

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Taken from institutional review board of Public Health Dentistry Department, SRM Dental College, Ramapuram.

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Influence of Kappa-Casein and Beta-Lactoglobulin Genetic Variants on Milk Productivity of Red-and-White Cattle

Velmatov, A.A¹, Dunin I.M.², A. Al-Isawi³, T.N. Tishkina⁴

¹PhD of Agricultural Sciences, Mordovian Research Institute of Agriculture. The Branch of the Federal State Budgetary Scientific Institution FANTS of the North-East, Russia, Republic of Mordovia, Saransk, Russia, ²Doctor of Agricultural Sciences, Professor, Academician of the Russian Academy of Sciences. All-Russian Scientific Research Pedigree Institute, Pushkin District, Lesnye Polyany, Moscow, Russia, ³PhD of Agricultural sciences, Iraqi Ministry of Agriculture, Baghdad. Iraq, ⁴PhD of Agricultural Sciences, Mordovian N.P. Ogarev State University, Mordovia Republic, Saransk, Russia

Abstract

The genotyping of red-and-white cattle for the genes of kappa-casein and beta-lactoglobulin in the farm has revealed the presence of three genotypes, namely AA, AB and BB. In first-calf heifers, the frequency of the AA genotype of the kappa-casein gene was 33.10%, AB was 56.69%, BB was 10.21%. A allele frequency was 0.61%, B - 0.39%. In first-calf heifers, the frequency of the AA genotype of the beta-lactoglobulin gene was 16.20%, AB was 53.52%, BB was 30.28%. A allele frequency was 0.43%, B - 0.57%. The AB AB genotype prevailed in the population of red-and-white cows (29.23%) according to the frequency of occurrence of complex genotypes of kappa-casein and beta-lactoglobulin. In terms of milk yield, cows with genotype BB of the kappa-casein gene surpassed cows of the same age with genotypes AB and AA by 153-644 kg. In terms of the content of fat and protein mass fraction, the superiority was 0.06-0.19% (P≥0.95; 0.99), and protein by 0.13-0.22% (P≥0.99; 0.999). In terms of milk yield, the superiority of cows with the AA genotype of the beta-lactoglobulin gene over cows of the same age with the AB and BB genotypes was 208-413 kg (P≥0.95). According to the mass fraction of fat and protein in milk, preference is given to cows with the BB genotype, whose milk contains 0.06-0.09% more fat and 0.08-0.17% protein (P≥0.95; 0.99) compared with cows with AB and AA genotypes. The yield of milk fat in first-calf heifers with genotype BB of kappa-casein was also higher by 14.3 kg and 43.5 kg (P <0.05) than that in animals of the same age with genotypes AB and BB. Heifers with the AA genotype of the beta-lactoglobulin gene had the highest milk fat yield of 328.36 kg. This is 13.87 kg (P <0.05) more compared to the BB genotype. In terms of milk protein yield, the difference in kappa-casein genotypes was: BB to AA 36.9 kg (P <0.01), BB to AB 14.3 kg.

Key words: breed, type, gene, kappa-casein, beta-lactoglobulin, milk production, fat, protein

Introduction

The use of genetic markers, which are responsible for the quality indicators of milk productivity in animal husbandry, will improve the economically useful characteristics of cattle. Kappa-casein and beta-lactoglobulin genes are some of these markers [1,2,3,4,5,6,7,8,9].

Kappa-casein is one of the genes that is directly related to indicators of milk protein content and technological properties of milk. It is believed that when breeding cattle, it is preferable to increase the frequency of the B allele of kappa-casein in the population. This allele allows to increase the protein content in milk, and also to improve the technological properties of milk in cheese production [10,11,12]. Many scientists are not yet able to determine exactly which of the A or B alleles of the beta-lactoglobulin gene will improve the biochemical and technological characteristics of milk [13,14,15,16].
Purpose of the study. The aim of this work is to study the polymorphism of kappa-casein and beta-lactoglobulin genes in the genomes of red-and-white cows of the being created Volga type and their influence on animals’ milk productivity.

Materials and Methods

The research was carried out with the red-and-white cows in the Federal State Unitary Enterprise “The 1st of May” at State Scientific Institution “Mordovian Research Institute of Agriculture”. The polymorphism of the beta-lactoglobulin and kappa-casein genes was assessed on the basis of polymerase chain reaction (PCR). For further research, based on analogues principle [17], according to the results of genotyping for the gene of beta-lactoglobulin and kappa-casein, 3 groups of red-and-white cows were formed. The first group included heifers with the genotype of beta-lactoglobulin and kappa-casein AA (control group), the second consisted of cows with the AB genotype, and the third involved animals with the BB genotype.

Blood obtained from the tail vein of animals was added to tubes with 100 mM EDTA to a final concentration of 10 mM.

Analysis of the beta-lactoglobulin gene locus

PCR was carried out by a programmable thermal cycler “Tertsik” (Russia) in a volume of 20 μL with primers Forward: 5’- GTCTTTGTGGCTGACACCCGACTACA-3’ and Reverse: 5’- CAGGACACCCGCCGTATATGAC-3’ designed by J.F. Medrano and E. Aguilar-Cordova, [1990] to amplify a 350 bp beta-lactoglobulin gene fragment.

To determine the allelic polymorphism of the kappa-casein gene, 20 μl of PCR samples were treated with 10 units restriction endonucleases Hfni I in 1 x buffer “O” and 1 x buffer “W” (made by SibEnzyme (Russia) at 37°C overnight.

The frequency of occurrence of the kappa-casein and beta-lactoglobulin genotypes was determined by the formula:

\[ p = \frac{n}{N} \]

p is the frequency of the genotype,

n is the number of individuals with a certain genotype,

N is the number of individuals.

The frequency of individual alleles was determined by the formula:

\[ p_A = \frac{n_{AA} + n_{AB}}{2N} \quad \text{and} \quad q_B = \frac{n_{BB} + n_{AB}}{2N}, \]

where

\[ p_A \] is frequency of A allele,

\[ q_B \] is frequency of B allele,

N is the total number of alleles.

Having conducted scientific and production experiments, the following indicators were studied:

- milk productivity of cows was determined by monthly control milking during the entire first lactation;
- fat and protein content was determined with “Klever-1M” device in farm dairy laboratory once a month.
The research results were processed by the method of biometric statistics \[18,19\] on a personal computer.

**Results**

Carried out genotyping of red-and-white cattle for kappa-casein and beta-lactoglobulin genes revealed the presence of three AA, AB and BB genotypes. In first-calf heifers, the frequency of the AA genotype of the kappa-casein gene was 33.10%, AB type was 56.69%, and BB type was 10.21%. A allele frequency was 0.61%, and B allele one was 0.39%. In first-calf heifers, the frequency of the AA genotype of the beta-lactoglobulin gene was 16.20%, AB type was 53.52%, and BB type was 30.28%. A allele frequency was 0.43%, and B allele one was 0.57%. The AB AB genotype prevailed in the population of red-and-white cows (29.23%) according to the frequency of occurrence of complex genotypes of kappa-casein and beta-lactoglobulin. Milk productivity study of cows with different genotypes of kappa-casein showed (Table 1).

### Table 1 - Milk productivity of cows with different genotypes of kappa-casein

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Genotype</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BB</td>
<td>AB</td>
</tr>
<tr>
<td>n</td>
<td>29</td>
<td>161</td>
</tr>
<tr>
<td>Milk yield, kg</td>
<td>6943.3±145.8</td>
<td>6790.5±267</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.90±0.02</td>
<td>4.84±0.02</td>
</tr>
<tr>
<td>Milk fat, kg</td>
<td>340.2±11.7</td>
<td>325.9±11.2</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.57±0.03</td>
<td>3.44±0.03</td>
</tr>
<tr>
<td>Milk protein, kg</td>
<td>247.9±7.1</td>
<td>233.6±6.7</td>
</tr>
</tbody>
</table>

Note: * P<0.05; ** P<0.01; *** P<0.001

Milk yield of first-calf heifers with genotype BB was higher than milk yield in animals with genotypes AA by 644 kg (P <0.01) and with genotype AB by 153 kg. Heifers with the BB kappa-casein genotype had higher levels of fat in milk than first-calf heifers with the AA and AB kappa-casein genotypes. The difference was 0.19% (P<0.001) and 0.06% (P<0.05) respectively. The milk fat yield in first-calf heifers with the BB genotype was 340.2 kg, which exceeds the same indicator in first-calf heifers with a homozygous AA genotype by 43.5 kg (P <0.001) and with a heterozygous AB genotype by 14.3 kg. In animals with genotype BB, the protein content in milk was 3.57%, which is 0.22% higher (P <0.001) than in animals with genotype AA and higher than in heifers with genotype AB by 0, 13% (P <0.01). In terms of the yield of milk protein, animals with the BB genotype also had a superiority. The difference in kappa-casein genotypes was: BB to AA 36.9 kg (P <0.01), BB to AB 14.3 kg.
Table 2 - Milk productivity of cows with different genotypes of beta-lactoglobulin

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Genotype</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BB</td>
<td>AB</td>
</tr>
<tr>
<td>n</td>
<td>86</td>
<td>152</td>
</tr>
<tr>
<td>Milk yield, kg</td>
<td>6471±79.6</td>
<td>6679.14±110.8</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.86±0.02</td>
<td>4.80±0.02</td>
</tr>
<tr>
<td>Milk fat, kg</td>
<td>314.49±3.16</td>
<td>320.60±3.2</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.37±0.02</td>
<td>3.45±0.03</td>
</tr>
<tr>
<td>Milk protein, kg</td>
<td>218.07±6.86</td>
<td>230.43±5.22</td>
</tr>
</tbody>
</table>

Note: * P<0.05; ** P<0.01; *** P<0.001

Milk yield per lactation in the group of first-calf heifers with the beta-lactoglobulin BB genotype was 6471 kg, AB genotype was 6679 kg and AA genotype was 6884 kg. Cows with the BB genotype were inferior to the same age animals with the AB genotype by 208 kg, with AA genotype by 413 kg (P <0.05) milk.

The highest fat content in milk (4.86%) was observed in heifers with the BB genotype of the beta-lactoglobulin gene, which is significantly higher in comparison with the AB genotype by 0.06% (P <0.05) and AA genotype by 0.09% (P <0.05). In terms of mass fat content, animals with A allele of the beta-lactoglobulin gene in their genome showed superiority (320.60-328.36 kg). They exceeded the same age animals by 6.11 and 13.87 kg (P <0.05), respectively. Animals with the A allele of the beta-lactoglobulin gene in their genome had significantly higher protein content in milk than the same age animals with the homozygous BB genotype by 0.08% -0.17% or by 12.36-25.62 kg.

The data in Table 3 show the effect of different combinations of kapp-casein and beta-lactoglobulin genes on cows’ milk production.

Table 3 - Milk productivity of cows with common genotypes for kappa-casein and beta-lactoglobulin genes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Milk yield, kg</th>
<th>Fat, %</th>
<th>Milk fat, kg</th>
<th>Protein, %</th>
<th>Milk protein, kg</th>
</tr>
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<tbody>
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<td></td>
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</tr>
<tr>
<td>Red-and-White</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA AA</td>
<td>7</td>
<td>6591.27±136.8</td>
<td>4.51±0.13</td>
<td>297.27±16.8</td>
<td>3.45±0.09</td>
<td>227.40±7.5</td>
</tr>
<tr>
<td>AA AB</td>
<td>51</td>
<td>6454.37±107.1</td>
<td>4.62±0.09</td>
<td>298.51±12.6</td>
<td>3.35±0.06</td>
<td>216.22±9.1</td>
</tr>
<tr>
<td>AA BB</td>
<td>36</td>
<td>6345.00±125.3</td>
<td>4.80±0.05</td>
<td>304.88±15.1</td>
<td>3.29±0.12</td>
<td>209.07±13.1</td>
</tr>
<tr>
<td>AB AA</td>
<td>28</td>
<td>6913.11±130.9</td>
<td>4.57±0.30</td>
<td>316.27±20.3</td>
<td>3.49±0.15</td>
<td>241.61±10.3</td>
</tr>
<tr>
<td>AB AB</td>
<td>83</td>
<td>6699.82±142.4</td>
<td>4.69±0.18</td>
<td>314.22±18.2</td>
<td>3.39±0.08</td>
<td>227.46±11.4</td>
</tr>
<tr>
<td>AB BB</td>
<td>50</td>
<td>6590.75±98.6</td>
<td>4.87±0.10</td>
<td>320.97±13.9</td>
<td>3.34±0.17</td>
<td>220.13±14.8</td>
</tr>
<tr>
<td>BB AA</td>
<td>11</td>
<td>6836.7±130.4</td>
<td>4.60±0.16</td>
<td>314.83±14.3</td>
<td>3.56±0.12</td>
<td>243.39±18.2</td>
</tr>
<tr>
<td>BB AB</td>
<td>18</td>
<td>6776.22±230.7</td>
<td>4.72±0.12</td>
<td>319.84±10.6</td>
<td>3.46±0.14</td>
<td>234.46±10.0</td>
</tr>
</tbody>
</table>
Studies have shown that red-and-white cows have a clear tendency for the influence of the beta-lactoglobulin genotype on milk yield. Thus, in the group of cows with the AB AA genotype of the kappa-casein and beta-lactoglobulin genes, the milk yield was 322.36 kg higher (P≤0.05) compared to animals with the AB BB genotype (Table 3).

The studies carried out made it possible to assess the productive capabilities of animals of the creating Volga type red-and-white dairy cattle with different genotypes of kappa-casein and beta-lactoglobulin.

Analysis of animals’ milk productivity found out a significant difference in fat content in groups with genotypes AAAA < AABB (P≤0.05) and AAAA < ABBB (P≤0.05), as well as in the amount of milk protein AABB < ABAA (P≤0.05).

**Discussion**

Carried out genotyping of red-and-white cattle for the genes of kappa-casein and beta-lactoglobulin revealed the presence of three genotypes AA, AB and BB. In first-calf heifers, the frequency of the AA genotype of the kappa-casein gene was 33.10%, AB genotype was 56.69%, BB genotype was 10.21%. A allele frequency was 0.61%, B - 0.39%. In first-calf heifers, the frequency of the AA genotype of the beta-lactoglobulin gene was 16.20%, AB was 53.52%, BB was 30.28%. A allele frequency was 0.43%, B - 0.57%. The AB AB genotype prevailed in the population of red-and-white cows (29.23%) according to the frequency of occurrence of complex genotypes of kappa-casein and beta-lactoglobulin.

The following table shows the reliability of the difference in the ratio of the genotypes of kappa-casein and beta-lactoglobulin in terms of milk productivity.

**Table 4 - Reliability of the difference in the ratio of the genotypes of kappa-casein and beta-lactoglobulin**

<table>
<thead>
<tr>
<th>Признак</th>
<th>Ratio</th>
<th>Difference</th>
<th>Td</th>
<th>v</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield, kg</td>
<td>AAAB ABAA</td>
<td>-458,74</td>
<td>2.7</td>
<td>71</td>
<td>P≤0,05</td>
</tr>
<tr>
<td></td>
<td>AABB ABBA</td>
<td>-568,11</td>
<td>3.1</td>
<td>76</td>
<td>P≤0,01</td>
</tr>
<tr>
<td></td>
<td>ABAA ABBB</td>
<td>322,36</td>
<td>2.0</td>
<td>77</td>
<td>P≤0,05</td>
</tr>
<tr>
<td>Fat, %</td>
<td>AAAA AABB</td>
<td>-0,295</td>
<td>2.1</td>
<td>63</td>
<td>P≤0,05</td>
</tr>
<tr>
<td></td>
<td>AAAA ABBB</td>
<td>-0,36</td>
<td>2.2</td>
<td>64</td>
<td>P≤0,05</td>
</tr>
<tr>
<td>Milk protein, kg</td>
<td>AABB ABAA</td>
<td>-32,54</td>
<td>2.0</td>
<td>76</td>
<td>P≤0,05</td>
</tr>
</tbody>
</table>
fat in cows with BB of kappa-casein was also higher than that of the same age animals with genotypes AB (by 14.3 kg) and BB (43.5 kg) (P <0.05). Heifers with the AA genotype of the beta-lactoglobulin gene had the highest milk fat yield of 328.36 kg. This is 13.87 kg (P <0.05) more compared to the BB genotype.

Studies of the effect of complex genotypes of kappa-casein and beta-lactoglobulin on milk productivity have shown that in red-and-white cows there is a clear trend in the influence of the genotype of beta-lactoglobulin on milk yield.

Conclusion

The studies carried out made it possible to assess the productive capabilities of animals of the created Volga type red-and-white dairy cattle with different genotypes of kappa-casein and beta-lactoglobulin.

Determination of the genotypes of kappa-casein and beta-lactoglobulin in young animals will improve selection, which will favorably affect the milk productivity and milk quality of red-and-white animals.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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Radiology - A Specific Tool in Identification: A Review

Vinoth Kumar S1, Monisha B. M2

1Associate Professor, Department of Radio Diagnosis, 2Assistant Professor, Skin and Std. Department, Vinayaka Missions Kirupananda Variyar Medical College and Hospitals, Salem – 636456, Tamilnadu

Abstract

Nowadays in Forensics, Radiology or Imaging plays a major role in identification of criminal and civil proceedings. Why Radiology here means, it is making bit sensible that to analyze the bony structures like skeletal remains and other body parts. The main aim of the manuscript is to unite the two disciplines in one part majorly “Radiology and Forensics”. The forensic application of diagnostic medical radiology can be applied in many fields; the prime target of evaluation is the osseous skeleton, but soft tissues which may offer several key findings. Essential methods consist of radiological methods such as conventional radiography, computed tomography, and magnetic resonance imaging, but other techniques such as 2D and 3D surface scanning along with printing are also employed.

Key Words: Radiology, Forensics, Investigations, Identification

Introduction

Forensic science deals with the identification of the dead using numerous techniques. Forensic odontology has a lot of scope in human identification. Methods like Rugoscopy, Bite marks, Photographs, Lip prints, Age Estimation and Sex Determination etc. are used for identifying the individuals.[1] Due to the increased use of imaging for forensic purposes as well as the establishment of specific research projects, the number of published studies in this field has increased rapidly in recent years. This new domain of research is interesting to radiologists as well as to forensic pathologists; radiologists have been involved in most forensic imaging projects from the beginning, underscoring the field’s integration of two distinct medical specialties. While radiologists read the obtained images, forensic pathologists focus on findings important for medico-legal reconstructions, which are not necessarily important from a clinical point of view. Pathologists are also able to explain certain phenomena visible on images due to their knowledge of thanatology. There was much speculation about the role of these methods and their relationship to forensic autopsy. Unclear study designs and unscientific terms were often used, leading to unsupported conclusions that were questioned. Confusion was further increased by the use of undefined or unclear terms such as “necro-radiology”, “forensic radiology”, “virtual autopsy”, and “minimally invasive autopsy”, which were rarely explained in the articles.[2]

Recent Methods of Radiology Imaging:

1) Intra Oral Periapical Radiography (IOPA)
2) Extra – Oral Radiography
3) Computed Tomography (CT)
4) Magnetic Resonance Imaging (MRI)
5) Postmortem Computed Tomography (PMCT)

Corresponding Author:
Dr. Vinoth Kumar S
Associate Professor, Department of Radio Diagnosis
Vinayaka Missions Kirupananda Vairiyar Medical College and Hospitals, Salem – 636456, Tamilnadu
Email id: hi_hi_sandy@yahoo.co.in
6) Multi-detector Computed Tomography (MDCT)

**Anatomical and Forensic Identification:**

Medico-legal cases, Natural disasters like tsunamis, earthquakes, explosions, etc. For confirming death in monetary issues, Septum deviations, Upper and Lower skeletal bone readings, Head and Neck bony remains identification, Teeth related identifications, etc., Digital radiography when used along with dental matching software fastens the forensic victim’s identification. Also, digital radiography reduces the exposure times by requiring 90% less radiation than that required to expose a standard type D film radiograph and 50% less radiation than that required in exposure of type E film radiographs.[3]

**MRI**

More recently, MRI has been used to increase forensic investigations, particularly in musculoskeletal, cardiovascular and angiographic fields and in forensic imaging of the living (such as cases of child abuse), survived strangulation and age estimation. Virtual Autopsy is one new technique that offers several advantages over the traditional approach and helps connect radiology with forensic medicine. The most important advantage is its non-invasive approach that doesn’t harm the body or tamper with forensic evidence. MRI involves no ionizing radiation; it is based on the principle of nuclear magnetic resonance. When a patient is placed in a magnetic field, the hydrogen protons in the body align with the field. A radiofrequency pulse is emitted from the scanner, exciting specific atomic nuclei and rotating the protons to a 180° position. As the energy from the pulse decreases, the protons return to their initial state within the magnetic field and generate an MRI signal that is digitally transformed into images. The interval between arrival in the initial state and signal emission is called the relaxation time. Contrast between anatomical structures is possible due to the specific relaxation time of atoms within each tissue. MRI offers high spatial resolution as well as excellent soft-tissue contrast, as it distinguishes muscles, fat, parenchyma, and neurological structures. [4-5]

**Conventional Radiography:**

Conventional radiography is the oldest radiological imaging method used in forensic medicine. In this technique, the body is investigated via direct exposure to X-rays; structures exposed to the beam are projected onto a radiographic image. The image is composed of different tonalities of black and white, corresponding to the number of X-rays that reach the detector. Contrast is possible due to the distinct absorption properties of body structures (bone is associated with high absorption and soft tissues display less attenuation). Conventional radiography employs two types of devices available in medical institutions: 1) analog equipment that uses radiological film for the impression of images, and 2) newer equipment that is completely digitized. In digital and digitized analog equipment, images are acquired in digital DICOM format, which is currently used for all imaging modalities. [2]

Conventional radiography is one of the most common imaging modalities in forensic medicine worldwide. Most forensic institutions possess their own X-ray devices, which are often used to evaluate the osseous system in cases of trauma or to characterize the presence of a foreign body. Radiography is advantageous, as it is simple to perform, rapid, and cost-efficient. Radiography is often implemented for infant corpses, for highly putrefied, charred, or otherwise altered bodies, and for bodies of unknown identity. Conventional radiography can also provide important information that is integrated with other complementary exams for age determination (Figure 1), not only of deceased but also of living persons. [6-7]
PMCT:

Currently, one of the most-used radiological modalities in modern forensic imaging is MDCT. This extensive use means that the term PMCT often refers to MDCT. Unlike conventional radiography, MDCT uses a computer to generate images that are saved in DICOM format. MDCT is based on the principle that the density of each tissue can be measured by calculating the attenuation coefficient of an X-ray beam passing through it. The body is examined through direct exposure to X-rays via a rotating tube. The attenuation values of the X-rays are expressed in Hounsfield units; these units are characteristic of various tissues and body fluids. Radiographic data are interpreted through the evaluation of various cross-sectional images. While 3D reconstructions are very clear and intuitive, enabling better understanding of the images for a medical layman, radiological assessment and diagnosis should always be based on axial views. Three-dimensional models are always at risk of artifacts, and the assessment of 3D models alone may cause discrete findings to be overlooked. [8]

CT:

CT is the ideal method for detecting radio-opaque foreign bodies. For example, it visualizes medical implants, projectiles and/or their fragments, and swallowed or aspirated foreign bodies. CT makes the discovery of small or fragmented objects much easier than does classic autopsy and allows rapid orientation for targeted extraction during autopsy. [9-11]
3D scanning:

Three-dimensional surface scanning is a technique that was developed for the car industry; it is extensively used for forensic investigations in Switzerland by police and medico-legal institutions. Its main fields of application are the reconstruction of traffic accidents, the correlation of a lesion and the presumed injuring-causing instrument, and the comparison of bite marks with dental models of the supposed perpetrator. [12-14]

Radiology in Age and Sex Determination:

Estimating age is the most important prerequisite for identification of the dead. Age estimation can be done by various factors like: Jaw bones, Tooth germs, Process of mineralization, Stages of crown development, Completion and their eruption into oral cavity, Volume of pulp chamber, Third molar development and eruption pattern, Root morphology, Maxillary Sinus height and width, Nasal Septum area, Pulp Tooth Area Ratio (PTR), Height and Width of the crown, Estimation of sutural closure, (Figure 2) etc.,[3]

Conclusion

Forensic radiology has modest and early origins. Previous evidence shows that medical principles have been applied to medico legal issues for thousands of years. It was not long after Roentgen discovered “a new kind of ray” that advanced forensic scientists established its value in forensic science. Radiologic imaging is better defined as the practice that lies at the many interfaces of medicine and law. Radiologic imaging plays a vital role at many of those connections, from the identification of dead to the confirmation and validation of precious sculptures.

Ethical Certificate: Since it is a Review article, we do not take ethical.

Conflict of Interest: Nil

Funding: Self

Acknowledgement: Nil

References


Concept of SARS-CoV-2 Vaccine Design to Fight COVID-19 Pandemic: A Review Insight

Viol Dhea Kharisma\textsuperscript{1,2}, Arif Nur Muhammad Ansori\textsuperscript{3}, Rasyadan Taufiq Probojati\textsuperscript{2}, Dora Dayu Rahma Turista\textsuperscript{4}, Yulanda Antonius\textsuperscript{5}

\textsuperscript{1}Student, Master Program in Biology, Faculty of Mathematics and Natural Science, Universitas Brawijaya, Malang, Indonesia, \textsuperscript{2}Researcher, Computational Virology and Complexity Sciences Research Unit, Division of Molecular Biology and Genetics, Generasi Biologi Indonesia Foundation, Gresik, Indonesia, \textsuperscript{3}Student, Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, \textsuperscript{4}Lecturer, Biology Education Departement, Faculty of Teacher Training and Education, Mulawarman University, Samarinda, Indonesia, \textsuperscript{5}Lecturer, Faculty of Biotechnology, University of Surabaya, Surabaya, Indonesia

Abstract
Cluster of pneumonia infection emerged in Wuhan, China due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Additionally, more than 190 countries have confirmed 82 million cases of SARS-CoV-2 infection. Currently, there is a SARS-CoV-2 epidemic, and no effective prophylactic methods are available. A vaccine is considered as an effective method to restrict an epidemic. Several vaccine designing techniques have been established, which is enabling researchers from various institutes for developing vaccine towards SARS-CoV-2 infections. In this review, we condense the development of vaccine research against SARS-CoV-2.

Keywords: COVID-19, SARS-CoV-2, Vaccine Design.

Introduction
A cluster of pneumonia infections emerged in Wuhan Hospital, China which started from December 2019 up to now. The infection was reported to be caused due to a type of beta-coronavirus, which named by World Health Organization (WHO) as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)\textsuperscript{1,2}. As per reports till March 26\textsuperscript{th} 2021, the pathogen had infected more than 120 million individuals and caused 2.7 million deaths worldwide\textsuperscript{1}.

SARS-CoV-2 had a material genetic of positive-stranded RNA and belongs to Betacoronavirus genera. Studies have revealed that SARS-CoV-2 genome consists of 29,700 nucleotides, and shares approximately 79.5% similarity to the genome of SARS-CoV\textsuperscript{3,4}. Furthermore, SARS-CoV-2 is known to have an ORF1ab polyprotein at the 5′-end which had a role in coding for viral non-structural proteins. Genes at 3′-end had been known to encode the structural proteins such as spike (S), nucleocapsid (N), membrane (M), and envelope (E)\textsuperscript{5,6}. Several studies have reported that SARS-CoV-2 binds to angiotensin converting enzyme 2 (ACE2) receptor on the host cell. The receptor played a key role in viral entry mechanism and related pathogenesis\textsuperscript{7,8} (Figure 1).

A vaccine is considered as an effective method which had high economic significance to restrict an infectious disease outbreak\textsuperscript{3,9}. The use of vaccines inducing neutralizing antibodies is the best option to increase the number of populations that are immune to SARS-CoV-2\textsuperscript{10}. Thus, the development of SARS-CoV-2 vaccine became an essential need. As per few reports, about 40 pharmaceutical companies and academic institutions in the world have currently assigned the
A program for developing vaccines towards SARS-CoV-2. Therefore, we summarize the ongoing advance research in development of SARS-CoV-2 vaccine.

**Figure 1. Structural part of SARS-CoV-2. The virion consists of spike glycoprotein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N).**

**Antigenic Retrieval and Selection from SARS-CoV-2 Structural Protein**

Various nonsrtructural protein is common to be utilized for vaccine development. However, spike protein (S protein) is widely being investigated to be used for developing a vaccine against SARS-CoV-2. Studies have revealed that it is a viral surface protein, which is directly recognized by the host immune system. Subsequently, it has been reported that S protein acts as a mediator for interaction between virus and host cell by binding to ACE2 receptor, and cause consequent pathogenicity. The homologous structure of S protein is being used for vaccine development, which has proven to be effective in overcoming the previous outbreaks including SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). The S protein monomer consists of 1273 amino acids in length and molecular weight about 140 kDa. Furthermore, it has ability to undergo the self-association mechanism and formed a homotrimer structure as a special feature of class I viral fusion protein. Structural studies showed that S protein is composed of two subunits, termed as S1 and S2 part. The S1 subunit can be organized into two domains, such as N-terminal domain (NTD) and C-terminal domain (CTD). In brief, the CTD is reported as receptor-binding domain (RBD). On the other hand, the S2 subunit had an essential part for membrane fusion which consist of internal membrane fusion peptide (FP), two heptad repeats (HR), membrane proximal external region (MPER), and transmembrane domain (TM). Recently, the S trimer structure of SARS-CoV-2 within the pre-fusion stage, and the RBD structure in convolution with ACE2 had been successfully determined.
Figure 2. Brief structure of SARS-CoV-2 spike glycoprotein.

The S trimer protein structure had been reported to act as an epitope and had ability to induce a high immunogenicity. Coleman et al. showed that the S trimer protein could be produced within baculovirus insect cells, and it could be transformed into nanoparticles\textsuperscript{16}. Furthermore, it was formulated with an adjuvant (alum) and injected into the mice. Moreover, the immunized mice were observed to obtain the data about titers level of specific antibodies after the nanoparticle administration\textsuperscript{16,17}. Recently, the Clover Biopharmaceutical has announced that they have designed SARS-CoV-2 S-trimer based-vaccine which processed into the preclinical testing stage\textsuperscript{9}.

RBD has been known to interact directly with ACE2 receptors of the host cells. Thus, many studies have suggested RBD for immunization to investigate the antibody formation. It estimated for having ability to inhibit the viral recognition of ACE2 receptor, and prevent the subsequent pathogenesis\textsuperscript{18,19}. Accordingly, many RBD-antigens based SARS-CoV-2 subunit vaccines are currently being developed. A study showed that the recombinant RBD, which was isolated from different types of virus serotypes, could trigger the multi-conformational by neutralizing antibodies towards SARS-CoV-2. This might be due to the presence of multiple epitopes that are recognized by specific immune systems\textsuperscript{20}.

As previously reported, S1 subunit has RBD as well as NTD, which suggests its role in binding S protein to the host receptor\textsuperscript{21}. Subsequently, these domains can be widely utilized for vaccine development. As comparison, previous studies showed that MERS-CoV S1 subunit with MF59 adjuvant could protect the human dipeptidyl peptidase 4 transgenic (hDPP4-Tg) mice against infections\textsuperscript{22}. Furthermore, FP in S2 subunit has been reported to be involved in membrane fusion which is essential step for viral pathogenicity\textsuperscript{23,24}. At present, Tianjin University is constructing RBD-FB-based vaccines and producing high titers of neutralizing antibodies in mice\textsuperscript{9}.

In other hand, N protein is abundantly expressed by the coronavirus and it has molecular weight about 50 kDa. It has been reported for promoting several functions such as nucleocapsid formation, initiation of viral budding transduction signal, RNA replication, and mRNA transcription\textsuperscript{25,26}. Furthermore, N protein
has been suggested to have high antigenicity as about 80% of patients with COVID-19 have been observed to produce antibodies specific to SARS-CoV N protein.27

Moreover, they have been observed to significantly reduce the viral load of vaccinia virus.28 In addition, previous studies reported that bronchitis virus-related N protein could induce the activation of cytotoxic T lymphocytes (CTLs) by attenuating infection and thereby, protecting lungs from viral pathogenicity.29,30

Moreover, M protein is a glycoprotein located in the transmembrane of virus and it has molecular weight about 25 kDa. In brief, it has a crucial role for viral assembly. Furthermore, previous report exhibited that it abundantly expressed on the viral surface.31,32 Full length M protein has been previously evaluated as a vaccine, which is reported could be neutralizing antibodies in patients with SARS. M proteins also consisted T cell epitope clusters and it was evaluated to be immunogenic33,34. In addition, it also identified for having high conserved domain.32,35

E protein has been reported to have about 76-109 amino acids from studies in different types of coronaviruses. Moreover, it has been demonstrated to have limited immunogenicity compared to S, N, and M proteins. Thus, E protein is not predicted as an immunogen. However, E protein knockout models have been observed to significantly reduce secretion of inflammatory cytokine such as interleukin 1β (IL-1β), tumor necrosis factor (TNF), and IL-6.36

Recent Developments in SARS-CoV-2 Vaccine Technology

Studies have shown that whole-cell killed or live-attenuated vaccine exhibit all antigenic components to the host and it could potentially trigger different immunological responses to fight the pathogen.37,38 Traditional vaccines could be developed using advanced technology to design the foremost SARS-CoV-2 vaccine that may have prompt applications in healthcare. Wuhan Institute of Virology, Chinese Academy of Sciences, Zhejiang University, and other companies have successfully isolated SARS-CoV-2 and they have started developing vaccines. Recently, Codagenix Inc. collaborated with Serum Institute of India Ltd. developed live-attenuated SARS-CoV-2 vaccine.9

Vaccine subunits that comprise of more than one antigen with high immunogenicity could trigger the host immune response. Good quality of vaccines should be safe and easy to produce; however, it commonly requires adjuvants to exhibit the strong immune response.39 Several companies have established programs to develop SARS-CoV-2 subunit-based vaccine, and most of them have been reported utilizing protein S as an antigen. The utilization of “molecular clamp” technology by University of Queensland and “Trimer-Tag” technology by Clover Biopharmaceuticals Inc. are several examples of subunit vaccines. Recently, Novavax Inc. announced nanotechnology-based vaccine candidates that have been evaluated in animal models while further testing in humans is needed. Furthermore, Pasteur Institute has collaborated with Chongqing Zhifei Biology Co. Ltd. and started developing SARS-CoV-2 subunit-based vaccine.9,40

Advanced technology in the synthesis, modification, and delivery of mRNA vaccines has been developed for past two decades. The mRNA vaccine is a notable alternative for conventional vaccine since it known for remarkable efficiency, fast production, low cost, and safe consumption.41,42 In general, the mRNA vaccine development consists of several steps, including antigen selection, sequence optimization, nucleotide modification, delivery system, evaluation of immune response, and evaluating vaccine safety.43 Recently, the mRNA vaccine against SARS-CoV-2 had been developed by Moderna and it is qualified for further analysis. Similarly, the mRNA vaccine based on protein S and RBD is being assessed into in vivo study under the collaboration of Fudan University, Shanghai Jiaotong University, and Bluebird Biopharmaceutical.9

Heterologous antigen expression is the principal action for live vector vaccines. It commonly produces stronger immunogenicity and trigger higher cellular
immunity. Houston-based Greffex Inc. has been successful in developing live vector-based SARS-CoV-2 vaccine using adenovirus vectors, and it is evaluated in animals. Moreover, Tonix Pharmaceuticals has recently announced the development of SARS-CoV-2 vaccine using Horsepox Virus (TNX-1800).

Another kind of vaccine, such as synthetic peptide-based vaccines are also developed. It generated from small fragments or peptides which synthesized through chemically process. However, it has been reported that it exhibited a low immunogenicity effect due to the low molecular weight and complex structure. As a result, synthetic peptide vaccines require a few modifications, such as structural modification, presence of a delivery system, and adjuvants formulation. Currently, researchers from Hong Kong University of Science and Technology are developing a peptide vaccine based on B and T cell epitopes from S and N proteins of SARS-CoV.

Conclusion

We hope that several vaccines that have been developed could be used as an effective treatment for COVID-19 outbreak. Different methodology of vaccine development had both advantages and disadvantages. Furthermore, current advancements in technology may assist researchers to contribute in development of SARS-CoV-2 vaccine.

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38. Ansori ANM, Kharisma VD, Antonius Y, Tacharina


How Do Patient Satisfaction in Dental Polyclinic? - Patient Characteristics and Quality of Dental Health Services

Vitri Nurilawaty¹, Ngatemi¹, Dwi Priharti¹, Tedi Purnama¹, Ruth Lasma Milan²
¹Lecturer, Departemen of Dental Health Ministry of Health Polytechnic Jakarta I, Indonesia,
²Dental Therapist of Bukit Duri Health Center, South Jakarta, Indonesia

Abstract

Excellent service is the demand of the community. Aspects of the quality of services provided will affect patient satisfaction, where patients will compare between dental treatment at dental health service facilities. In improving the quality of service, the management of complaints from patients is needed. This study aims to determine the relationship between patient characteristics and the quality of dental health services on patient satisfaction. Method: this type of analytic observational study with cross sectional design. The research was conducted on patients with dental polyclinic at the Health Center of Bukit Duri Village, Tebet District, South Jakarta. Data collection using a questionnaire. Data analysis using Chi-Square. Results: showed that the variable age had a p value of 0.691, gender with a p value of 0.013, a source of funding with a p value of 0.921 and the quality of dental health services with a p value of 0.001 on patient satisfaction. Conclusion: There is a significant relationship between gender and the quality of dental health services on patient satisfaction at the dental clinic.

Keywords: Patient satisfaction; service quality; dental polyclinic

Introduction

The health sector is the main service sector in various countries because it plays a vital role in maintaining and improving public health to achieve the goals of a country. Currently the service industry in the health sector is one of the promising business opportunities with high competition and fast growth.¹,²

The quality of health services is a factor that can increase patient satisfaction. Excellent service is the demand of society, in line with the increasing needs and awareness in state life as a result of technological advances. High quality is a requirement, not only in business activities but also in health activities.³,⁴

Aspects of the quality of service provided will affect patient satisfaction. Patients will compare or ask other people’s recommendations for dental treatment instructions. The aspects seen from the quality of service include the physical appearance of the dental clinic (tangible), reliability aspects, responsiveness, assurance, and empathy aspects.⁵,⁶

In improving the quality of service, the management of complaints from patients is needed. Many complaints from patients are received by the health center management either directly or through the suggestion box that has been provided as a channel to measure patient satisfaction.⁷,⁸

Oral and dental health services are professional health services aimed at the community, family and individuals, both sick and healthy. Dental health services are carried out to maintain and improve the degree of public health in the form of improving dental health, prevention of dental disease, treatment of dental diseases.
and restoration of dental health by the government which is carried out in an integrated, integrated and sustainable manner.9,10

Public health center is a functional organization that organizes health efforts that are comprehensive, integrated, equitable, acceptable and affordable to the community and uses appropriate technology and focuses on services for the wider community, in order to achieve an optimal health degree. Many Public health center still do not have adequate facilities for public health services. There are still a lot of dental equipment that is still not owned by the Public health center, because Public health center usually only provide basic / minor treatments, so that many cases are referred or handled minimally.11,12

Bukit Duri Health Centre is one of the Public Health Center in South Jakarta which is visited by many people because of its strategic location and adequate health personnel. In an effort to improve health services, including dental health services, the Bukit Duri Health Center has implemented an ISO 9001-2008 quality management system. It is proven that the data on patient visits at the dental polyclinic at the Bukit Duri Health Center showed that the number of patients at the dental polyclinic in 2018 was 2214 patients.

**Method**

This research is an analytic observational study with a cross-sectional design. The research was conducted at the Bukit Duri Health Centre, Tebet district, South Jakarta from March to April 2019. The sampling technique used purposive sampling. The number of samples in this study using a minimum sample of 30 respondents.13 With inclusion criteria: new patients who went to a dental polyclinic; The age of the respondent is at least 20 years old and willing to be the respondent.

The collection of service quality data using a questionnaire has been tested for its validity and reliability and patient satisfaction is measured by providing a smile card / pin.

The stages of the activity include: before conducting a questionnaire, the respondent is given an informed consent first as an agreement that he is willing to be a research respondent. Then give a service quality questionnaire and then give an assessment card / pin to enter it into the patient satisfaction box. Data analysis was performed using the SPSS statistical program for univariate analysis and presented in the form of a frequency distribution after bivariate analysis with chi-square.

**Result**

<table>
<thead>
<tr>
<th>No.</th>
<th>Variable</th>
<th>N</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 - 40 Years</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>41 - 65 Years</td>
<td>13</td>
<td>43.3</td>
</tr>
<tr>
<td></td>
<td>&gt; 65 Years</td>
<td>2</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Man</td>
<td>11</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>19</td>
<td>63.3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>32</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>Sources of financing</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>General</td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>BPJS</td>
<td>26</td>
<td>86.7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1 shows that the majority of respondents in the study were from the age group of 20-40 years (50%) with female gender, namely 17 respondents (63.3%) and 26 respondents (86.7%) used funding sources with BPJS.
Table 2. Frequency distribution of patient satisfaction

<table>
<thead>
<tr>
<th>No.</th>
<th>Patient Satisfaction</th>
<th>N</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Very satisfied</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Satisfied</td>
<td>14</td>
<td>46.7</td>
</tr>
<tr>
<td>3</td>
<td>Not satisfied</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2 shows that the respondents who stated very satisfied as many as 15 people (50%), respondents who are satisfied as many as 14 people (46.7%) and respondents who are not satisfied as many as 1 person (3.3%).

Table 3. Frequency distribution of dental polyclinic service quality

<table>
<thead>
<tr>
<th>No.</th>
<th>Service quality</th>
<th>N</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Very satisfied</td>
<td>17</td>
<td>56.7</td>
</tr>
<tr>
<td>2</td>
<td>Satisfied</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>Not satisfied</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3 shows that the respondent 17 people (56.7%) stated that the service quality was very satisfied, 12 respondents (40%) stated that the service quality was satisfied and 1 person (3.3%) stated that the service quality was not satisfied.

Table 4. Results of the chi-square analysis of respondent characteristics and quality of dental health services on patient satisfaction

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient satisfaction</th>
<th>Total</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Satisfied</td>
<td>Very satisfied</td>
<td>Not satisfied</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 - 40 years</td>
<td>6</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>41 - 65 years</td>
<td>7</td>
<td>53.8</td>
<td>5</td>
</tr>
<tr>
<td>&gt; 65 years</td>
<td>1</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>46.7</td>
<td>15</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>9</td>
<td>81.8</td>
<td>2</td>
</tr>
<tr>
<td>Women</td>
<td>5</td>
<td>26.3</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>46.7</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 4 shows the relationship between respondent characteristics and the quality of dental health services and patient satisfaction. The table indicates that there is a significant difference in the patient satisfaction level among different age groups and genders. The chi-square test results show that the age group (p = 0.691) and gender (p = 0.013) do not have a significant relationship with patient satisfaction. However, the gender has a significant relationship with patient satisfaction.
Cont... Table 4. Results of the chi-square analysis of respondent characteristics and quality of dental health services on patient satisfaction

| Sources of financing |  
|---------------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| BPJS                | 12   | 46.2 | 13   | 50  | 1   | 3.8 | 26  | 100 |
| General             | 2    | 50   | 2    | 50  | 0   | 0   | 4   | 100 |
| Total               | 14   | 46.7 | 15   | 50  | 1   | 3.3 | 30  | 100 |

| Quality of dental health services |  
|-----------------------------------|------------------|-----------------|-----------------|-----------------|-----------------|
| Satisfied                        | 6    | 50   | 6    | 50  | 0   | 0   | 12  | 100 |
| Very satisfied                   | 8    | 47.1 | 9    | 52.9| 0   | 0   | 17  | 100 |
| Not satisfied                    | 0    | 0    | 0    | 0   | 1   | 100 |
| Total                            | 14   | 46.7 | 15   | 50  | 1   | 3.3 | 30  | 100 |

Table 4 shows that what has a significant relationship with patient satisfaction is the variable quality of dental health services with a p value of 0.001 and gender with a p value of 0.013, while other variables, namely age and funding sources, do not have a significant relationship with patient satisfaction because p value > 0.05.

**Discussion**

Patient satisfaction is the state when the patient’s wants, hopes and needs can be met. Respondent characteristics obtained respondents indicated that the majority were 20-40 years old as many as 15 people (50%), female gender as many as 19 people (63.3%), BPJS financing as many as 26 people (86.7%).

The results of research on the quality of dental polyclinic services found that most of the respondents stated that the service quality was very satisfied as many as 17 people (56.7%). Meanwhile, regarding the satisfaction of dental polyclinic patients, it was found that the majority of respondents who stated that they were very satisfied were 15 people (50%).

The results of the study on the relationship between respondent characteristics and patient satisfaction showed a p value of 0.013, meaning that it had a significant relationship between gender and service quality.

This is in accordance with Suhamiartici. Sembelet al stated that men are easily satisfied with dental and oral care so that they do more dental and oral care. Budiman et al. stated that gender is related to patient satisfaction with Jamkesmas services at Public helath center. Gender affects satisfaction, where men have greater demands so they tend to be dissatisfied than women. In addition, gender is a predisposing factor that affects behavior.

The results of the study on the relationship between the quality of dental health services on patient satisfaction showed a p value of 0.001, meaning that there was a significant relationship between the quality of dental health services and patient satisfaction. This is due to the professionalism of dentists and dental nurses in providing services and having complete facilities and infrastructure. Ability, knowledge, skills of staff in handling every service provided so as to foster trust and a sense of security for customers.

The results of this study are in line with the results of Rizal research, there is a relationship between the quality of health services as seen from the guarantee and the level of patient satisfaction. Sembel et al. research which was conducted at the Shoulder Health Center in Manado, showed that medical services showed 89.1% of patients were very satisfied. Research Dahyanto et al. in the patient at Hospital, proved the level of satisfaction in the very satisfied category.
Conclusion

Based on the results of the study, it can be concluded that there is a significant relationship between gender and the quality of dental health services on patient satisfaction at the dental clinic.

Acknowledgement: This study was done by self-funding from the authors. The authors thank to all participants and research assistants

Conflict of Interest: The authors declare that they have no conflict of interests.

Ethical Clearance: All participants were signed the informed consent prior to the data collection.

References

17. Montol SA, Maramis FR, Engkeng S. Hubungan Antara Status Demografi Dengan Kepuasan Dalam Pelayanan Pasien Jamkesmas Di Wilayah...


A Study Survey of Awareness of Jordanian Chemists about Chemical Hazards

Waed R Alahmad, Tala H. Sasa, Nawal H Bahtiti, Ahmed Abu-Rayyan

Abstract

This study is an attempt to identify the level of awareness of safety measures practiced in the survey was conducted between September and December 2020 among chemists graduated in Jordan in several working sectors (teaching, Laboratory work and in manufactures). A total of 245 eligible subjects were asked to participate in the study. The majority were females (71.0%), their ages ranged from 18 to 40 years, and most of them were between 18-22 (62.9%). As for educational qualifications, 65% were students. 77.1% of them had a good knowledge of chemistry. Most of the accidents were in the laboratory by females (68%), 78% of the accidents were by females in the household. The age of 18-22 (58.9%) caused most of the accidents in the laboratory. Also, the age of 18-22 (69.9%) caused most of the accidents in the household. 55.5% of laboratory accidents were from students. As result females and students were the major reason for chemical accidents. There is no significant difference in the average attitude towards chemical safety by age. There is no significant difference in the average attitude towards chemical safety as per education.

Keywords— Awareness, chemical safety; safety perceptions; laboratory safety.

Introduction

The amount and variety of chemicals used in workplaces, households, and others have led to an increase in chemical accidents. Chemical accidents include fires, explosions, or leaks that can cause illness, injury, disability, or death. These accidents affect the environment, disrupt societies, and the economic burden in many countries. Hazardous chemicals are controlled and managed through relevant laws and regulations to prevent chemical accidents.

A chemical incident is defined as the uncontrolled emission of a toxic material, which results in harming the health and the environment surrounding it. Chemical incidents can occur because of natural activities, or because of accidental or intentional activities. These incidents can be rapid and severe or have a slow onset when there is a ‘silent’ release of a chemical. Also, they can also range from slight releases to full-scale main crises (1). The term “chemical incident” might refer to anthropogenic or technological events, including:

- an explosion at a place of work that stores or uses chemicals.
- contamination of a chemical with food or water supply.
- an oil spills.
- a leak during transportation from a storage unit.
- deliberate release of chemicals in conflict or terrorism.
- an outbreak of disease that is associated with chemical exposure.

Chemists are dealing with dangerous materials that
can be irritant, explosive, flammable, radioactive, or a health hazard. Accidents in industrial factories, chemical laboratories & chemical storages, or even in houses have been reported worldwide for several reasons, such as an absence of personal protective equipment (PPE), limited experience, mishandling of chemicals, and lack of knowledge about the proper actions to be taken in emergency cases \(^{(1)}\). A list of chemical incidents illustrated in table 1.

### Table 1: Chemical Incidents

<table>
<thead>
<tr>
<th>Date</th>
<th>Chemical Incidents</th>
</tr>
</thead>
<tbody>
<tr>
<td>December 6, 1917</td>
<td>Halifax, Canada. The Halifax Explosion. A ship loaded with about 9,000 tons of high explosives destined for France caught fire as a result of a collision in Halifax harbour, and exploded. The explosion killed about 2,000 and injured about 9,000.</td>
</tr>
<tr>
<td>October 4, 1918</td>
<td>T. A. Gillespie Company Shell Loading Plant explosion. An ammunition plant in Sayreville, New Jersey exploded, killing approximately 100 people, destroying 300 buildings and causing $18 million in damages.</td>
</tr>
<tr>
<td>March 1, 1924</td>
<td>1924 Nixon Nitration Works disaster. A plant for processing ammonium nitrate in Edison, New Jersey exploded, killing 24 people, injuring 100 and destroying several buildings.</td>
</tr>
<tr>
<td>July 17, 1944</td>
<td>Port Chicago Disaster. A munitions explosion that killed 320 people occurred at the Port Chicago Naval Magazine in Port Chicago, California.</td>
</tr>
<tr>
<td>Nov 27, 1944</td>
<td>RAF Fauld Explosion. Explosion of between 3500 and 4000 tonnes of ordnance in an underground munitions store that killed 70 people.</td>
</tr>
<tr>
<td>August 9, 1965</td>
<td>Little Rock AFB, Searcy, Arkansas. 53 contract workers were killed during a fire at a Titan missile silo. The cause of the fire was determined to be a welding rod damaging a hydraulic hose carrying Aerozine 50 fuel. This allowed the hypergolic fuel vapors to spread throughout the silo, which were then ignited by an open flame.</td>
</tr>
<tr>
<td>April 13, 1976</td>
<td>Lapua Cartridge Factory explosion. An explosion in a munitions factory in Lapua, Finland kills 40 workers.</td>
</tr>
<tr>
<td>May 5, 1983</td>
<td>“6 Martie” Ammunition Factory in Zărnești, Romania. An explosion in the production facilities inside the factory completely destroyed two buildings, killing 37 people and injuring more than 300.</td>
</tr>
<tr>
<td>April 10, 1988</td>
<td>Ojhri Camp, Rawalpindi, Pakistan. A military storage center exploded, killing more than 90 people.</td>
</tr>
<tr>
<td>July 11, 2011</td>
<td>Evangelos Florakis Naval Base explosion, Cyprus. The disaster occurred when 98 containers of gunpowder exploded; 13 people were killed, among them the captain of the base, three commanders, twin brothers who were serving there as marines, and six firefighters. 62 people were injured, and the explosion knocked out the island’s power station for days.</td>
</tr>
</tbody>
</table>
The likelihood of these events can be minimized if chemicals are used and stored properly and under strict safety regulations and rules. As a result, regulations and laws have been developed by different organizations for using chemicals with potential hazards, through Safety Data Sheets (SDS) or labels. Labels are assigned to each chemical according to the potential hazard it may cause, and chemists should be familiar with the meaning of each label to know how it should be handled. Crucially, the United Nations Conference on the Environment and Development (UNCED) has recognized that a Globally Harmonized System (GHS) of classification and labeling of chemicals was needed.

Proper comprehension and interpretation of the dangerous chemicals & chemical labels is a very important factor for preventing accidents anywhere. Most of the studies that were conducted were aimed at students of chemistry and related branches of work in chemistry laboratories, chemical laboratory safety among college students in Trinidad and Tobago.

Regulatory standards - such as the Process Safety Management (PSM), the Risk Management Plan Rule Standard in the USA, and the Seveso III Directive in Europe - require locations where hazardous chemical products are stored and used to comply with their requirements. These regulatory standards should prompt a process safety protocol for any chemical-physical laboratory in industry and academia.

In academic physical and chemical laboratories, where toxic and flammable chemical products are handled, there should be qualified personnel. However, according to Olewski and Snakard (2017), student and research staff turnover is an important reason why compliance with these regulations is difficult in short-term experiments in academic research laboratories. Also, process safety information should be available when performing the routine analysis. However, when research is conducted, process safety information is usually part of the research. This creates a difficult problem from a safety standpoint if poor process safety information procedures are in place during the research. Ultimately, poor information on process safety for short-term experiments can occur due to the high turnover of students and postdoctoral researchers.

It is recommendations to the creation of the Chemical Hygiene Plan, involving: i) lab planning and inspections, ii) the creation of safety policies, and iii) training for students and staff.

To ensure safety and security in scientific laboratories, the following requirements must be met:

1) Risk management: It is the preventive aspect of laboratory work and aims to prevent or reduce risks to individuals and facilities, reduce losses and avoid recurring accidents.

2) Laboratory practices: It is the practical aspect that includes all practical activities related to the science course. It is imperative that these activities are sound and properly performed according to the instructions regulating them. Failure to perform tasks due to lack of knowledge, carelessness, or haste may cause accidents.

3) First aid: It represents the therapeutic aspect that is no less important than the preventive and practical side of safety procedures in the laboratory. In many cases, good behavior in an emergency and providing first aid to injured people in the laboratory can be critical.

The aim of this work was to evaluate chemist knowledge in workplaces regarding the safe use of chemicals and chemicals products in Jordan.

Methods

Study design

The survey was conducted between September and December 2020 among chemists graduated in Jordan in several working sectors (teaching, Laboratory work and in manufactures). A total of 245 questionnaire were conducted.

It is divided into the following secondary questions:

1) What is the level of awareness of safety measures (i.e., laboratory risk management, appropriate laboratory
practices, and first aid for laboratory injuries).

2) Are there statistically significant differences in the degree of awareness of safety procedures used by the participants due to practical experience at (α ≤ 0.05)?

3) Are there statistically significant differences in the degree of awareness of the safety procedures used by the participants due to the educational level at (α ≤ 0.05)?

**Survey instruments**

The questionnaire was developed based on the literature reviews of comparable studies. The questionnaire consisted of 20 items distributed into the following five sections: Personal Information, Knowledge of Chemicals, Attitude towards Chemical Laboratory Safety, Chemical Laboratory Safety Practices, Emergency Equipment and Procedures.

**Statistical analysis**

Statistical analysis was developed using STATA software program, version 16 (Stata Corporation. College Station, Tx).

**Results**

**Subjects characteristics**

A total of 245 eligible subjects were asked to participate in the study. The majority were females (71.0%), their ages ranged from 18 to 40 years, and most of them were between 18-22 (62.9%). As for educational qualifications, 65% were students. 77.1% of them had a good knowledge of chemistry. 65.0% had good knowledge of hazardous chemicals, for an issue (how many chemical accidents have you been involved in), most of the answers were between 1 to 3 accidents (89.8%).

The frequency and the percent are illustrated in Table 2.

**Table 2 Demographic, professional and knowledge of chemical hazards characteristics of the responders**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>71</td>
<td>29.0</td>
</tr>
<tr>
<td>female</td>
<td>174</td>
<td>71.0</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-22</td>
<td>154</td>
<td>62.9</td>
</tr>
<tr>
<td>23-30</td>
<td>44</td>
<td>18.0</td>
</tr>
<tr>
<td>31-39</td>
<td>15</td>
<td>6.1</td>
</tr>
<tr>
<td>ABOVE 40</td>
<td>32</td>
<td>13.1</td>
</tr>
<tr>
<td><strong>educational qualification</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Currently student</td>
<td>160</td>
<td>65.3</td>
</tr>
<tr>
<td>Post graduate students</td>
<td>19</td>
<td>7.8</td>
</tr>
<tr>
<td>Teachers</td>
<td>14</td>
<td>5.7</td>
</tr>
<tr>
<td>Lab technicians</td>
<td>12</td>
<td>4.9</td>
</tr>
<tr>
<td>Lab managers</td>
<td>6</td>
<td>2.4</td>
</tr>
<tr>
<td>Instructors (master or doctor)</td>
<td>15</td>
<td>6.1</td>
</tr>
<tr>
<td>Others</td>
<td>19</td>
<td>7.8</td>
</tr>
</tbody>
</table>
Table 2 Demographic, professional and knowledge of chemical hazards characteristics of the responders

<table>
<thead>
<tr>
<th>Knowledge of chemistry</th>
<th>Minor</th>
<th>42</th>
<th>17.1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>189</td>
<td>77.1</td>
</tr>
<tr>
<td></td>
<td>Expert</td>
<td>14</td>
<td>5.7</td>
</tr>
<tr>
<td>Interest in chemical hazardous</td>
<td>YES</td>
<td>163</td>
<td>66.5</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>82</td>
<td>33.5</td>
</tr>
<tr>
<td>How many chemical accidents where you involved in</td>
<td>1-3</td>
<td>220</td>
<td>89.8</td>
</tr>
<tr>
<td></td>
<td>4-6</td>
<td>12</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>MORE THAN 6</td>
<td>12</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>BASICALLY ALWAYS</td>
<td>1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Three questions 7, 8 and 9 were asked, then linked to basic information (age, gender, and education), and a chi-square test was administered to these questions.

Q7-where was most of these accidents?
Q8-what was the type of accident?
Q9-What is the major reason for the chemical accident?

The result shows that:

Most of the accidents were in the laboratory by females (68%), 78% of the accidents were by females in the household. The age of 18-22 (58.9%) caused most of the accidents in the laboratory. Also, the age of 18-22 (69.9%) caused most of the accidents in the household. 55.5% of laboratory accidents were from students. As result females and students were the major reason for chemical accidents. 60% of instructor masters or doctors saw that absence of protection as the main cause for the chemical accident. The persons whose age 18-22 saw that the limited experience as the main cause reason for the chemical accident.

The second part concerns the types of situations that are considered chemical accidents. For them, the answer was agreed or disagree, or neutral, Cronbach’s alpha was applied, and it gives a score of 0.671 for these questions.

T-tests and analysis of variance were performed on these questions to answer whether there were statistically significant differences in the levels of study structures that could be attributed to age, gender, and education. Table 3 shows the mean, standard deviation, and position of the position elements of chemical safety.

A t-test was performed for independent samples to test that there were no differences in the levels of the study structures that could be attributed to gender, and there was a significant difference in the mean attitude towards chemical safety of males (2.7981) and females (2.3046) $PV = 0.000 < \alpha = 0.05$. An analysis of variance was performed to explore the effect of age on levels of study constructs, Sig. $> 0.05$ no sig difference. There is no significant difference in the average attitude towards chemical safety by age. There is no significant difference in the average attitude towards chemical safety as per education. Table 3 shows Mean, standard deviation and attitude for items of Attitude Toward Chemical Safety.
Table 3 Mean, standard deviation and attitude for items of Attitude Toward Chemical Safety

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Attitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8694</td>
<td>1.02793</td>
<td>disagree</td>
</tr>
<tr>
<td>2.5102</td>
<td>1.27592</td>
<td>disagree</td>
</tr>
<tr>
<td>2.9633</td>
<td>1.28782</td>
<td>neutral</td>
</tr>
<tr>
<td>2.4476</td>
<td>0.78557</td>
<td>disagree</td>
</tr>
</tbody>
</table>

The third part; Chemical Safety Practices –

Alpha Cronbach (0.787), Table 4 gives the mean, standard deviation and position of the elements of chemical safety practices, a t-test was performed for independent samples to test that there are no differences in study levels that show that can be attributed to gender, there is a large difference in the average practices Chemical integrity of females (2.6494) and males (2.4742) p-value = 0.005 <α = 0.05, an analysis of variance was performed to explore the effect of age on levels of study constructs. There is no significant difference in the average chemical safety practices by age. There is no significant difference in the average chemical safety practices according to education.

Table 4 Mean, standard deviation, and attitude for items of Chemical Safety Practices

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std. Deviation</th>
<th>ATTITUDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5347</td>
<td>0.59006</td>
<td>ALWAYS</td>
</tr>
<tr>
<td>2.6694</td>
<td>0.54405</td>
<td>ALWAYS</td>
</tr>
<tr>
<td>2.5918</td>
<td>0.59086</td>
<td>ALWAYS</td>
</tr>
<tr>
<td>2.5986</td>
<td>0.44955</td>
<td>ALWAYS</td>
</tr>
</tbody>
</table>
It is well known that preventing or reducing accidents in laboratories is a collective responsibility that requires efforts on the part of staff and users. That is, everyone is responsible for reducing the occurrence of the accident, especially those who conducted the experiment. Accidents usually occur due to negligence, lack of common sense, failure to implement instructions, or errors in conducting experiments.

Prevention of laboratory accidents requires a range of measures including the application of safety awareness requirements after appropriate training, the use of personal safety tools such as wearing glasses and a lab coat during the laboratory period, the use of the least amount of chemicals possible, and experimentation with non-hazardous or less hazardous materials. Whenever possible, anticipate the sequence of events in action. Sources of safety awareness include university courses, faculty, laboratory safety guides, and websites (4).

Correct laboratory practices were significantly more likely in researchers who recognized that their exposure to chemicals was low, but who had significant exposure to biological hazards, who agreed with the statement that colleagues handled chemicals by following safety procedures, and who recognized that they had received adequate training in accident management and first aid. Our results showed large gaps in knowledge and a paucity of preparedness in adhering to safety processes to prevent and contain risks related to the use of chemical compounds in research laboratories (5).

Residents’ awareness of Personal Protective Equipment (PPE) against chemical accidents. Overall, 88% of the population indicated that they need to be prepared to use PPE for chemical accidents, while only a small portion (9%) of respondents answered otherwise, which means that PPE is absolutely necessary for chemical accidents (6).

The online safety training available is usually not standardized or accredited courses and is a general limitation. Conversely, similar scores across all demographic workshop groups that have received cross-curricular training indicate chemical laboratory safety, or have attended a bachelor’s degree, indicate that these resources are effective in improving students’ attitude toward chemical laboratory safety (1).

**Conclusion**

This result can be explained by the fact that strict rules apply to students enrolled in chemistry laboratories. Students are not allowed to enter chemistry labs without wearing personal protective equipment. Chemicals are kept in fume hoods, so students are not allowed to take them to their seats.

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**Ethical Clearance**: Taken from Applied Science private University.

**References**


Services of Specialist Doctor at Type C Regional General Hospital based on Regulation of the Minister of Health No. 3 of 2020 on Hospital Classification and License

Wahyudi¹, Irma Indra Wahyuni¹, Muhammad Firdan Resaldi¹, Mokhamad Khoirul Huda²

¹Master of Law Students at Hang Tuah University, Surabaya, Indonesia, ²Professor Faculty of Law, Hang Tuah University, Surabaya Indonesia

Abstract

The problem occurs when the number of human resources for health workers, mainly the number of specialist doctors, is not comparable to the number of hospitals in Indonesia, particularly in type C regional general hospitals. Currently, many private hospitals are competing to provide better and quality services. When they are sick, many people choose a hospital with a much better and modern service. It becomes a concern for the government to meet its citizens’ basic needs in the health sector. The problem that lies behind the researchers to write is Regulation of the Minister of Health No.3 of 2020 on Hospital Classification and License to find out a legal review of specialist doctor services at type C regional general hospital. Based on the analysis of the Regulation of the MinisterNo. 3 of 2020 on hospital classification and license, the regulation does not regulate the minimum requirements for specialist doctors in each hospital classification. It causes an unequal distribution of specialist doctors in type C regional general hospital and results in the unequal quality of health services throughout Indonesia.

Keywords: Regional General Hospital, Law, Specialist Doctor, Type C Hospital Classification

Background of Study

Based on Regulation of the Minister of Health No. 3 of 2020 on Hospital Classification and License, it is necessary to carry out a legal analysis of the need for specialist doctors in every hospital, especially in type C hospitals. The legal analysis is to improve the degree of equitable public health in distributing specialized healthcare services and improving regional healthcare services. As quoted from kompas.com, specialist doctors’ ratio to a population is 14.6 per 100,000 population where this ratio exceeds the target ratio set. Still, the distribution of specialist doctors is not evenly distributed.¹ This condition raises many problems, and it becomes worse when it is in an emergency. There is no standby-specialist doctor at the hospital, so the patient must wait for doctors from other hospitals. The lack of concern for health services has resulted in unfulfilled health services standards and rampant lawsuits by the public.

On the other hand, hospitals are obliged to improve quality and service by especially presenting specialist doctors to make the public believe hospital services’ quality.² However, the problem occurs when the number of human resources, especially medical specialist doctors, is not comparable to the number of hospitals throughout Indonesia. The specialist doctors are located in major provinces in Indonesia and are piled up in type A and B hospitals. The unequal distribution of specialist doctors results in the quality of health services throughout Indonesia. Based on the Regulation of Minister of Health No. 23 of 2019 on the criteria for
determining the needs and selecting candidates for civil servants in 2019, regional governments must submit the number of civil servants to the central government. However, as stated in article 31 paragraph (2) of Law No. 23 of 2014 on Regional Government, the government has the autonomous right to handle the health sector to accelerate public welfare improvement. It is an increase in the human development index marked by an increase in health, education, and community income, even though type C and D hospitals require specialist doctors. The four basic specialist doctors needed are pediatrician, surgeon, internist, obstetricians and gynecologists, and an anesthetist as the supporting specialist doctors.

Hospital as one of the health service facilities is part of health indispensable resources in supporting the health efforts. Essentially, the hospital functions as a place to cure diseases must carry out health service efforts, including promotive, preventive, curative, and rehabilitative, and health restoration, as the government’s responsibility in increasing the level of community welfare. The right to get healthcare services in government hospitals must be protected to ensure health financing for the poor and emergency financing in hospitals because of disasters and extraordinary incidents.

According to Law No.36 of 2009 on Health Article 1 paragraph (1) (from now on referred to as the Health Law), health is a healthy state of physical, mental, spiritual, and social, enabling everyone to have productive life socially and economically. Health is a prerequisite for good economic growth because unhealthy people cannot work correctly, effectively, and productively, and they will spend their finance on their health treatments. The problem of healthcare and the importance of health maintaining is an integral part of the safety of productive human life as a fundamental right for every person guaranteed by the 1945 Constitution, as stated by article 28H, that everyone has the right to healthcare services. Based on article 34 paragraph (3), the state is responsible for providing proper health service and public service facilities.

Nowadays, medical science and technology are developing rapidly; hence, in this case, medical personnel and health workers must work harder also to improve their capacity by providing optimal, humane, and quality services. Nevertheless, the power of technology must be utilized carefully and responsibly to ensure its application is carried out efficiently and humanely. Satisfying and quality service, the friendliness of experienced senior doctors in handling health cases becomes a consideration for prospective patients to choose a particular hospital. According to Mosadeghrad (2013), the excellent quality of health services is indicated by appropriate service methods, good communication, joint decision making, and cultural sensitivity. Based on the description above, the researchers aim to outline the main issues on the general description of the distribution of specialist doctors, a legal review of the needs for specialist doctor services based on Regulation of the Minister of Health No. 3 of 2020 on Hospital Classification and License, and service quality and distribution of specialist doctors in type C regional general hospital (RSUD).

Discussion

Hospital

The hospital definition is clearly stated in Article 1 of Law No. 44 of 2009 on Hospital and Article 1 of Regulation of the Minister of Health No. 3 of 2020 on Hospital Classification and License. It is stated as follows, “Hospitals are health service institutions organizing comprehensive individual health services that provide inpatient, outpatient and emergency services.” Meanwhile, the Hospital in several references is explained as something with some elements, including physical buildings and other infrastructures, patients and doctors; the existence of services; the presence of sick people; the existence of treatment and medical action as professional practice.

To achieve its objectives, functions, and roles requires a transparent form of regulation; the hospital requires adequate legal instruments. Based on Law No. 44 of 2009 on Hospitals, article 1 paragraph (2), government, local government, or the private sector
can establish hospitals. Meanwhile, the Regulation of the Minister of Health on Hospital Classification and License, article 16 paragraph (1), stated the distinction of hospital classification. General hospitals’ classification is divided into type A general hospital, type B general hospital, type C general hospital, and type D general hospital. Furthermore, in Article 17, it is explained in detail about the difference in hospital classification as follows:

1. Type A general hospital, as referred to in Article 16 paragraph (1) letter (a) is a general hospital that has many beds of at least 250 (two hundred and fifty) units.

2. Type B general hospital, as referred to in Article 16 paragraph (1) letter (b), is a general hospital that has many beds at least 200 (two hundred) units.

3. Type C general hospital, as referred to in Article 16 paragraph (1) letter (c), is a general hospital that has many beds at least 100 (one hundred) units.

4. Type D general hospital, as referred to in Article 16 paragraph (1) letter (d), is a general hospital that has many beds at least 50 (fifty) units.

Hospital classification is further explained again in the Draft of Government Regulation of the Republic of Indonesia in implementing Law No. 11 of 2020 on Job Creation of Health Sector in Hospitals. The government determines hospitals’ classification based on health service facilities, supporting facilities, and human resources. As stated in article 16 of the Draft of Government Regulation, hospital classification arrangement remains only based on the number of beds. It is still the same as the previous regulation in Article 17 of the Regulation of the Minister of Health No. 3 of 2020. However, it is still in the discussion stage, which has not yet been passed into a Government Regulation.

Articles 18 and 19 of Law No. 44 of 2009 on Hospitals explained that hospitals are different based on the type of service and management. Based on the types of services provided, hospitals are categorized into general hospitals and specialized hospitals. The general hospital provides health services in all fields and types of disease, while a specialized hospital only provides primary services in one particular field or type of disease based on scientific discipline, age group, organ, or other specificities.10

**Type C Regional General Hospital**

Regional General Hospital belongs to local, district/city, or provincial government. It provides health services and must have clearly defined standard services following the public’s expectations and needs.11 The process of service delivery strongly influences the quality of health services in hospitals. Therefore, the improvement of quality factors such as physical facilities, available human resources, medicines, and medical devices, including professionalism, becomes the critical factor in quality health services and results in equitable distribution of health services to the whole community.12

Type C Regional General Hospitals is commonly facing a fact on the lack of specialist doctors. According to the Board for Development and Empowerment Human Resources of Health, at the end of 2019, there were 350 Type C Regional General Hospitals in Indonesia. In performing its functions, the Type C Regional General Hospitals requires at least basic specialist doctors (internist, obstetricians and gynecologists, surgeons, and pediatrician) and supporting specialists (radiology specialists, anesthetists, and clinical pathologists).13 The evaluation of the Board for Development and Empowerment Human Resources of Health of the Ministry of Health showed as many as 216 hospitals had met the needs for specialists, while the other 134 hospitals have not met the need yet either basic specialists or supporting specialist doctors. The non-fulfillment of specialist doctors in 134 related regional general hospitals can cause sub-optimal service for type C regional general hospitals.

The Need for Specialist Doctor Services at Type C Regional General Hospital
The need for specialist doctor services in Type C Hospitals was once regulated in several Regulations of the Minister of Health, initially regulated in the Regulation of the Minister of Health No. 56 of 2014 on Hospital Classification License. As stated in article 43, the medical workers at the type C regional hospitals consist of at least 9 (nine) general practitioners for basic medical services; 2 (two) general dentists for oral dental, medical services; 2 (two) specialist doctors for each type of basic specialist medical service; 1 (one) specialist doctor for each supporting specialist medical service; and 1 (one) specialist dentist for each type of oral dental specialist medical service.

Regulation of the Minister of Health No. 56 of 2014 subsequently changes to the Regulation of the Minister of Health No. 30 of 2019. In Article 19, it is emphasized that class C general hospital is a general hospital that has facilities and medical service capability of at least 4 (four) basic specialists and 4 (four) specialist medical support. The last amendment of Regulation of the Minister of Health on Hospital Classification and License issued in early 2020 is the Regulation of the Minister of Health No. 3 of 2020 on Hospital Classification and License. Based on this amendment, the medical services provided in Type C Hospital are divided into 3; general medical services, specialist medical services, and subspecialty medical services.14

The difference between the Regulation of the Minister of Health No. 3 of 2020 from the previous one is the removed part or ambiguity on what types of subspecialists are included in the basic subspecialty category, what is included in other subspecialty groups. The specialist doctors for basic medical services, specialist doctors for medical support services, and specialist doctors for medical services other than basic specialists and subspecialists are no longer mentioned in detail also. The operating license for determining the type of hospitals no longer requires based on the assessment results of the fulfillment of the Hospital classification criteria such as buildings and infrastructure, service capability, human resources, and equipment, but only based on assessing the number of beds. In this case, it becomes a challenge, an opportunity, or even a threat for type C regional general hospitals. There will be disadvantaged parties and vice versa by enacting the Regulation of the Minister of Health No. 3 of 2020.

Quality of Service of Specialist Doctors at Type C Regional General Hospital

According to Parasuraman et al., there are five aspects to view the service quality of specialist doctors in type C regional general hospital: reliability, responsiveness, assurance, empathy, and tangibility. Reliability is the ability to provide services reliably and accurately. Responsiveness is the willingness to help service users and provide services on time. Assurance is the knowledge and friendliness of service providers and their ability to inspire service users’ trust and comfort. Empathy is a complex cognitive and affective response to the emotional distress of others. Tangibility is the physical facilities, equipment and appearance of service personnel.15 Government must realize public service to the community as one of the community’s rights, including the right to Empathetic services. Health is a right and an investment. Every citizen has the right to health, including the poor, therefore, there must be a system to regulate its implementation to fulfill citizens’ rights to stay healthy. Health services are the rights of everyone guaranteed by the 1945 Constitution of the Republic of Indonesia, and it must be placed as the primary effort to improve the highest public heal16,17

Based on several studies, specialist doctor services at type C regional general hospital were not optimal since many people do not experience excellent service. The quality of fast and precise service at the type C regional general hospital has not been achieved. There are still many convoluted service procedures, and the community is still having difficulty getting the information services needed.18 The unequal distribution of specialist doctors in various type C regional general hospital type C, then the amendment to the Regulation of the Minister of Health No. 3 of 2020 from the previous regulation is a challenge, opportunity, and threat for type C regional general hospital. It becomes a consideration.
in improving the quality of health services for type C regional general hospital.\textsuperscript{19} Accordingly, the Specialist Doctor Utilization Program can be a solution to the need for equal distribution of specialist doctors in type C regional general hospital to optimize the quality of health services for the community.

The regional government’s role should be further improved in the health sector to maintain the distribution of specialist doctors. Based on article 9 paragraph (3) of Law No. 23 of 2014 on Regional Government, concurrent government affairs are government affairs shared between the Central Government and Provincial and Regional Governments. Concurrent government affairs submitted to the Regions are the basis for the implementation of Regional Autonomy.\textsuperscript{20} The health sector is a compulsory concurrent government affair related to basic services that fall under the region’s authority.

\textbf{Conclusion}

Regulation of the Minister of Health No. 3 of 2020 only determines the classification of hospitals based on the number of beds, while the number and qualifications of specialist doctors are only adjusted to the results of the analysis of the workload, needs, and capacity of hospital services. It is no longer specific to determine the distribution of specialist doctors, especially in the type C regional general hospital, where usually it is a lack of specialist doctors. It results in health services that are not optimal for specialist doctors at type C regional general hospital. The quality of service is a crucial thing to get attention more. Hence, Regulation of the Minister of Health No. 3 of 2020 should not eliminate the minimum requirements for basic specialist doctors owned by hospitals, and the Draft of Government Regulation on Job Creation in the Health Sector in the Hospital should restate the details of the number and specifications of specialist doctors in each hospital. It is expected to be a solution to the need for equal distribution of specialist doctors in type C regional general hospital to optimize the community’s quality of health services.

\textbf{Ethical Clearance}: Nil

\textbf{Conflict of Interest}: Nil

\textbf{Source of Funding}: Self-Funding

\textbf{Acknowledgement}: Nil

\textbf{References}


The Effects of Telehealth During Pregnancy on Maternal Knowledge and Postpartum Mental Health in the Covid-19 Pandemic

Wahyul Anis¹, Rize Budi Amalia¹

¹Lecturer Staff at School of Midwifery, Faculty of Medicine, Universitas Airlangga, Surabaya

Abstract

Background: The Covid-19 pandemic caused changes in health services, especially Antenatal Care (ANC) because pregnant women are at risk of contracting Covid-19 so telehealth is one of the methods developed to make ANC more effective and efficient. This study aimed to analyze the relationship between telehealth during pregnancy on knowledge and mental health of postpartum mothers.

Methods: The research method uses a case-control where there are two groups of samples. The case group consisted of 30 postpartum mothers who received telehealth (routine online monitoring and counseling) during pregnancy, while the control group consisted of 27 postpartum mothers who did not receive telehealth during pregnancy. Samples were taken using a random sampling technique with inclusion criteria are postpartum mothers who received online monitoring and counseling during pregnancy routinely by volunteer mothers. The exclusion criteria were postpartum mothers who did not routinely receive online monitoring and counselling. Statistical analysis used Wilcoxon Mann Whitney, Chi-Square, and Fisher Exact tests.

Conclusions: The telehealth has influenced the maternal knowledge but it will necessary to develop a telehealth strategy that contribute to the mental health of mother during the postpartum period.

Key-words: telehealth, knowledge, mental health, postpartum

Introduction

The first Covid-19 case was discovered in Indonesia on March 2, 2020. During this pandemic, it was reported that health services at the community level stopped, more than 75% of integrated service post (Posyandu) were closed, more than 41% of home visits and less than 10% of facilities primary health care (Puskesmas), antenatal care (ANC) services have also stopped. Services were stopped because of public health worker was concern about Covid-19 transmission because they face it directly¹. Telehealth is one of the innovative methods in health care that aims to monitor a person’s health because it does not allow direct examination and counseling. Telehealth for pregnant women is very important during the Covid-19 pandemic to minimize pregnant women contracting Covid 19 during direct visits to health facilities. During pregnancy, support is needed not only from the family, the community but also from health providers, this monitoring and support can be done via telehealth.

The technical guidelines for maternal and newborn health services during the Covid-19 pandemic issued by the Ministry of Health, have explained several things about telehealth, including that the District / City Health Office is expected to facilitate the use of technology to replace routine direct services. The communication technology used can be a special call center for...
Mother and Children Health, Short Message Service (SMS), Whatsapp messenger, or telemedicine/telehealth applications.

Mental health is very important, especially for postpartum mothers. The decrease in hormones during childbirth can cause postpartum mothers to be susceptible to psychological and emotional problems such as postpartum blues, depression to psychosis. Prevention of this problem is by having a good support system from families, society, and health providers. Knowledge is very important to evaluate the effectiveness of counseling by health providers. It is hoped that good knowledge can make a mother more empowered and improve their health degree. Therefore, we are interested in further researching the effects of telehealth during pregnancy on the knowledge and mental health of postpartum mothers at Krembangan Selatan dan Tenggilis Public Health Center Surabaya, Indonesia.

Materials and Methods

An observational case-control study was undertaken at Krembangan Selatan and Tenggilis Public Health Center, Surabaya, Indonesia. Ethical approval was obtained from the Faculty of Public Health, Universitas Airlangga Surabaya, Indonesia number 02/EA/KEPK/2020.

The study population was postpartum mothers in the target areas of Krembangan Selatan and Tenggilis Public Health Center. The sample in the case group was postpartum mothers who received telehealth (online monitoring and counseling) during pregnancy, while the control group was postpartum mothers who did not receive telehealth during pregnancy. Inclusion criteria are postpartum mothers who were recorded at the Krembangan Selatan and Tenggilis Public Health Centers and received online monitoring and counseling (telehealth) during pregnancy. The exclusion criteria were postpartum mothers who did not routinely receive online monitoring and counseling (telehealth) during pregnancy. The sample size was 30 postpartum mothers in the case group and 27 postpartum mothers in the control group.

This study used an online questionnaire form. The questionnaire contains questions that examine the level of knowledge and mental health of postpartum mothers. The knowledge component was questioned about pregnancy, childbirth, and postpartum that the mother should know, while the mental health questionnaire uses the Edin Burgh Scale. Data were analyzed descriptively with frequency distribution and statistical tests Wilcoxon Mann Whitney, Chi-Square, and Fisher Exact tests.

Results and Discussion

Telehealth is a method of communication in the health sector that aims to monitor a person’s health because it does not allow for direct examination and counseling. Telemedicine has proven to be a method that can be used to provide sustainable care and can increase maternal satisfaction when receiving services.

Telehealth is an important service, especially in the care of pregnant women during the Covid-19 pandemic because it can minimize pregnant women contracting Covid-19 when making direct visits to health facilities. Pregnant women are a vulnerable group to contracting the Covid-19 virus, so many recommendations aim to explain the importance of social distancing and self-isolation for this group. Telehealth will facilitate pregnant women to continue monitoring and management through online communication.

The digital era greatly facilitates health workers in providing health services through telehealth, for example by telephone, virtual communication media, and other applications that provide for online health services. Telehealth carried out in this research is a monitoring and counseling which have done by mother’s companion volunteer. They were midwifery students who have graduated from a midwifery diploma school and currently continuing their education to the professional level. Assistance activities carried out starting from early pregnancy until the postpartum period is complete.
Table 1. Characteristic of the sample

<table>
<thead>
<tr>
<th>Category</th>
<th>Group</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-35 years</td>
<td>27</td>
<td>51.92</td>
<td>25</td>
</tr>
<tr>
<td>&gt;35 years</td>
<td>3</td>
<td>60.00</td>
<td>2</td>
</tr>
<tr>
<td>Job</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Work</td>
<td>5</td>
<td>16.67</td>
<td>11</td>
</tr>
<tr>
<td>Housewife</td>
<td>25</td>
<td>83.88</td>
<td>16</td>
</tr>
</tbody>
</table>

It can be explained that most of the postpartum mothers in both groups were between 20-30 years old and were mostly not working or as housewives.

The Canadian Community Health Survey results show that a mother between 35-39 years old has a lower risk of developing postpartum depression and mothers between 40-44 years old have a greater risk of postpartum depression⁴. A study from Gebregziabher explained that housewives had 0.24 times less incidence of postpartum depression than working mothers, but mothers with low economic status had a 13 times greater risk of developing postpartum depression than the good one⁵. Occupation is a part of the demographic that related to the level of knowledge on postpartum mothers. Maternal age has a significant relationship to maternal knowledge and ability to carry out beneficial practices during the puerperium⁶.

Table 2. Level of knowledge of postpartum mothers

<table>
<thead>
<tr>
<th>Group</th>
<th>Knowledge</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Sufficient</td>
<td>Lack</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Case</td>
<td>17</td>
<td>56.67</td>
<td>10</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>29.63</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>86.30</td>
<td>23</td>
</tr>
</tbody>
</table>

α : 0.05
More than half (56.67%) of the respondents in the case group had a good level of knowledge, while 48.15% of respondents in the control group had sufficient knowledge. Analysis results using the Wilcoxon Mann Whitney statistical test obtained a P-value of 0.037 so that it can be explained that there are different levels of knowledge between the case and control groups.

Table 2 explains that the knowledge level of postpartum mothers in the case group is better than the control group. In the case group, the information given is according to the mother’s need especially about pregnancy, childbirth, and postpartum. This information has given via WhatsApp message, telephone, and video call. The questionnaire used to measure the mother’s knowledge contain what pregnant women should know about pregnancy, childbirth, and postpartum.

Table 3. Mental health status of postpartum mothers

<table>
<thead>
<tr>
<th>Group</th>
<th>Mental health</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Depression risk</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Case</td>
<td>22</td>
<td>73.33</td>
<td>8</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>55.56</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>128.89</td>
<td>20</td>
</tr>
</tbody>
</table>

α : 0.05

In the case group, only a small proportion (26.67%) of postpartum mothers were at risk for depression during the puerperium, while in the control group nearly half (44.44%) of respondents were at risk of experiencing depression during the puerperium. Chi-Square test obtained a P-value = 0.26, it can be explained that there is no difference in the mental health of postpartum mothers both those who get telehealth during pregnancy.

The risk of mental health disorders of postpartum mothers is great influenced by hormonal changes during childbirth and the lack of support from people around them, especially husbands and families. Health providers, especially midwives, assist women during the life cycle of women so that midwives have an important role in preventing mental health problems in women during pregnancy until the postpartum period.

Chi-square test results that there is no relationship between telehealth during pregnancy and the mental health of postpartum mothers. Postpartum mothers feel more comfortable when monitoring and counseling have given directly through home visits. Most mothers are not used to getting online or telehealth counseling. The insignificant results could also be due to the insignificant sample size because many studies are explained the importance of telehealth in mental health. Some of the factors that influence the occurrence of postpartum depression are marital status, unwanted pregnancy, infant disease, and low social support so the importance of good communication and health counseling during postpartum care.
Home-based telehealth is believed to provide effective care for mothers at high risk because it provides psychological and social comfort for pregnant women, but to achieve this requires good communication and information technology systems. The main activity of telehealth by monitoring high-risk pregnant women is an innovative way to provide a better experience for mothers. The mother is in a comfortable and private environment, it can help reduce anxiety and consider the efficiency of costs incurred. The results of experimental studies to analyze the importance of telehealth through telemonitoring in cases of metabolic syndrome explain that telemonitoring can help reduce the risk of disease, improve mental health and physical abilities so that they are more productive. Telehealth with the aim of monitoring has been applied to pregnant women who are at high risk of developing hypertension during pregnancy. Telemonitoring in pregnant women requires careful planning and it is important to collaborate with multidisciplinary knowledge by midwives, obstetricians, psychologists, nutritionists, and others to achieve the expected goals. Telemonitoring has been shown to prevent preterm labor.

Midwives and mother’s companion volunteers are very important to consider planning and strategies when implementing telehealth so that it is expected to have an impact on reducing the incidence of postpartum depression in mothers. Interprofessional collaboration is needed so that the care provided is more comprehensive.

<table>
<thead>
<tr>
<th>Level of knowledge</th>
<th>Mental health</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Depression risk</td>
<td>N</td>
</tr>
<tr>
<td>Good</td>
<td>14</td>
<td>3</td>
<td>100.00</td>
</tr>
<tr>
<td>Sufficient</td>
<td>7</td>
<td>3</td>
<td>100.00</td>
</tr>
<tr>
<td>Lack</td>
<td>1</td>
<td>2</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>8</td>
<td>100.00</td>
</tr>
</tbody>
</table>

α : 0.05

It can be explained that almost half (46.67%) of postpartum mothers who are well informed also have normal health during the postpartum period. Fisher Exact test obtained a P-value of 0.212, it can be concluded that there is no relationship between knowledge and the mental health of postpartum mothers after receiving telehealth during pregnancy.

Table 4 explains that postpartum mothers in the case group with good knowledge have good mental health during the postpartum period, but the statistical analysis is not significant. This could be because other factors affect the mental health of postpartum mothers. Several studies have shown that family support is a major factor in reducing the incidence of depression in postpartum mothers, but there is still low public awareness about the problems and impacts of mental health in pregnancy and childbirth, that efforts are needed to increase public awareness through knowledge of mental health.
Table 5. The relationship between the level of knowledge and mental health in the control group

<table>
<thead>
<tr>
<th>Level of knowledge</th>
<th>Mental health</th>
<th>P-value</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Depression risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Sufficient</td>
<td>9</td>
<td>33.33</td>
<td>4</td>
<td>14.82</td>
</tr>
<tr>
<td>Lack</td>
<td>0</td>
<td>0.00</td>
<td>6</td>
<td>22.22</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>55.55</td>
<td>12</td>
<td>44.45</td>
</tr>
</tbody>
</table>

α : 0.05

The results of statistical analysis using the Fisher Exact test obtained a P-value = 0.009, it can be concluded that there is a relationship between knowledge and the mental health of postpartum mothers who do not get telehealth during pregnancy.

Table 5 explains that in the control group, it was found that mothers with less knowledge were at risk of depression than mothers with sufficient and good knowledge. The results of statistical analysis using chi-square are significant, it can be explain that there is a relationship between knowledge and the mental health of postpartum mothers. Research on knowledge and mental health during a pandemic also suggests that good knowledge can be useful in reducing anxiety and depression.\(^1\)

This study results explained that providing online care through telehealth at Krembangan Selatan and Tenggilis Public Health Center shows the relationship between telehealth during pregnancy with knowledge and mental health in postpartum mothers. It still needs to do further research about strategies used in implementing telehealth because mothers in this work area are not familiar with telehealth. Factors that influence maternal satisfaction during telehealth are very important to studied, so that telehealth will provide an optimal contribution to maternal health both physically, mentally, and socially.

Research in Poland explains that midwives are not ready to accept new tasks in perinatal care, such as assessing and treating mothers who experience mental health problems during pregnancy and childbirth. Special training is needed to increase knowledge and skills related to screening and management of depression in mothers.\(^1\) Currently, there have been many studies on mental health during pregnancy and childbirth, but there have not been many implementations and efforts to reduce mental health problems. It is very important to pay attention to the mental health of mothers. Increasing maternal knowledge is an effort to reduce mental health problems in postpartum mothers.

**Conclusion and Acknowledgement**

The telehealth contributes to increasing maternal knowledge especially when there are restrictions on offline pregnancy visits because of the Covid-19 pandemic but it is necessary to develop strategies so that telehealth can more effective to improve the mental health of mothers during pregnancy and postpartum.

**Conflict of Interest:** None
Source of Funding: The research was funded from Universitas Airlangga

Ethical Clearance: Taken from the Faculty of Public Health Universitas Airlangga Surabayacommitee

References


Discharge Plan for Parents having Children Suffering from Head Trauma

Walaa Abd Allah El-Tayar¹, Eman Amin Mohammed¹, Salma El Sayed Hassan¹, Hyam Refaat Tantawi¹

¹Department of Pediatric Nursing, Faculty of Nursing, Ain Shams University, Cairo, Egypt

Abstract

Background: Effective discharge planning is a vital component to maintain care continuity for head trauma pediatric patient. Despite this, there is a critical gap in the process and quality of discharge planning in Egypt.

Aim of this study was to assess the effect of discharge plan on parents having children suffering from head trauma.

Design: A quasi-experimental design was used.

Settings: This study was conducted in Emergency Department, Pediatric Intensive Care Unit (PICU) and Neurological Department at children Hospital affiliated to Ain Shams University and El-Menshawy Hospital in Tanta affiliated to Ministry of Health.

Subject: A convenient sample consist of 40 parents accompanied their children suffering from head trauma who attended in the previously mentioned settings.

Tools: 1)- Pre-designed questionnaire sheet which included two parts: part I: concerned with characteristics of studied parents and characteristics of the studied children, Part II: knowledge of the parents regarding caring of their children suffering from head trauma, 2)- Observational check list and 3)- Child Medical Record.

Results: All of studied parents reported that no one gave them any information about head trauma. There was a marked improvement in parents’ knowledge and reported practices post implementation of discharge plan. There were strong positive correlations between studied parents’ total knowledge and their total reported practices related to care of children after head trauma pre/post discharge plan. There was highly statistical significance difference between parents’ total knowledge regarding care of head trauma in children and their level of education post implementation of discharge plan. There was statistical significance difference between parents’ total reported practices regarding care of head trauma in children and their level of education post implementation of discharge plan.

Conclusion: All of research hypothesis were accepted and implementation of discharge plan led to significant improvements in parents’ knowledge and reported practices regarding care of their children suffering from head trauma.

Recommendations: Discharge plan for all parents having children suffering from head trauma must begin with parents at their first day of admission.

Key words: Head trauma, Discharge plan, Parents, Pediatric Patient

Introduction

Traumatic head injury is recognized as a significant public health issue that needs urgent intervention due to its role in high pediatrics’ mortality and long-term disabilities, which involve impaired physical, psychological, cognitive, emotional, and social functions, resulting in complete reliance on parents for all everyday activities.¹ & ²
Management of pediatric patients with mild head trauma targets to concentrate on early detection of intracranial injuries. These intracranial injuries lead to bad neurological outcomes if not readily detected. Clinical management of moderate to severe head trauma targets to maintain physiological state of the pediatric patient to prevent secondary brain injury, following identifying of intracranial injuries that can profit from neurological intervention and neurosurgical strategies.3

Pediatric patient return home immediately after being medically stable. This doesn’t imply that the child is recovered completely. Instead, it intelligibly means that, a specialist has concluded that the child’s condition is stable and the hospital-level treatment is no longer needed. A discharge plan is a series of actions and activities carried out by hospital clinicians to ensure that patients move smoothly and safely from acute care to subsequent care or home environments.4

After the child returns home, parents have to continue engaging in the care scenario as continuity of care and rehabilitation becomes their responsibility since the post-release period is a highly vulnerable period for pediatric head trauma patients. Intensive steps to enhance pediatric patients’ and parents’ hospital discharge planning are being considered to avoid poor discharge behaviors and treatment discontinuity and reduce the likelihood of poor health outcomes, readmission, and other adverse outcomes.5, 6

**Aim of the Study**

This study aims to assess the effect of discharge plan on parents having children suffering from head trauma. This will be achieved through:

- Assessing knowledge and reported practice of parents related to care of their children suffering from head trauma.
- Designing and implementing discharge plan for parents related to care of their children suffering from head trauma.
- Evaluating the effect of discharge plan for parents related to care of their children suffering from head trauma

**Research hypothesis:**

The current study hypothesized that there is a positive relation between discharge plan for parents having children suffering from head trauma and knowledge and reported practice related to head trauma.

**Research Design:**

A quasi-experimental design was utilized in carrying out the study.

**Technical design**

Technical design includes the research design, setting, subjects, and tools for data collection.

**Research Setting:**

The study was conducted in emergency department, pediatric intensive care unit (PICU) and neurological department at children Hospital affiliated to Ain Shams University and El-Menshawy Hospital in Tanta affiliated to Ministry of Health.

**Research Subjects:**

A convenient sample consist of 40 parents accompanied their children suffering from head trauma who attended in the previously mentioned settings, regardless their characteristics.

**Tools for data collection:**

Data was executed using the following Tools:

**I-Pre- Designed Questionnaire Sheet (Before and After):**

This sheet was developed by researcher after reviewing the related literatures in a simple Arabic language to suit all parent’s knowledge related to their awareness about care of their children suffering from head trauma. It was consisted of two parts:
Part 1: It was concerned with characteristics of studied subjects:

- Characteristics of studied parents include name, age, gender, educational level, and occupation.

- Characteristics of the studied children include name, age, gender, ranking, date of the trauma, causes of the trauma, and imaging studies include CT.

Part 2: It was concerned with knowledge of the parents regarding caring of their children suffering from head trauma which include definition, causes, types, signs and symptoms, diagnosis, management, preventive and safety measures.

II- Observational Check List:

It was used to assess parents reported practice regarding care of their children suffering from head trauma which include:

Care of child with posttraumatic seizure, consisted of 7 items according to checklist adapted from Hockenberry & Wilson, 7.

Administer of oral medication, consisted of 23 items according to checklist adapted from Leifer, 8.

Administer of ear drops, consisted of 10 items according to checklist adapted from Leifer, 9.

Administer of topical medication (Ointments and creams), consisted of 8 items according to checklist adapted from Pope, 10.

Administer of nose drops, consisted of 7 items and nasal sprays consisted of 7 items according to checklist adapted from Perry et al., 11.

Applying eye ointments and eye drops, consisted of 12 items according to checklist adapted from Mason & Stevens, 12.

Measuring axillary temperature, consisted of 11 items according to checklist adapted from McKinney et al., 13.

Apply tape water compression, consisted of 15 items according to checklist adapted from McKinney et al., 14.

Perform scalp wound care at home, consisted of 17 items according to checklist adapted from Schmitt, 15.

Perform cast care at home, consisted of 20 items according to checklist adapted from James et al., 16.

Maintain skin integrity at home, consisted of 17 items according to checklist adapted from The Royal Children’s Hospital Melbourne, 17.

Perform eye care at home, consisted of 8 items according to checklist adapted from Macqueen et al., 18.

Perform oral care, consisted of 17 items according to checklist adapted from Beatty, 19.

Preventing of falling for child from (0 to 2 years), consisted of 20 items according to checklist adapted from McWilliams, 20.

III- Child Medical Record.

Medical record used to assess child history, diagnosis, growth and development, investigations, consultation, treatment, and prognosis.

Operational design:

Field Work

The actual field work was implemented over a period of 6 months from the first of September 2019 up to the end of February 2020. The researcher was attending in the agreed study setting during morning shift 4 days/week (Saturday to Tuesday) from 8.00 am to 2.00 pm. and actual field work was divided into four phases:

1-Assessment phase

2-Planning phase

3-Implementing phase

4-Evaluating phase
Validity and Reliability:

Assessment and certainty of the tools of the study were performed by pool of five experts in pediatric nursing to enable validating of format, layout, consistency, accuracy and relevance of the tools. The reliability was conducted for the developed tools. Cronbach’s Alpha for questionnaire was 0.921 and for reported practices was 0.968.

Exploratory phase:

A pilot study was proceed on 10% of the parents of the study sample (four parents) to test the validity and feasibility of the tools, time consumed to fill in the tools. The parents involved in the pilot study were banned from the study sample.

Administrative design:

An official sanctioned permission to carry out the study was obtained through an issued letter from Dean of faculty of nursing, Ain Shams University to hospital directors of the previously mentioned settings.

Statistical Design:

The obtained data was organized, tabulated, categorized and statistically analyzed by using statistical package for social sciences (SPSS). The significance of the results was considered as follows: When \( P > 0.05 \) there is no statistical significance difference and \( P \leq 0.001 \) there is a statistical significance difference.

Ethical considerations:

The ethical considerations in the study included the following:

All the gathered data was used for research purpose only. The study sample was edified about the purpose and intended results of the study and they was assured that the study is free of hazards, they uncoerced for participation and they have the right to withdraw from the study at any time and without given any reason. They were assertive also that anonymity and confidentiality were guaranteed. Informed consent was obtained from parents preceding data collection.

Results

This study was designed to assess the effect of discharge plan on parents having children suffering from head trauma.

<table>
<thead>
<tr>
<th>Items</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age / years</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 20 &lt; 30 )</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>( 30 &lt; 40 )</td>
<td>19</td>
<td>47.5</td>
</tr>
<tr>
<td>( 40 \leq 50 )</td>
<td>11</td>
<td>27.5</td>
</tr>
<tr>
<td>( \bar{x} )</td>
<td></td>
<td>35.3 ± 7.3</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (father)</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Female (mother)</td>
<td>32</td>
<td>80</td>
</tr>
<tr>
<td><strong>Level of education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Read and write</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Secondary education</td>
<td>21</td>
<td>52.5</td>
</tr>
<tr>
<td>High education</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td><strong>Job</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not working</td>
<td>26</td>
<td>65</td>
</tr>
<tr>
<td>Free job</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Governmental job</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 1 showed that less than half (47.5%) of the studied parents aged from 30 < 40 years with mean age 35.3 ± 7.3 years and most (80%) of them were females. Moreover, this table revealed that, more than half (52.5%) of the studied parents held secondary education and 65% of them were not working.

Table 2: Distribution of the Studied Children according to their Characteristics (No=40).

<table>
<thead>
<tr>
<th>Items</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age / years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5</td>
<td>9</td>
<td>22.5</td>
</tr>
<tr>
<td>5 &lt; 10</td>
<td>17</td>
<td>42.5</td>
</tr>
<tr>
<td>10 &lt; 15</td>
<td>11</td>
<td>27.5</td>
</tr>
<tr>
<td>15 &lt; 20</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td>(\bar{x} \pm SD)</td>
<td>8.5 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28</td>
<td>70</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Ranking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Second</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Third &amp; more</td>
<td>10</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 2 revealed that more than two fifth (42.5%) of the studied children aged from 5 < 10 years with mean age 8.5 ± 4.4 years and more than two thirds (70%) of them were males. Also, it was clear from this table that, half (50%) of the studied children were ranked as the first child in their families.

Table 3: Distribution of the Studied Parents according to their Sources of Knowledge about Head Trauma (No=40).

<table>
<thead>
<tr>
<th>Sources of knowledge</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No one give me any information in hospital.</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3 showed that all (100%) of the studied parents reported that no one gives them any information in hospital about head trauma in hospital.
Figure (1): Distribution of the Studied Parents according to their total Knowledge about head trauma in children pre/post discharge plan.

Figure (1) illustrated that, there was a marked improvement in parents’ knowledge post implementation of discharge plan with highly statistically significant difference ($\chi^2 = 42.7$ and $P$ value = 0.000), where the majority (92.5%) of the studied parents had satisfactory total knowledge regarding to care of children after head trauma post implementation of discharge plan compared to only (20%) pre implementation.

Figure (2): Distribution of the Studied Parents according to their total reported practices related to care of children after head trauma pre/post discharge plan.
Figure (2) illustrated that, there was a marked improvement in parents’ reported practices post implementation of discharge plan with highly statistically significant difference ($\chi^2 = 54.8$ and $P$ value = 0.000), where the majority (87.5 %) of the studied parents had adequate total reported practices regarding to care of children after head trauma post implementation of discharge plan compared to only 5% of them pre implementation.

Table (4): Correlation between the Studied Parents’ total knowledge and their total Reported Practices related to Care of Children after Head Trauma Pre/Post Discharge Plan (No=40).

<table>
<thead>
<tr>
<th>Total parents’ reported practices</th>
<th>Total parents’ knowledge</th>
<th>Pre discharge plan</th>
<th>Post discharge plan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>p</td>
</tr>
<tr>
<td>Pre discharge plan</td>
<td></td>
<td>0.772</td>
<td>0.000**</td>
</tr>
<tr>
<td>Post discharge plan</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table (4) illustrated that, there were strong positive correlations between studied parents’ total knowledge and their total reported practices related to care of children after head trauma pre/post discharge plan.

Table (5): Relation between the Studied Parents’ Characteristics and their total Knowledge regarding Care of Head Trauma in Children Post Discharge Plan (No=40).

<table>
<thead>
<tr>
<th>Parents’ characteristics</th>
<th>Parents’ total knowledge</th>
<th>( \chi^2 )</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unsatisfactory</td>
<td>Satisfactory</td>
<td></td>
</tr>
<tr>
<td>Age / years</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>20 &lt; 30</td>
<td>2</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>30 &lt; 40</td>
<td>0</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>40 ≤ 50</td>
<td>1</td>
<td>9.1</td>
<td>10</td>
</tr>
<tr>
<td>Gender</td>
<td>Fisher Exact Test</td>
<td>0.502</td>
<td></td>
</tr>
<tr>
<td>Male (father)</td>
<td>3</td>
<td>9.4</td>
<td>29</td>
</tr>
<tr>
<td>Female (mother)</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Level of education</td>
<td>40</td>
<td>0.000**</td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Read and write</td>
<td>2</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Secondary education</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>High education</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Job</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not working</td>
<td>2</td>
<td>7.7</td>
<td>24</td>
</tr>
<tr>
<td>Free job</td>
<td>1</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Governmental job</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

** Highly statistical significant difference
This table illustrated that, there was highly statistical significance difference between parents’ total knowledge regarding care of head trauma in children and their level of education post implementation of discharge plan ($\chi^2 = 23.46$ and $P\ value = 0.000$). While, there was no statistical significance difference between parents’ total knowledge regarding care of head trauma in children and their age, gender and job post implementation of discharge plan ($P\ value = 0.147, 0.502 & 0.812$ respectively).

Table (6): Relation between the studied parents’ characteristics and their total reported practices regarding care of children with head trauma post discharge plan (No=40).

<table>
<thead>
<tr>
<th>Parents’ characteristics</th>
<th>Parents’ total reported practices</th>
<th>$\chi^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inadequate</td>
<td>Adequate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Age / years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 &lt; 30</td>
<td>2</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>30&lt; 40</td>
<td>1</td>
<td>5.3</td>
<td>18</td>
</tr>
<tr>
<td>40 ≤ 50</td>
<td>2</td>
<td>18.2</td>
<td>9</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (father)</td>
<td>5</td>
<td>15.6</td>
<td>27</td>
</tr>
<tr>
<td>Female(mother)</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Read and write</td>
<td>2</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Secondary education</td>
<td>2</td>
<td>9.5</td>
<td>19</td>
</tr>
<tr>
<td>High education</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Job</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not working</td>
<td>4</td>
<td>15.4</td>
<td>22</td>
</tr>
<tr>
<td>Free job</td>
<td>1</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Governmental job</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

** Highly statistical significant difference

This table illustrated that, there was statistical significance difference between parents’ total reported practices regarding care of head trauma in children and their age, gender and job post implementation of discharge plan ($P\ value = 0.417, 0.563 & 0.662$ respectively).

Discussion

Head trauma is a critical public health problem which has a relatively high rate of emergency department
visits worldwide. It is one of the most frequent causes of pediatric death and acquired disabilities. Long-term disabilities can transpire across many areas of child functioning including: cognitive abilities, psychomotor skills, language, executive functioning, emotional and behavioral functioning, social competences, academic and occupational performance and quality-of-life, resulting to a high burden for pediatric patients and their families.\(^\text{21}\)

The findings of the current study revealed that, more than two fifths of the studied parents aged from \(30 < 40\) years. This finding was in agreement with the study of Halawa et al.,\(^\text{22}\) who study entitled “Epidemiology of Non-Fatal Injuries Among Egyptian Children: A Community-Based Cross-Sectional Survey” found that, about one third of the studied mothers were in the age group \(30 < 40\) years.

As regards characteristics of parents under study, results revealed that, more than three quarters of studied parents were females. This finding was consistent with O’Brien et al.,\(^\text{23}\) who conducted a study entitled “A Comparison of Student and Parent Knowledge and Perceived Confidence about Brain Injury and Concussion” and reported that, females parents proportion were more than two thirds, while males parents proportion were more than one third.

Concerning to parents’ level of education, the finding of the present study showed that, more than half of the studied parents held secondary education. This finding was not in accordance with Black et al.,\(^\text{24}\) who carried out a study entitled “Parental Report of Significant Head Injuries in Children Aged 3–17 Years: United States, 2016” reported that, the percentage of children suffered from head injury whose parents had high school education were higher compared with those whose parents had secondary education or less. These results might be due to the differences in the study settings.

This study showed that, more than two thirds of studied parents were not working. This finding was incongruent with Foster et al.,\(^\text{25}\) who performed a study entitled “Parent Perspectives and Psychosocial Needs 2 years Following Child Critical Injury: A qualitative Inquiry”, observed that, proportion of paid employment was the majority of the studied sample.

Regarding the characteristics of the studied children, the present study clarified that, the most affected age group was \(5 < 10\) years. This finding was contrasting with Amram et al.,\(^\text{26}\) who found in a study entitled “Socio Economic Status and Traumatic Brain Injury amongst Pediatric Populations: A Spatial Analysis in Greater Vancouver”, that, the majority of injuries occurred in children between 15 and 18 years of age.

The findings of the present study revealed that male children were more affected than female children. This was parallel to study done by Egbohou et al.,\(^\text{27}\) who carried out a study entitled “Epidemiology of Pediatric Traumatic Brain Injury at Sylvanus Olympio University Hospital of Lomé in Togo” described that, males have a higher risk of head injury than females. This result might be due to males and females are different in growing environment, neurodevelopment, and sociological attributes.

Considering parents’ source of knowledge about head trauma, all of studied parents’ reported that, no one give them any information about head trauma in hospital. From the researcher point of view, this could be due to low awareness of health care providers on importance of discharge plan in addition to, some job obstacles include but not limited to, lack of staff, and poor communication among different healthcare professionals. This finding was supported by that of Sarsfield et al.,\(^\text{28}\) who performed a study entitled “Evaluation of Emergency Medicine Discharge Instructions in Pediatric Head Injury” reported that, children sustaining head injury were inadequately instructed to restrict athletic activities upon discharge. In the same context, Camp et al.,\(^\text{29}\) who carried a study entitled “Emergency department visits for children with acute asthma: discharge instructions, parental plans, and follow-through of care--a prospective study” mentioned that, parents not obtained any specified asthma strategies to demote the repercussion of upper respiratory tract infections. Also
Kirk et al.,30 studied “Supporting Parents following Childhood Traumatic Brain Injury: A Qualitative Study to Examine Information and Emotional Support Needs across Key Care Transitions.” clarified that, parents had disappointed information and emotional support needs across the care pathway from the time of the accident up to their child’s return to home. They lacked information related to the consequences of the traumatic brain injury on their child, existing and future treatment/rehabilitation plans, helping their child and managing their behavior, and accessing services/support.

According to the studied parents’ total knowledge and total reported practices, the current study clarified that, there were an improvement post discharge plan compared to its implementation. These findings were corresponding to those of Mohamed et al.,31 who conducted a study entitled “Effect of Discharge Plan for Children Undergoing Chemotherapy and their Caregivers on Improving Practice and Coping Pattern” affirmed that, caregivers who take receipt of discharge plan instructions showed improving in knowledge and practice with regard to care of their children. In the same context Rashad et al.,32 very recently carried out a study entitled “Effectiveness of Maternal Training Program on Improvement of Care Provided to Their Children with Cerebral Palsy at Zagazig University Hospitals” reported that, slightly less than on quarter of the studied mothers had satisfactory reported practice score before implementation of the educational module compared to half of them after implementation of educational module. On the other hand, these findings were not in accordance with Thomas et al.,33 who carried out an observational study entitled “Parental Knowledge and Recall of Concussion Discharge Instructions” reported that, nearly fifth of parents who received verbal instructions were muddled about when to return to the emergency department after evaluation for head injury, moreover one quarter of parents misremembered discharge advice related to concussion.

As regards the correlation between the studied parents’ total knowledge of and their total reported practices regarding care of head trauma in children, the result of the current studied clarified that, there were a strong positive correlations between studied parents’ total knowledge and their total reported practices post implementation of discharge plan. This result indicated that the improvement in parents’ knowledge about head trauma led to improve parents’ reported practices. Previously, Hassan et al.,34 carried out a study entitled “Discharge Plan for Mothers to Cope with their Children Suffering from Bronchial Asthma” mentioned that, there were a strong positive correlations between total knowledge of the studied parents and their total reported practices post implementation of discharge plan. Additionally, Mahmoud & Sabea,35 recently conducted a study entitled “Dietary Counseling Program for Mothers of Children with Cerebral Palsy” mentioned that, mothers’ knowledge and reported practices toward their children with cerebral palsy improved significantly post implementation of the dietary educational program.

As regards the correlation between the characteristics of the studied parents and their total knowledge and total reported practices regarding care of head trauma in children, the result of the current studied indicated that, there were a positive correlations between parents’ level of education and their total knowledge and total reported practices post implementation of the discharge plan. Meanwhile, there were no correlations between parents’ age and job. These findings were close resemblance to those of Megahed et al.,36 who carried out a study entitled “Knowledge, Attitude and Practice of Rural Mothers towards Home Injuries among Children Under 5 years of age in Menouf District- Menouf Governorate, Egypt” reported that, there was a statistically significantly higher percent of satisfactory knowledge amidst mothers who held high education. There was a significant positive correlation between mothers’ practice and either their education.

**Conclusion**

All of research hypothesis were accepted and the implementation of the discharge plan led to significant improvements in parents’ knowledge and reported practices regarding care of their children suffering from head trauma.
head trauma.

**Recommendations:**
- Discharge plan for parents having children suffering from head trauma must begin with parents at their first day of admission.
- Reinforce effective discharge plan through planning, educational programs for health care providers, performance review, and standardized and policy-driven protocol.
- Periodical educational training program for all parents in safety measures should be conducted to decrease incidence of head trauma among pediatric population.

**Conflict of Interest**: The authors declare that they have no conflict of interest.

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Pap Smear in A Sample of Iraqi Women with Positive Visual Inspection by Acetic Acid

Wasan Fawzi Sanad1, Halah Abdulwahhab Mohammed2, Bothayna Ahmed Alwan3
1Specialist Obstetrics and Gynecology, Fallujah Teaching Hospital, Ministry of Health, AlAnbar, Iraq, 2Specialist Obstetrics and Gynecology, Ministry of Health, Baghdad, Iraq, 3Specialist Obstetrics and Gynecology, Abu Garib Hospital, Ministry of Health, Baghdad, Iraq

Abstract

Background: Organized cervical cytology screening program are not feasible in many developing countries where cervical carcinoma is important cause of mortality among adult women. This study compared visual in section of the cervix after application of 3-4% acetic acid (Visual inspection by acetic acid, or colposcopy) with cytology as methods for the detection of cervical carcinoma and its precursors.

Method: Prospective study was done in Baghdad city. 547 patients all of them had Visual inspection by acetic acid those with positive Visual inspection by acetic acid send for Pap smear and those with positive Pap smear send for Histopathology to see the sensitivity and specificity of Visual inspection by acetic acid by 3-4% acetic acid.

Result: Visual inspection by acetic acid was positive 35.5% and Negative 64.5%, Pap smear was positive 32.0% and Negative 68.0%.

Visual inspection by acetic acid:

Sensitivity → 92.6%.
Specificity → 46.5%.
Positive predictive → 44.8%.
Negative predictive value → 93.0%.
Accuracy rate → 61.2%.

This result mean Visual inspection by acetic acid is significant and sensitive test for detection of premalignant change of carcinoma of cervix.

Conclusion: There was no significant demographic change in pap smear in Iraqi general VIA can be used in screening of cervical cancer for premalignant and malignant condition Because of high negative predictive value this will be reassure the patients probably they don’t have CIN or cancer.

Keywords: Positive Visual Inspection, Acetic Acid,

Introduction

In countries that do not have access to cervical cancer screening and prevention programs, cervical cancer remains the second most common type of cancer (17.8 per 100,000 women) and cause of cancer deaths (9.8 per 100,000) among all types of cancer in women.

Human papillomavirus (HPV) is central to the development of cervical neoplastic and can be detected...
in 99.7% of cervical cancers. The most common histologic types of cervical cancer are squamous cell (69% of cervical cancers) and adenocarcinoma (25%) (1).

Cervical cancer can be prevented by A. Visual inspection of the cervix & pap smear, in countries where Pap smear screening is routine, it is recommended that females who have had sex seek regular Pap smear testing. Guidelines on frequency vary from every three to five years. If results are abnormal, and depending on the nature of abnormality, the test may need to be repeated in sex to twelve months. If the abnormality requires closer scrutiny, the patient may be referred for detailed inspection of the cervix by colposcopy. The patient may also be referred for HPV DNA testing, which can serve as an adjunct to Pap testing. Additional biomarkers which may be applied as ancillary test with Pap test are evolving (2).

Many health care providers are under the false impression that only sterile water, or no lubricant at all, should be used to lubricate the speculum. This may result in unnecessary discomfort. A number of studies have shown that using a small amount of water-based gel lubricant does not interfere with, obscure, or distort the pap smear. Further, cytology is not affected nor some STD test (3).

B: Vaccination

HPV vaccines are typically given to women age 9 to 26 as the vaccine is only effective if given before infection occurs. The vaccines have been shown to be effective for at least 4-6 years (4).

C: Condom

One study suggests that prostaglandin in semen may fuel the growth of cervical and uterine tumors and that affected women may benefit from the use of condoms (5).

Pre-cancerous lesion

It classifies mild dysplasia as CIN1, moderate dysplasia as CIN2, and severe dysplasia and CIS as CIN3, more recently, CIN2 and CIN3 have been combined into CIN2/3. These results are what a pathologist might report from a biopsy (6).

Cervical cancer

Is a severe and potentially life-threatening illness with adverse effects on the physical and psychological well-being of patients (7). The development of abnormal cells in the cervix (uterine neck) is cervical cancer. More than 92,000 deaths in the female population due to cervical cancer were reported in agreement with WHO. The number of cervical cancer deaths is 10.3 percent in total (8). 158,000 new cervical cancer cases have been reported, and 95,766 have been reported in Southeast Asia. Cervical cancer is the most prevalent disease in Indonesia among women 0.8% (9).

Aim of Study

The aim of this study is to compare between naked eye visual inspection with acetic acid and cervical cytology as a screening test for cervical intraepithelial neoplasia.

Materials and Methods

Statistical prospectivestudy was carried out in Baghdad city (one hospital) and six health centers and oncology teaching center during the period from the 1st of May 2019 to the 1st of June 2020. The study included 547 women ages 20-55 years, who were attending the outpatient department for different gynecological problems.

Exclusion criteria for the study:

1. Virgin.
2. Pregnant woman.
3. Previous total hysterectomy.
4. Women with severe cervicitis until they had completed treatment.
5. Moderate to severe vaginal bleeding at time of examination.
6. During menstruation.
The patients included in the study were given information about the study, and agreed to participate. Questions asked to the patients are listed in paper.

Freshly prepared 5% acetic acid solution (5ml of glacial acetic acid with 95 ml of distilled water). The woman is asked to lie in a modified lithotomy position on to the examination table after she has emptied her bladder.

Cusco’s speculum was carefully inserted in the vagina, and avoids use antiseptic solution for Sterilization of genetilia. Inspection of the cervix. Then screening by VIAdone for all patient and those who had abnormal changes by visual inspection and the cervix looks unhealthy then we did tests Pap smear sampling was performed. A Pap smear sample was done for 294 patients using conventional or liquid based by Bruch.

The patient should avoid douching or using vaginal medicines, spermicidal foams, creams, or gels pessaries. The patient should not have sexual intercourse for 1 to 2 days before Pap test, because these may hide abnormal cells and cause unclear results. After completing the Pap smear, on the same day and it involve gentle application of 5% acetic acid using cotton swab to avoid bleeding. The woman is informed that she might feel a slight stinging sensation. After 1–2 minutes a naked eye evaluation was performed under 100- watt illumination. The transformation zone is carefully checked for any dense non movable acetowhite areas in the mucosa. If acetowhite areas are identified on the cervix after 1-2 minute, the test is positive. Criteria for categorization

Results

VIA test outcome Criteria

- Negative (-)
- Positive (+).
- Quire 100 randomly selected patients with negative acetowhite area but complain of IMB or PCB or having unhealthy cervix during examination.
- No aceto white lesions.
- Acetowhitening on endocervical polyps, nabothisan cysts.
- Prominent white line like acetowhitening of the squamo columnar junction.
- Faint, translucent, ill defined, irregular acetowhite lesions on the cervix.
- Definite, angular, geographic acetowhite lesions far away from the squamo columnar junction.
- Opaque, dense, dull, definite, well-defined acetowhite lesions touching the squamo columnar junction or close to the external os.
- Large, circumferential, well-defined, thick, dense acetowhite lesions.
- Growth on the cervix turns acetowhite.
- Those with negative result reassured and returned back to the program of screening by Pap smear every three year.
- Those with quireor positiveresult we did for her pap smear and then we do comparison between both results of pap and VIA.

We did colposcopy for those with abnormal finding either by pap or by VIAThen a punch biopsy was taken from transformation zone of the cervix of 94 patients with positive aceto white areas and pap smear with positive result. These samples were kept in container with 10% formaldehyde, and sent for histopathological examination to the pathologist specificity, predictive values) with their 95% confidence interval. Finding with p value <0.05 were considered significant and our P value =0.0001 tables (1-5).

Result of Pap smear: Normal LSIL HSIL Cancer

Result of VIA: Negative, positive, quire

Result of Histopathology: Normal, N.S.C CIN1 CIN2 CIN3 Cancer.
Table 1: Patient complaints

<table>
<thead>
<tr>
<th>Complaints</th>
<th>Yes</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>373</td>
<td>68.2</td>
</tr>
<tr>
<td></td>
<td>174</td>
<td>31.8</td>
</tr>
<tr>
<td>Bleeding (Postcoital, Intermenst)</td>
<td>Yes</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>55.2</td>
</tr>
<tr>
<td></td>
<td>174</td>
<td>31.8</td>
</tr>
<tr>
<td>Pain &amp; Dysparunia</td>
<td>Yes</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>182</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>34.9</td>
</tr>
<tr>
<td></td>
<td>174</td>
<td>31.8</td>
</tr>
<tr>
<td>Discharge &amp; Itching</td>
<td>Yes</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>252</td>
<td>46.1</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>22.1</td>
</tr>
<tr>
<td></td>
<td>174</td>
<td>31.8</td>
</tr>
</tbody>
</table>

Data was taken from 547 patients, 68.2% of them had complaints while 31.8% had no complaints. Some of them had multiple complaints, those with bleeding (postcoital, intermenstual) 13.0%, those with pain & dysparunia 33.3%, those with discharge & itching was 46.1%.

Table 2: VIA and Pap smear findings

<table>
<thead>
<tr>
<th>VIA findings</th>
<th>Yes</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>194</td>
<td>35.5</td>
</tr>
<tr>
<td></td>
<td>353</td>
<td>64.5</td>
</tr>
<tr>
<td>Pap smearresult</td>
<td>Yes</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>94</td>
<td>32.0</td>
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<tr>
<td></td>
<td>200</td>
<td>68.0</td>
</tr>
</tbody>
</table>

Table 3: Histo findings

<table>
<thead>
<tr>
<th>Histo findings</th>
<th>Yes</th>
<th>%</th>
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<tbody>
<tr>
<td>CINI</td>
<td>71</td>
<td>75.5</td>
</tr>
<tr>
<td>CINII</td>
<td>22</td>
<td>23.4</td>
</tr>
<tr>
<td>CINIII</td>
<td>1</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Table 4: VIA findings

<table>
<thead>
<tr>
<th>VIA findings</th>
<th>Pap smear positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>87</td>
<td>92.6</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>7.4</td>
</tr>
</tbody>
</table>

P=0.0001 (Significant using Pearson Chi-square test at 0.05 level).

Diagnosis by VIA

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>92.6</td>
</tr>
<tr>
<td>Specificity</td>
<td>46.5</td>
</tr>
<tr>
<td>Positive Predictive Value</td>
<td>44.8</td>
</tr>
<tr>
<td>Negative Predictive Value</td>
<td>93.0</td>
</tr>
<tr>
<td>Accuracy Rate</td>
<td>61.2</td>
</tr>
</tbody>
</table>

Table 5: Patient Complain, VIA and Pap positivity

<table>
<thead>
<tr>
<th>Histo findings</th>
<th>VIA Positivty</th>
<th>Pap smear Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>CINI</td>
<td>64</td>
<td>90.1</td>
</tr>
<tr>
<td>CINII</td>
<td>22</td>
<td>100</td>
</tr>
<tr>
<td>CINIII</td>
<td>1</td>
<td>100</td>
</tr>
</tbody>
</table>

*Significant positivity using Pearson Chi-square test at 0.05 level.

Discussion

VIA sensitivity 92.6%, specificity 46.5%, VIA positivity for hormonal contraception user 22.4% & Pap smear positivity for hormonal contraception users 16.3% & for smoker VIA positivity is 33.3% & Pap smear positivity is 24.2%. And this mean that smoking and hormonal contraception is not significant risk factor for cervical cancer in our community, the most striking thing in our study is significant VIA & Pap smear positivity for patient complaining of (post coital & intermenestrual bleeding) is 78.9% & 51.5% respectively.

Hormonal contraception is implicated on cervical cancer development. Appleby et al, in analyzing 24 studies worldwide that included 16,573 women with cervical cancer and 35,509 without cervical cancer, concluded that the relative risk of cervical cancer is increased in current users of oral contraceptives, and that increased risk is higher in current smokers’ women (10).
Male Muslim circumcision and circumcision decrease incidence of HPV infection, also Muslim population have the same culture and same sexual habit, many studies show that notably orthodox and Jewesses are almost immune to cervical cancer, the strict avoidance of coitus during and after menstruation when cervical epithelium might become vulnerable,

While in Washington Pap smear is a robust test among HIV-positive women regardless of immune status. Results should be cautiously interpreted when using HPV among those younger, immunosuppressed, or on HRT <2 years, and when using VIA among those ≥40 years, of 500 enrolled, 498 samples were collected. On histology, there were 172 (35%) normal, 186 (37%) CIN1, 66 (13%) CIN2, 47 (9%) CIN3, and 27 (5%) indeterminate. Pap (ASCUS+) was the most sensitive screening method (92.7%), combination of both Pap (HSIL+) and VIA positive was the most specific (99.1%), and Pap (HSIL+) had the highest AUC (0.85). In multivariate analyses, (10)

Xi et al. in 2009 reported an analysis of 1,050 women HPV16 and/or HPV18 positives for viral DNA load, enrolled into the ASCUS-LSIL Triage Study. The authors concluded that higher HPV16 and HPV18 DNA load was associated with status of current, but not former, smoker. Among current smokers, the viral load did not appear to vary appreciably by the intensity and duration of cigarette smoking, in accordance, (11) with previous study of Gunnell et al. in 2006, that in testing for HPV16 DNA presence in first archival cervical smears from 375 cases of in situ cervical squamous carcinoma (CIS) and in 363 controls, it was found that current smokers with a high HPV16 viral load at time of first smear were at a particularly increased risk (27-fold) compared with current smokers without HPV-infection(12).

Many developing countries do not have ample resources to implement cytology-based prevention programs, which necessitates well-organized laboratories to collects material and specialized personnel apt to render a diagnosis(13).

in India, Goel in 2003 -2005 (16.27%) had a positive Pap, VIA was positive in patients (13.81%), In India Cancer cervix has been considered preventable but not prevented because it has a long preinvasive state and availability of screening programs and treatment of preinvasive lesions is effective. It has been well established that well-organized screening by cytology has substantially reduced the incidence of morbidity and mortality from cervical cancer in developed countries(14).

In Iran, Ghaemmaghami 2004 VIA test sensitivity and specificity were 95.7% and 44.0% respectively, while they were 10% and 92% for cytology tests. The results of this study indicate that although VIA is a sensitive screening test for detection of cervical dysplasia, it cannot be used by itself. Applying VIA along with Pap smears helps to detect a higher number of cases with cancer precursor lesions, which is very comparable to our research result sensitivity by VIA 92.6%, and specificity 46.5(15)

In Lahore the sensitivity of VIA was 93% and of Pap smear was 83%. Corresponding specificities were 90% and 97%. VIA was more sensitive than Pap smear which was statistically significant (P value < 0.05). The PPV of VIA was 62.5% versus 83% for Pap smear in our result PPV of VIA was 44.8% which is statistically significant (P value < 0.001) The NPV of VIA was 98% versus 97% for cytology in our result NPV 93% Accuracy rate 61.2%, our P =0.0001(significant) There was no significant difference between the negative 81 predictive values (NPV) of both tests (P value equals 1). Overall, VIA demonstrated an accuracy of 90% as compared to 96% for cytology, these results indicate that VIA is more sensitive as compared to Pap smear. The detection rate of early lesions of cervix using VIA is comparable to Pap smear in validity and usefulness. (16)

In Bangladesh However, the specificity of VIA was slightly lower (97.87%) than that of cytology (98.58%). The PPV of VIA was 73.91% versus 71.42 % for Pap smear82. Also, by Rana et al (2010), the sensitivity for VIA was 93% which is significantly higher than that for Pap. Smear (83%). There was no statistically significant
difference between the sensitivity of low and high threshold VIA, high threshold VIA were the same 80% and for pap smear was 77.5%\(^{(17)}\).

**Conclusion**

There was no significant demographic change in pap smear in Iraq. In general VIA can be used in screening of cervical cancer for premalignant and malignant condition. Because of high negative predictive value this will be reassure the patients probably they don’t have CIN or cancer. thors declare that there are no potential conflicts of interest related to the study.

**Source of Funding:** Nil

**Ethical Clearance:** This research has exemption as it a routine treatment (no new materials were used).

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Ecological Relationship between Poverty and Nutritional Status of Toddler in Indonesia in 2018

Wasis Budiarto1, Ratna Dwi Wulandari1, Nikmatur Rohmah2, Agung Dwi Laksono3

1Lecturer, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia. Universitas Airlangga Campus C Mulyorejo, Surabaya, Indonesia 60115, 2Lecturer, Faculty of Health Science, Muhammadiyah University of Jember, East Java, Indonesia. Gumuk Kerang, Karangrejo, Sumbersari, Jember, East Java, Indonesia 68124, 3Researcher, National Institute of Health Research and Development, the Indonesia Ministry of Health, Jakarta, Indonesia. Percetakan Negara 29, Jakarta, Indonesia 10560

Abstract

Data in the world states that 462 million are underweight, 47 million children under the age of 5 are wasting, 14.3 million are very thin, and 144 million are stunting. Previous study reports about 45% of child deaths <5 years are related to malnutrition—the study aim to analyzing the relationship between poverty and nutritional status of the toddler in Indonesia ecologically. The study used secondary data from the official report of the Indonesia Ministry of Health. The study takes all provinces as samples. Moreover, the study used the percentage of poor people as an independent variable. On the other hand, the researchers analyzed three other variables as dependent variables: the proportion of underweight toddlers, the proportion of stunting toddlers, and the proportion of wasting toddlers. The study examined the data using a scatter plot to determine the relationship. The results show that the greater the percentage of an underweight toddler in a country, the higher the proportion of underweight toddler in that region. Meanwhile, the more significant the proportion of the deprived population in an area, the more considerable proportion of stunting in that area. Moreover, the higher the poor people in the province, the higher the proportion of wasting in that province. The study concluded that poverty has a relationship with nutrition status of the toddler in Indonesia.

Keywords: poverty, underweight, stunting, wasting, ecological analysis, public health.

Introduction

Malnutrition can include wasting, stunting, and underweight. Data in the world states that 462 million are underweight, 47 million children under the age of 5 are wasting, 14.3 million are very thin, and 144 million are stunting. About 45% of child deaths <5 years are related to malnutrition1. Therefore, reducing child stunting is the first goal in the global nutrition target by 20252. Despite social and economic developments, the worldwide burden of malnutrition remains too high. There is an important relationship between nutritional status, human resources, and financial status. Poor nutrition causes a decrease in individuals’ physiological, mental capacities, hinders productivity levels, and is vulnerable to poverty. There is a two-way relationship between malnutrition and poverty. Malnutrition results in conditions of poverty by reducing the economic potential of the population3. Discussion on nutritional status is crucial because it is an indicator of the growth of children under five.

We can measure toddler growth through body weight, height, and body mass index. Impaired growth of children under five is a sign of an obstacle to nutritional status. Malnutrition can cause various losses, including...
developmental delays in children aged 3-6 years, quality of life of children, physical function, emotional function, social function, communication, fine motor skills, and problem-solving. The overall prevalence of developmental delay was 35.4\%.\(^4\)-\(^7\)

The prevalence of child stunting in Indonesia has remained high over the last decade. At the national level, child stunting is around 37\%.\(^2\). Another study states that the prevalence of stunting, wasting, and very thin is 9.1; 3.8; and 3.8\%.\(^6\). The prevalence of stunting was 39.4\%, while the percentage of households consuming foods high in protein and calcium was 41\%.\(^8\). This phenomenon shows that nutritional status is still a significant problem in achieving the global nutrition target in 2025. Several factors supporting stunting and wasting rates in Indonesia require a deeper study.

Previous studies have suggested that the percentage of poor people is positively related to the prevalence of stunting\(^9\). Households that occupy a livable house and have completed basic education negatively correlate with the prevalence of stunting in Indonesia. The situation means that this variable is a protective factor for a province to have short children under five. Meanwhile, non-exclusive breastfeeding for the first 6 months, low socioeconomic status of the family, premature birth, short birth length, short mothers, low maternal education are the determining factors for child stunting in Indonesia\(^2\). Education and income reduce the likelihood of being underweight by 10-30\%\(^{10}\). It is estimated that 90 percent of children, especially girls and young women, experience some form of poverty\(^{11}\). Physical, economic, demographic, social, and environmental factors are the main contributors to food insecurity\(^{12}\).

In contrast to other studies, it is stated that there is no correlation between short-term changes between measures of income and overall height\(^{13}\). Similarly, a study was found that found positive deviations of feeding with the nutritional status of children under five in low-income families \(^{14}\). Overall, the factors that represent the ecology of internal and external nutrition need to be considered to reduce stunting rates\(^{12}\)—the study aim to analyzing the relationship between poverty and the nutritional status of the toddler in Indonesia ecologically.

Materials and Methods

Study Design

The author used an ecological interpretation method in the research. Environmental research relies on collective comparisons rather than human comparisons. The data examined in the ecological analysis is aggregate data at a given community or level; in this case, it is at the provincial level. Aggregate measures, environmental measurements, and global measures should also be used as factors in an ecological survey. In epidemiology, ecological research aims to make biological inferences about individual risk effects or ecological inferences about group effects\(^{15,16}\).

Data Source

Secondary data from the 2018 Indonesia Basic Health Survey and the 2018 Indonesia Health Profile report were included in this analysis. Both papers are official documents of the Republic of Indonesia’s Ministry of Health. The province is the study’s unit of research. The thesis looked at every part of Indonesia (34 provinces).

Data Analysis

The dependent variable in this study is the nutrition status of the toddler. The nutritional status of a toddler consists of underweight, stunting, and wasting. Underweight is a classification of toddler nutritional status based on body weight index for age (Z-score < -2.0). Meanwhile, stunting is a classification of the nutritional status of children under five based on indicators of length/height per age (Z-score < -2.0). Moreover, wasting is a classification of the nutritional status of toddlers based on indicators of body weight per length/height (Z-score < -2.0)\(^{17}\).

In this analysis, poverty was used as an independent variable. The percentage of a province’s impoverished
population was used to define poverty (September 2018). The data was evaluated bivariate using a scatter plot in the analysis. The linear fit line was used in the analysis to assess the association between toddler poverty and nutrition status. The author carried out the research with the assistance of the IBM SPSS 21.

Findings

Table 1 is a descriptive statistic of the poverty

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Range</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of the poor population</td>
<td>34</td>
<td>23.88</td>
<td>3.55</td>
<td>27.43</td>
<td>10.6076</td>
<td>5.70346</td>
</tr>
<tr>
<td>Proportion of underweight</td>
<td>34</td>
<td>16.50</td>
<td>13.00</td>
<td>29.50</td>
<td>19.2088</td>
<td>4.53581</td>
</tr>
<tr>
<td>Proportion of stunting</td>
<td>34</td>
<td>25.10</td>
<td>17.60</td>
<td>42.70</td>
<td>30.2618</td>
<td>5.30260</td>
</tr>
<tr>
<td>Proportion of wasting</td>
<td>34</td>
<td>40.30</td>
<td>31.90</td>
<td>72.20</td>
<td>49.4706</td>
<td>9.20351</td>
</tr>
</tbody>
</table>

Source: The 2018 Indonesia Basic Health Survey and the 2018 Indonesia Health Profile

Figure 1 depicts a scatter plot of the number of impoverished citizens and the proportion of underweight toddlers by Indonesian province. The graph displays the two variables’ penchant for a positive relationship. The more significant the poor people proportion in a region, the higher the underweight toddler percentage.
Figure 2. Scatter plot of the percentage of the poor people and the proportion of stunting by the province in Indonesia, 2018

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

Figure 2 depicts a scatter plot of the number of disadvantaged people and the proportion of stunting in Indonesia by region. Figure 2 illustrates the two variables’ propensity for a favorable interaction; as a result of the condition, the more significant the deprived population proportion of the region, the greater the stunting toddlers proportion in that region.

Figure 3. Scatter plot of the percentage of the poor people and the proportion of wasting by the province in Indonesia, 2018

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile
Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

Figure 3 shows the scatter plot of the percentage of poor people and the wasting of toddlers in Indonesia’s province. The scatter plot shows the tendency for a positive relationship between the two variables. The situation means that the higher the poor population in the province, the higher the wasting toddlers proportion in that province.

Discussion

Several previous studies have informed that socioeconomic status is related to the nutritional status of children under five, both underweight, stunting, and wasting. Previous studies have found that poverty is closely related to the nutritional status of children under five\(^1\). Poverty is known to be closely associated with the ability of families to provide food needs in the family. Families with poor conditions for a long time will significantly affect the nutritional status of children under five to become worse\(^1,2\). In general, better socioeconomics is a strong determinant of better health output\(^2\).–2\)

Meanwhile, poverty in the family is also known to be related to the low level of education of family members, especially their parents’ education\(^2\). The intense maternal education situation makes them less aware of the nutritional status of children, both because of limited knowledge and because of financial limitations\(^2\)–26.

On the other hand, poverty is also closely related to poor sanitation, especially in urban slum settlements. This situation worsens the low nutritional status of children under five due to the risk of infection and disease caused by poor sanitation. The low-income family lives, including diarrhea and worms\(^27,28\).

Intervention by the government in this poor group of people is needed. This intervention ensures that there is no deterioration in the nutritional status of children under five due to food availability in inadequate families. In Indonesia, the government has issued several policies to strengthen food security for low-income families. Among them is distributing rice to the poor and providing direct cash assistance, including subsidizing contribution assistance for National Health Insurance\(^29\).

Study Limitation

Since the data used is statistical data at the regional level, this study, undertaken using the ecological analysis method, has drawbacks in its use as a policy basis. More research at the personal level is needed to collect more reliable knowledge before deciding on an intervention strategy\(^30\).

Conclusion

The study concluded that poverty has a relationship with the nutritional status of the toddler in Indonesia. The higher the poor population in the province, the higher the proportion of underweight, stunting, and wasting in that province.

Conflict of Interests: Nil

Source of Funding: Self-funding

Ethical Clearance: The study was conducted by utilizing secondary data from published reports. For this reason, the study not required an ethical clearance in the implementation of this research.

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References

5. Yuliasti Eka Purnamaningrum I. The Effect of


Culture, Ethnic, Lifestyle, and Diabetes

Windy Tri Yuana1,2, Sri Widati3, Oedojo Sudirham3
1Researchers at Tanah Bumbu Unit for Health Research and Development, National Institute of Health Research and Development, Tanah Bumbu, South Kalimantan 72171, Indonesia, 2Master’s Student, 3Senior Lecturer, Department of Health Promotion and Behavioral Science, Faculty of Public Health, Universitas Airlangga, Surabaya 60115, Indonesia

Abstract
Diabetes is a social health problem that is large with special attention in the community and among individuals. Nevertheless, the efforts to spread paradigm of health society to inputsocial determinant of health including analysis and contextual of social factors that are considered out of the scope of the health research is seldom conducted. As a result, the conceptualization of dynamic health inequality of diabetes is superficial. By using a holistic anthropologist lens has the potency to offer insight intothe character of social determinant which is wider. The individual factor that is traditionally focused on the health of society and qualitative exploration structural, deeply local context, social environment, and culture, and their interaction and intersexuality, as the key factors, while considering how to reach alterations. The systematic study aims to describe that culture, ethnicity, and lifestyle have much impact on the patients of diabetes mellitus. The method of the systematic study is the article’s investigations which are listed by PubMed with the keywords “culture, ethnic, lifestyle, and diabetes”. Twenty-nine articles are got from the result of an investigation and after a selection has been done and got five articles to be analyzed furthermore. The result of the review of the literature study shows that culture, ethnicity, and lifestyle are able to influence the case of diabetes illness to the individual or group. On a certain ethnic group besides economic problem, the characteristic of certain body starts from size and shape of the body make someone can suffer from diabetes, in addition, the motoric activity of the body and physical activity can influence the case of diabetes.

Keywords: Culture, Ethnic, Life Style, and Diabetes.

Introduction
Health paradigm as a national movement in the context of health development toward Indonesia Sehat 2015 is an effort to increase national health which is pro-active. The goal of the effort is to push society to be independent to keep in their health and realize how important health service that is promotive and preventive without ignoring curative and rehabilitative efforts is.(1) The lifestyle change which affects the change of the eat behavior pattern can cause degenerative illness. Diabetes Mellitus is one of the degenerative illness. It is a group of metabolic illness which is marked by increasing of blood sugar levels. The increase can occur because of the abnormality of the secretion of insulin, insulin work, or both of them.(2,3)

Corresponding Author:
Windy Tri Yuana,
Tanah Bumbu Unit for Health Research and Development, National Institute of Health Research and Development, Jl. LokaLitbang,KomplekPerkantoranPemerintah Daerah Kabupaten Tanah Bumbu, Tanah Bumbu, South Kalimantan 72171, Indonesia
windytriyuana@yahoo.co.id

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among them undergo routine treatments. Environment factors and unhealthy lifestyles, such as eating too much, overweight, lack of activities, and stress have an enormous role as the cause of Diabetes Mellitus. Besides that, Diabetes Mellitus can occur because of genetic factors (Mahendra, et al, 2008).

Around 60% of the number of diabetes patients are in Asia. (4) Indonesia is in the fourth position and has the most cases of Diabetes Mellitus in the world. (5) In 2000 in Indonesia there are 8.4 million Diabetes Mellitus Patients and it is estimated to get 21.3 million in 2030. (6) In diabetes map InternationalDiabetes Federation list Indonesian over 20 years old is 125 million and by the assumption of Diabetes Mellitus prevalence 4.6%. Based on the pattern of current population growth, it is estimated that in 2020 there will be 178 million of the population over twenty-year-old within assumption prevalence of Diabetes Mellitus 4.6% will be 8.2 million Diabetes Mellitus patients. (7)

Diabetes Mellitus become the highest cause of death in a certain age group. It makes Indonesia is on the fourth world position as a country that has the most diabetes Mellitus cases. In Jawa Timur, is got that the highest prevalence is in Surabaya where is 6.2%. (6) The result is predicted that it will increase in 2030 and become 21.3 million people. Diabetes Mellitus is on top five of the biggest illness that spreads in PuskesmasPutat Jaya with 3.555 cases (6.37%).

In ancient times, Diabetes Mellitus is considered an “old illness” because more cases are found over forty-year-old, but now there has been a shift in disease. The illness transitions show that Diabetes Mellitus does not only attack elderly age but also attacks children and adolescents. The research conducted by the Child Endocrinology Coordination Work Unit throughout Indonesia in 2012 showed that the number of patients with diabetes in children and adolescents under 20 years-old was 731 people. Diabetes Mellitus in children and adolescents can be caused by some factors, one of them is the genetic factor in which children and adolescents with Diabetes Mellitus parents are at risk of developing Diabetes Mellitus.

The effort of preventions can be done to adolescents, especially those who are at risk in order to for them to not suffer from Diabetes Mellitus in the future, so the prevalence of Diabetes Mellitus cases can be brought down. Nutritional needs during adolescence are relatively bigger than other periods because adolescence is a period of growth and development. Nutritional needs in adolescence need attention because changes in lifestyle and eating habits at this time have an impact on the need for and intake of nutrients. (9)

Diabetes Mellitus (DM) is a disease, in which the condition of glucose levels in the blood exceeds normal limits. This is because the body is unable to release or use insulin adequately. Insulin is a hormone released by the pancreas and is the main substance responsible for maintaining blood sugar levels in the body to keep it in balance. Insulin functions as a tool that helps sugar move into cells so that it can produce energy or be stored as an energy reserve. (10)

Diabetes mellitus is characterized by chronic hyperglycemia. The patients with Diabetes Mellitus will be found by various symptoms, such as polyuria (lots of urination), polydipsia (lots of drinking), and polyphagia (lots of eating) with weight loss. Hyperglycaemia cannot be detected because Diabetes Mellitus does not show symptoms (asymptomatic) and often referred to as the “Silent Killer” of human beings and cause vascular damage before the disease is detected. Diabetes Mellitus can cause metabolic disorders that cause macrovascular and microvascular pathological disorders in the long-term. (11)

So far, diabetes has focused on diet only, but there are important things that have not been noticed, namely culture, ethnicity, and lifestyle, apart from genetic factors. Culture, ethnicity, and lifestyle can affect food consumption patterns which can result in overweight conditions and can trigger of arising of Diabetes. The original culture of ethnicity as well as the cultural changes experienced due to modernization can play an important role in the prevalence of diabetes in a certain
ethnic group. Therefore, the aim of the literature study is to provide an overview of how culture, ethnicity, and lifestyle can have no small effect on the occurrence of Diabetes.

**Method**

In this literature review, was conducted an in-depth study of articles published in various journals indexed by Pubmed. The keywords used in the article search were “culture, ethnicity, lifestyle, and diabetes”. In choosing articles, flow charts are used. The articles selected for analysis (inclusion criteria) were (1) articles containing descriptions of how a culture, ethnicity, and lifestyle can affect diabetics; (2) articles containing the location of diabetics; and (3) articles containing data on diabetics. Meanwhile, the exclusion criteria were articles written other than in English or Indonesian. Data taken from each research article includes information about diabetes, the location of diabetics, and data on diabetics.

![Article selection diagram](image)

**The Result**

A total of 29 articles in the form of titles and abstracts were found in the search, as shown in Picture 1. The articles were checked, then were chosen 17 articles to be selected furthermore based on the inclusion and exclusion criteria. After eliminating the unsuitable articles, there were 5 articles published from 2013 to 2015 for further analysis (Table 1).
In this systematic study, the following data were collected on the relationship between energy value and nutritional intake with body shape and size parameters (weight, height, waist, and hip line) which were carried out in 2014 and 2016 on 148 female volunteers aged 57-88 from Mazovian and Lublin Province (Poland). The sample selection targeted elderly age with different body types. The exclusion criteria were: multi-organ failure, cancer, and disability. Then among the subjects, a survey was conducted, which included demographic data, lifestyle, health status, and vitamin and mineral supplement intake. WHR and BMI index, total protein intake, animal protein intake, vitamin E intake (p ≤ 0.05), and fat, phosphorus and thiamine intake (p ≤ 0.1). The results showed that the place of residence, physical activity, chronic disease, diet use, fluctuations in body weight, BMI, and WHR differed depending on the somatotype in female groups. Endomorphic subjects had a significantly bigger waist and hip line and diastolic blood pressure compared to other somatotypes. The somatotypes only have a significant effect on total protein, animal protein, and vitamin E intake, and ectomorphic elderly women may be particularly susceptible to nutritional deficiencies. Due to the risk of macronutrients, vitamin and mineral deficiencies in the diets of the women examined, the mean age of the subjects was 68 years. Most of the subject’s education is high school. The majority of women surveyed live in cities of more than 100,000 residents. Significant differences were found between somatotypes and residence (p < 0.001). Ectomorphic females are most prevalent in cities with under 100,000 population. Mesomorphic and endomorphic subjects live mainly in cities with a population of over 100,000. Among the villages, the most dominant are endomorphic. Statistically significant relations between somatotype and physical activity, chronic disease, and application of a special diet were observed. All women with ectomorphic physiques engage in regular physical activity such as recreational activities. The majority of mesomorphic women are active, unlike the endomorphic ones who rarely do physical activity. The chronic disease most often occurs in an endomorphic physique. The application of a special diet is most popular among endomorphic women, less popular with mesomorphic subjects, and least popular among ectomorphic subjects.
Endomorphic women were more likely to experience fluctuations in body weight than those in other groups. All are ectomorphic and mostly mesomorphic are thin. Conversely, the highest number of women who were overweight or obese was found in the endomorphic group. They also had significantly more frequent WHRs above 0.8 (the tendency for ectomorphic abdominal obsession ($p < 0.001$)). Diastolic blood pressure differed significantly in somatotypes and was significantly higher in endomorphic women than in ectomorphic ones ($p = 0.046$). Average daily energy intake, total protein, animal and vegetable protein, fat, saturated fatty acids, monounsaturated and unsaturated fatty acids, cholesterol, sucrose, and fiber were estimated based on a 3-day diet. Statistically significant higher total protein intake was found in endomorphic subjects, than lower in mesomorphic ones, and low in ectomorphic women ($p \leq 0.05$). There was a statistically significant effect on the somatotype of animal protein intake. Ectomorphic and mesomorphic consume less than endomorphic.

In the study group, the tendency to increase total fat intake among endomorphs was also higher compared to other types ($p \leq 0.1$). There are no other statistically significant differences in macronutrient intake between the somatotypes. The highest propensity for higher intakes of amines ($p \leq 0.1$) and phosphorus ($p \leq 0.1$) was observed in women with endomorphs compared to the other groups. Intakes of other vitamins and minerals are similar in all subjects regardless of somatotype.\textsuperscript{(12)}

Ethnic society was stripped from their land and forcibly resettled and reserved, which currently have the highest unemployment rates in the United States. For years, diabetes rates have been very low among Native Americans. In the 1950s, the Cornell University Medical Team provided and performed physical examinations for most members of the Many Farms Navajo community and found several cases of type 2 diabetes. In 1998, Hall, Hickey, and Young \textsuperscript{11} revisited many farms and collected the comparison data. They found that members are now 10 times more likely to have diabetes than they were 30 years ago. The main changes in society included dependence on subsidized food, consisting mainly of refined flour, cheese, lard, and refined sugar. These factors coupled with the dramatic reduction in physical activity resulted from changes to traditional work patterns and greater access to fast food. Healing rituals restore body, mind, and spirit to a state of balance for a balanced and harmonious life between food, activity, prayer, sleep, and social relationships. Widely in the Southwest and Mexico, they are known to be hypoglycemic to nopal (prickly pear cactus), garlic, onions, and hintonia (copalquin). Some of these ingredients also contain toxins. Hintonia, for example, contains alkaloid pyrrolizidine which can cause severe liver damage. \textsuperscript{29,30} Other ethnic groups have followed suit. Rural South Africa Americans may believe that “blood sugar” is caused by an imbalance in eating (lots of sugar and starchy foods) and is exacerbated by stress. Common treatments include prayer, trusting in God, and the use of bitter foods and herbs (lemon juice, garlic, juniper berries) to neutralize the blood. Older African Americans may reverse the typical pattern of older ethnic members using more traditional therapies, including prayer, belief in God, and the use of bitter foods and herbs (lemon juice, garlic, juniper berries) to neutralize blood. Older African Americans with diabetes were found to use popular remedies less often. Chinese Americans may have incorporated diabetes into the traditional Chinese medical system. Diabetes may be considered a “hot” or yang illness that can be neutralized by “cold” or yin remedies. Ginseng is one of the cooling herbs believed to restore energy and cure diabetes. Mexican-American and Puerto Rican patients may believe that diabetes results from their consuming too much sugar or as God’s will or punishment. This population reports the following symptoms indicating increased blood glucose: weakness, headache, nervousness, leg pain, joy, and anger. In one study of elderly Puerto Ricans with diabetes, more than half used herbs and prayer to treat diabetes, reporting that the latter provided a peacefulness that improved their diabetes.\textsuperscript{(13)}

Culture and family challenges to diabetes management within foreign-born Chinese American families are identified as “participant” statements.
The family’s symptoms challenged family harmony. Increased irritability as a diabetes symptom was frequently described as a challenge to family harmony. Participants noted that patients “get angry easily” particularly when their blood glucose was high. Emotional variability held particular resonance for Chinese immigrants because social ease, avoidance of overt expression of strong negative emotions, and accommodation of family members’ expressed and unexpressed needs were culturally valued. Emotional fluctuations were most often attributed to the disease.

The meaning of rice in the Chinese family diet was a culturally multifaceted and historically nuanced story about sustaining holistic health and well-being and partaking of symbolically vital food. Patients and families were challenged by being asked to restrict rice and change from familiar white “fragrant” rice to foreign “chewy” and “tasteless” brown, red, or black rice. These challenges were persistently noted by participants who felt called upon to cope with this change in communal meals. The importance of rice was taken for granted in group discussions. Participants agreed that the amount of rice provided in institutional meals - such as airplanes or hospitals is very small, “If you don’t eat rice can you sustain your daily living?” Additionally, participants expressed significant suffering because of restrictions on rice, a symbolically comforting food. Many found that disease-related food restrictions disregarded cultural concerns for balancing foods (e.g., “hot” and “cold”) understood to have specific medicinal properties according to traditional Chinese medicine (TCM). Even for those who did not specifically incorporate TCM in their diet management, the metaphor of balance was powerfully invoked. Participants additionally feared that diabetic food restrictions, if strictly followed, might lead to emotional imbalance and depression. Pleasurable food was generally appreciated as crucial to mental health and balance: “If I say every time, ‘This and that you can’t eat’ some people would develop negative feelings and say, ‘There’s no meaning to life now.’” Patients’ difficulties in following an appropriate diet at Chinese restaurants led some to withdraw from socializing over meals. Spouse: “Now when I asked her to go to dim sum, she would say, ‘I am not going. You can go. I don’t want to go alone, right? (Why?) It’s meaningless to go by myself.”

For many Chinese participants, social interaction was an integral part of a meal. Attending dim sum or Chinese breakfast alone was meaningless because meals were sustaining only if shared. Difficulties in managing the social elements of meals were intensified in ritual meals. Birthdays, weddings, or Chinese New Year’s banquets, with multiple courses and desserts, were unavoidable, yet socially fraught: “Gee, I couldn’t even eat a bowl of sweet dessert soup! How miserable.” The social context of ritual meals provided layered concerns for patients and families. The presence of family reminded patients of their responsibility to observe diabetes restrictions, as a duty to family. “Since they tell you, you don’t dare do it. You are well aware that the bowl of sweet dessert soup may do you a lot of harm.” Reciprocally, family members felt obliged to care for patients’ disease and yet at the same time to create social ease and pleasure. It’s better to die. You restrict me like this and don’t let me eat.” Disagreements arose about whether all family members should observe the patient’s dietary restrictions. A few participants argued in favor of family restraint because the diabetic diet supported general health and observing restrictions demonstrated camaraderie with the patient. Participants also argued against such family restraint as unnecessarily restrictive. He agreed to his wife’s strict restrictions on family dinners but would not relinquish his breakfast pork buns. Families were additionally challenged when patients and spouses held differing expectations about what family members should learn about the disease. Some patients felt neglected because spouses were “not very aware” of the risks and demands of the disease. (14)

These results suggesting that beliefs about diabetes held by low-education African Americans and American Indians differ from all other groups is also consistent with Kawachi and colleagues’ contention that the combination of educational attainment and ethnic minority status has the potential to create distinct contexts for health disparities. Our results, when combined with the broader literature, suggest that
educational attainment and ethnicity both require careful consideration when developing and implementing culturally-appropriate diabetes education (and possibly broader health education) programs. Our multi-ethnic sample did not include individuals from other ethnic groups, like Latinos, that experience elevated rates of diabetes. The sample consisted of adults aged 60 years and older. Previous research suggests that age is associated with different EMs of diabetes among African Americans, presumably capturing either period or cohort effects. Consequently, the generalizability of the findings to young adults is unknown. Theoretically informed to ensure comprehensive coverage of diabetes-related beliefs. The comparative sample design with similar adults from three distinct ethnic groups provides a solid foundation for examining cultural differences in diabetes beliefs. The structure and content of diabetes beliefs held by Whites, African Americans, and American Indian older adults in rural North Carolina are very similar. Similarity across ethnic groups is particularly evident in the Symptoms and Consequence domains of diabetes beliefs. Where ethnic differences do exist, they appear to be driven primarily by socioeconomic factors rather than “ethnicity”.

71 percent of American Samoa females were obese and another 19 percent were overweight, leaving fewer than 10 percent of American Samoa females within the range of normal BMI. The reductions of physical activities that come with a transition away from an agricultural economy, other factors are directly connected to these increases in obesity and its related disease such as diabetes. Food choices have changed from a traditional plant and fish-based diet to one with a heavy reliance on highly processed imported foods, resulting in higher consumption of calories, protein, simple carbohydrates, cholesterol, sodium, and saturated fat. Fast-food consumption has also increased and as of this writing, the main American Samoan island of Tutuila has several fast-food outlets, including two MC Donald’s. in 2011 the local franchises began advertising Samoan burger: a big mac sandwich to which a fried egg is added.

Discussion

Somatotype can be defined as the present morphological state of the individual. The ectomorphic body type is characterized by a slim physique, weak bones and muscles, sloping arms, relatively short torso, and long limbs. The chest is narrow and flat, arms rounded, thighs and shoulders weak, fingers long and delicate and skin is dry. This body type is characterized by rapid energy expenditure, low-fat cell count, and as well as slow muscle growth. The ectomorphic physique requires less intensive training, longer interruptions, higher protein intake, and an adequate resting period. Ectomorphs are considered introverted, ill-tempered, irritable, with a tendency to schizophrenia. A mesomorphic physique is a muscular body type with a strong skeleton, broad shoulders and chest, firm limbs, massive pelvis, and very fast muscle growth. A mesomorphic person is regarded to be energetic, active, dynamic, and aggressive. In contrast, the endomorphic type is characterized by a rounded physique, a large number of fat cells, a larger waist circumference than the chest, a large head, a broad face, and a short neck. Besides endomorphs have rounded shoulders, relatively short and weak limbs and fingers, small feet and hands, and strong bones. Results are presented in a three-digit form. An individual who has only endomorphic features has the assigned symbol 7-1-1, while a person with only mesomorphic characteristics 1-7-1 and ectomorphic one 1-1-7. This means that there are 343 possible combinations.

Because females have a higher total fat mass, there are more females in an endomorphic state. The change happens as at both sexes except mesomorphic somatotype group increased until 50-year-old at the females and thereafter decreased, and Male endomorphy remained almost unchanged at the somatotype components. dried after age 30. The largest difference of all somatotype components was between the age groups 18–40 years. The mesomorphy continued to increase until the 6th decade and then decreased. The number of ectomorphy decreased until age 50 years and then there is no more
change to be observed.\(^{(18)}\)

Somatotype, diet, and female’s nutrient status 395 of endomorphic components, stability in one mesomorphy, and a little increase in ectomorphic components. It is believed that somatotype may be important in age studies like diabetes type 2 and Alzheimer’s disease where the differentiation of the big body is observed. The somatotype technic can be equipped to observe and study the change of physic in this case illness.\(^{(19)}\)

For reasons which are mentioned above and the relation between body composition and energy and the order of nutrition in this research between somatotype and diet and nutrient status of females, age 57-88 has been checked. Subjects and method of the design of the study and the research which was conducted in 2014 and 2008 2016 about 148 females 57-88-year-old from Mazovian and Lubin Province (Polandia). The result of the study in this paper is the first in Poland to show the effect of somatotype to indicators which are chosen from the health status, diet behavior, and national status nutrition of elderly females. The relations between somatotype and blood pressure has been shown in this research. Found that female endomorphy have diastolic blood pressure which is higher than mesomorphic and ectomorphic. The same correlation has been shown where the endomorphic subjects are characterized by higher blood pressure than another type.\(^{(18)}\)

The government of California State, for example, has the competency of established culture orientation to have contractor medical plans. 9 culture competencies include awareness and sensitivity of different cultures: knowledge of cultural values, beliefs, behaviors; and skill to work with a culturally diverse population. Cultural competency needs both at the practitioner level and agency. Contents and instruments have been available to evaluate practitioners and agency of cultural competency and to create service of cultural competence.\(^{(20)}\)

A cultural assessment is a focused and systematic appraisal of beliefs, values, and practices conducted to determine the context and substance of client needs and then to best adapt (or construct) and evaluate health interventions. Unlike physical assessments, cultural assessments are necessary for each of the three phases of professional practice: problem identification, intervention, and evaluation. Cultural assessments are not exhaustive of all aspects of culture, but rather are focused on those elements relevant to the presenting problem, necessary intervention, and participatory evaluation.

Needs to consider cultural factors in the treatment of diabetes patients has been identified for several decades. However, it is not close to problem-solving effectively. The main reason is that patient’s culture is often seen as a problem, the cause-obstacle to care. Our problematize are patients and culture. Next, by thinking that culture is what another has, we mind that culture can keep away from ethnic patients.\(^{(21)}\)

However franchise of fast food is a new phenomenon, Samoa non-high fat food has been part of the island diet since the early 20s century, while canned meat imported by the US army become a precious class that used in food exchange - an important golden rule for many cultural events.\(^{(22)}\) The level of practice decreases because of the movement of agricultural and sub-system when giving birth lose the source of core physical activity regularly.\(^{(23)}\) Although epidemiology research is in progress and perhaps gives the evidence of the impact of new genetic, modernization, American culture, and the change of another lifestyle in the last thirty-forty years all of them contribute to the levels of epidemic of obesity and diabetes.\(^{(23)}\)

Samoan Concepts of Health, Body Image and Individuals Previous body image studies among Samoan adults have demonstrated the acceptability of large body size. Traditionally as well as in the past, larger body size was seen as beautiful and correlated with social prestige.\(^{(24)}\)

**Conclusion**

The general data of respondents on the five articles above show that most female respondents have a higher risk to have diabetes than males. It is caused by stress levels of psycho-social and people with colored skin had higher diabetes prevalence than white people because of
socioeconomic problems. Until now, we have assumed that when we exercise self-control and strict dietary regulation of what enters the body according to our knowledge, we will avoid diabetes and based on the writer’s opinion that while someone’s knowledge of disease is pretty good then they will avoid the disease, but in the five articles above, there are not so many factors that influence, namely culture, ethnicity, and lifestyle. Therefore, in-depth research is needed in Indonesia to find out whether culture, ethnicity, and lifestyle really influence the prevalence of diabetes considering there are so many tribes in Indonesia.

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**Ethical Clearance:** This study was approved by Health Research Ethics Committee, number 537/HRECC.FODM/XII/2020, Faculty of Dental Medicine, University of Airlangga, Surabaya.

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Assessment of Cardiovascular Risk in a Patient with Rheumatoid Arthritis

Xamrayev Xamza1, Goyibov Salim2, Tagaeva Dilnoza2

1Assistant Professor 2Researcher, Department of Internal Medicine Samarkand State Medical Institute, Samarkand, Amir Temur Street 18, Uzbekistan

Abstract

Purpose: to study cardiovascular risk on the mSCORE scale in patients with rheumatoid arthritis (RA).

Methods: We examined 140 RA patients aged 35 to 60 years. The diagnosis of rheumatoid arthritis was made on the basis of the ACR (1987) and ACR / EULAR (2010) criteria. The mSCORE scale (SCORE / EULAR) was used for early detection and prediction of cardiovascular risk.

Results: In patients with RA, hereditary risk factors (RF) for cardiovascular diseases were detected in 31.4%, abdominal obesity in 35%, arterial hypertension (AH) in 63.6%, hypercholesterolemia in 25.7%, and hypodynamia in 37.9% of patients. Smoking accounted for 10.8% of patients in the study, as the majority (82%) of the surveyed were women. At the same time, AH was observed 1.5 times, hypercholesterolemia 2.1 times more often in men than in women. According to the results of the study on the mSCORE scale, 52 (37.1%) had low risk, 64 (45.7%), a high risk was in 14 (10%), and a very high risk was in 10 (7.2%) patients.

Conclusion: In RA patients, the use of the mSCORE scale enables early diagnosis of cardiovascular risk and timely correction of risk factors.

Keywords: rheumatoid arthritis, cardiovascular risk, mSCORE scale

Introduction

The risk of cardiovascular disease (CVD) in rheumatoid arthritis (RA) is an important problem in modern medicine. RA occupies one of the leading places among rheumatic diseases and belongs to diseases of high medical and social significance, due to the significant prevalence and progressive nature of the course, leading to early disability in patients of working age. According to multicenter studies, cardiovascular morbidity and mortality in patients with rheumatoid arthritis is higher than in the general population[Myasoedova et al. 2010; Zulfiqar et al. 2018]. The immediate cause of death in RA patients is diseases associated with atherosclerotic vascular lesions, namely: coronary heart disease (CHD), cerebrovascular accident (CVA), congestive heart failure[Liao2017; Mackey et al. 2018]. Arterial hypertension (AH) is the most important risk factor for cardiovascular complications; however, in patients with RA, there is a wide scatter in the prevalence of AH (from 16% to 76%), which is associated with an underestimation of the clinical characteristics of patients and the characteristics of antirheumatic therapy[Crowson et al. 2018; Khanna et al. 2019].

The development of cardiovascular complications in RA patients is associated with the influence of traditional risk factors, systemic inflammation, and side effects of non-steroidal anti-inflammatory drugs taken[Magda et al. 2016; Metsios et al. 2020]. In the pathogenesis of these diseases, NSAIDs, by suppressing the activity of cyclooxygenase (COX), can lead to a decrease in the systemic and renal synthesis of vasodilator prostaglandins, which causes an increase in vascular
tone and fluid retention, accompanied by an increase in blood pressure (BP) and decompensation of chronic heart failure (CHF) [Castañeda et al. 2016; Krüger 2019]. The association of RA and cardiovascular risk is the subject of close attention of rheumatologists and cardiologists. Studies have shown that the prevalence of RA is about 0.5–1% of the population and cardiovascular disease is the leading cause of mortality in these patients. In a recently published meta-analysis, the authors found that the risk of mortality from cardiovascular disease (ischemic heart disease and stroke) is 50% higher in RA patients compared to the general population [England et al. 2018; Saidova et al. 2018]. However, it is clear that early atherosclerosis observed in this group of patients cannot be explained only by traditional cardiovascular risk factors. The solution to this problem involves the assessment of the prevalence of CVD, cardiovascular risk factors and metabolic disorders; identification of groups of patients predisposed to the development of cardiovascular complications (CVC); study of the effect of antirheumatic drugs on the cardiovascular system; development of a set of preventive and therapeutic measures aimed at reducing the risk of CVC; creation of a system of dynamic control and monitoring of the development of cardiovascular pathology in this category of patients [Zegkos et al. 2016; Gómez-Vaquero et al. 2018; Saidova et al. 2018].

Material and Methods

We examined 140 RA patients aged 35 to 60 years. The diagnosis of rheumatoid arthritis was made on the basis of the ACR (1987) and ACR / EULAR (2010) criteria. When determining the frequency of cardiovascular risk factors in patients with rheumatoid arthritis, heredity, smoking, rheumatoid factor, hypercholesterolemia, abdominal obesity, C-reactive protein, as well as the incidence of arterial hypertension (AH), coronary heart disease and diabetes mellitus were assessed. The mSCORE scale (SCORE / EULAR) was used for early detection and prediction of cardiovascular risk. SCORE chart: 10-year risk of fatal cardiovascular disease (CVD) in countries at low CVD risk based on the following risk factors: age, sex, smoking, systolic blood pressure, and total cholesterol. To calculate the mSCORE risk index, the value initially collected from the SCORE risk index was multiplied by a factor of 1.5 for RA patients that met two out of the following three criteria: a disease lasting more than 10 years, RF and/or anti-CCP positivity, and finally, patients with extra-articular manifestations. The study did not include patients with coronary heart disease and diabetes mellitus.

Results

According to the results of the study, 90 (64.3%) of 140 patients with RA were women, whose average age was 51.21 ± 5.63 years, and 50 (35.7%) were men, whose average age was 52.89 ± 5.56. It was determined that 46 patients were 35–49 years old, and 94 patients were 50–60 years old. The results showed that 101 (72.1%) patients were seropositive and 39 (27.9%) were seronegative RA.

The clinical course of the disease in patients was assessed using the pain syndrome scale - VAS and the DAS-28 activity index. On the VAS scale, 25 (17.8%) patients had moderate pain, 73 (52.1%) had severe pain, and 42 (30%) had very severe pain. According to the DAS-28 index, 61 (43.6%) patients had II - moderate activity, and 79 (56.4%) - III - high activity.

In patients with RA, hereditary risk factors (RF) for cardiovascular diseases were detected in 31.4%, abdominal obesity in 35%, AH in 63.6%, hypercholesterolemia in 25.7%, and hypodynamia in 37.9% of patients. Smoking accounted for 18.5% of patients in the study, as the majority (64.3%) of the surveyed were women. At the same time, AH was observed 1.5 times, HCS 2.1 times more often in men than in women.

When analyzing the incidence of risk factors in one patient, 113 (80.7%) patients had a risk factor, of which 1 risk factor was detected in 21.4%, 2 risk factors were detected in 25%, and patients with 3 or more risk factors were 34.2%. No risk factors were observed in 19.3% of patients. When risk factors were analyzed by age,
patients aged 50-60 had higher rates than patients aged 35-49. It was noted that AH is 2.3 times more common in patients aged 50-60 years than in patients aged 35-49 years.

Cardiovascular risk scores in patients with rheumatoid arthritis were determined using the mSCORE scale. On the basis of performance criteria, patients were considered to have “low risk” less than 1%, “moderate risk” from 1 to 5% (1% ≤ 5%) and “high risk” from 5 to 10% (5% ≤ 10%), ≥ 10% are in the “very high risk” group. According to the results, 52 (37.1%) had a low risk, 64 (45.7%) had a medium risk, 14 (10%) had a high risk, and 10 (7.2%) had a very high risk. patients (Table 1).

Table 1: mSCORE indicators for cardiovascular risk in patients with rheumatoid arthritis

<table>
<thead>
<tr>
<th>Indicators</th>
<th>mSCORE (n =140)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n =140)</td>
</tr>
<tr>
<td>Patient age</td>
<td>54.04±8.82</td>
</tr>
<tr>
<td>Low risk &lt;1%</td>
<td>52(37.1%)</td>
</tr>
<tr>
<td>Intermediate risk ≤5%</td>
<td>64(45.7%)</td>
</tr>
<tr>
<td>High risk ≤10%</td>
<td>14(10%)</td>
</tr>
<tr>
<td>Very high risk &gt;10%</td>
<td>10(7.2%)</td>
</tr>
</tbody>
</table>

The increased risk of death from cardiovascular disease is associated with the age of the patient and the presence of additional risk factors. Coronary atherosclerosis and the associated complications largely determine the clinical course and outcome of some rheumatic diseases [19]. In this context, it is important to reduce CVD by assessing RF, overall cardiovascular risk and changing all available RFs [Zulfiqare et al. 2018]. Of the examined patients, 64 were 35-49 years old, 76 patients were 50-60 years old. When these rates were analyzed by age, a low risk of cardiovascular disease was found in 53.1% aged 35-49 years, 23.7% aged 50-60 years old, the intermediate risk aged 35-49 years old was 40.6% and 50% at the age of 50-60. In the group of patients aged 50-60 years, the risk <10% was 2 times higher than in patients aged 35-49 years, and a very high risk >10% was found only in patients aged 50-60 years (tab. 2).

Table 2: The incidence of cardiovascular risk in patients with rheumatoid arthritis depending on age

<table>
<thead>
<tr>
<th>Indicators</th>
<th>mSCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35-49 years old (n=64)</td>
</tr>
<tr>
<td>Low risk &lt;1%</td>
<td>34 (53.1%)</td>
</tr>
<tr>
<td>Intermediate risk ≤5%</td>
<td>26 (40.6)</td>
</tr>
<tr>
<td>High risk ≤10%</td>
<td>4 (6.3%)</td>
</tr>
<tr>
<td>Very high risk &gt;10%</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: * - reliability of differences in indicators at the age of 35-49 years (* -P <0.001)
Discussion

According to international guidelines, every patient with a SCORE risk of >1% should be informed of the presence of risk factors and should work with a physician to address these factors as well as complications from cardiovascular disease [Weijers et al. 2018]. The risk factors can occur in humans over a number of years, and this condition is often the result of non-compliance with healthy birth principles [Kamilova et al. 2019]. It is therefore difficult to change the lifestyle that has been practiced over the years and it often requires an individual approach [Targocski-Stepniak et al. 2018].

Early detection and assessment of risk factors for cardiovascular diseases in patients with RA using the mSSORE scale increases the effectiveness of the principles of prevention of cardiovascular diseases by raising public awareness, eliminating risk factors, and promoting a healthy lifestyle [Ozen et al. 2016; Kamilova et al. 2019].

The results obtained showed that 10-year CVD risk indicators depend on arterial hypertension and its level, and risk factors. Assessment of CVD risk using the SCORE scale allows for the timely identification and monitoring of people with high and very high risk of cardiovascular diseases, and also increases the effectiveness of therapeutic and preventive measures [Van der Heijde et al. 2013; Turdiev et al. 2019].

Conclusion.

Thus, determination of CVD risk factors in patients with RA, the use of the mSCORE scale makes it possible to early diagnosis of cardiovascular risk, development of preventive measures and timely correction of risk factors.

Conflict of interests. The authors declare no conflicts of interest.

Ethical clearance – Taken from the ethics committee under the Ministry of Health of the Republic of Uzbekistan

Source of Funding – Self

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11. Mackey R.H., Kuller L.H., Moreland L.W. Update on Cardiovascular Disease Risk in Patients with Rheumatic Diseases. Rheum Dis Clin North Am,


The Effect of Chemical Weapon Exposure on Semen Fluid Analysis and the Determination of SOD and MDA Levels in Peshmerga against ISIS War

Yasin Kareem Amin
Assist. Prof. Hawler Medical University, General Directorate of the Institute of Forensic Medicine, Kurdistan Region-Iraq

Abstract

The identification of chemical weapons as “weapons of mass destruction” highlights their possible damaging consequences on many civilians’ health. The effect of chemical weapon agents has been documented to cause reproductive toxicity and have adverse effects on semen, leading to infertility in those exposed to those chemical agents. This study analyses the semen fluid and follows up the exposed Peshmerga forces to the chemical weapon during the ISIS war. A prospective cohort study in which 58 exposed Peshmerga in three chemical attacks, compared with the same number of the non-exposed Peshmerga. Semen fluid analysis was performed for all, following up after one year. The serum level of both MDA and SOD was measured. An individual’s BMI was calculated. The participants’ mean age in this study is (32.5) years in the case group while (35.5) years in the control group, with a standard deviation of 5 years in both groups. All participants are male, and no gender effect be seen. All chronic illnesses have been excluded from the study. The mean body mass index is 22.4, with a standard deviation of about 3.1 kg/m. Hypospermia developed in 18% of the exposed group (20% of the total participants), oligospermia (20% overall, only 3% of these in the non-exposed), and decreased motility in 19% of all participants, but 16 of this 19% where the exposed group with significant relative risk results, while morphology not changed in both groups, also the findings showed that significant elevation in MDA level and SOD activity. Chemical weapons can significantly affect semen fluid analysis and interpretation through quantitative disturbances in the volume and account of sperm, while the percentage of abnormal sperm counts has been increased considerably in peoples exposed to chemical weapons. However, the morphology of semen fluid sperms has not changed to the same degree, and its function cannot be estimated. Even when functioning, the genetic errors on future generations may need further testing.

Keywords: Exposure, Peshmerga, semen fluid analysis, MDA, and SOD.

Introduction

Infertility has been identified as the failure to function to attain pregnancy one year after regular, unprotected intercourse. In recent years, reproductive medicine and specifically male infertility have developed unexpectedly in the domain of appropriate evaluation, etiological factors such as anatomy, genetic, environmental aspects. Semen analysis is very important for evaluating male infertility. Samples can be collected by masturbation after a period of abstinence of two to three days. Semen volumes and sperm concentrations might be considerably different among fertile men. Several samples might be required before it is possible to conclude that the results are abnormal. Semen fluid volume greater than two ml is generally accepted as a reference value for any semen analysis, the sperm concentration is more than 20 million/ml, total count...
more than 40 million/ejaculate, motility of more than 50% motile, with 25% rapid progressively motile, and morphology to be more than 15% normal, vitality (live) more than 75% of the collected sample, and the leukocytes must be less than one cell in each million/ml. A normal sperm concentration is greater than 20 million per milliliter as a result; however, men with low sperm counts may be fertile. The accessory sex glands’ ability to dilute the concentrated epididymal spermatozoa during ejaculation with fluids. Sperm concentration is affected by other reproductive organs’ functioning, so it is not a quick indicator of testicular sperm product. Nevertheless, multiplying sperm concentration by semen volume yields the total number of sperm ejaculated. In the absence of ejaculation, spermatozoa accumulate young and older adults’ sperm concentrations in semen fluid, for example, maybe similar. Still, their total sperm counts may vary; as people get older, both the volume of seminal fluid and total sperm production decrease, as a minimum, in some populations. The time since the last sexual activity. The epididymis is then flushed out in the urine after running into the urethra. The increased length of abstinence and penultimate abstinence period does not affect sperm vitality or chromatin. Semen is formed during ejaculation from a concentrated suspension of spermatozoa held in the paired epididymis, fluid secretions from the accessory sex organs mix with and dilute, containing two main quantifiable attributes. The total number of spermatozoa is the first attribute, and it underlines sperm production by the testes and the post-testicular duct systems potential. The total fluid volume, which reflects secrecy activity of the glands, is the second attributed by all the accessory glands; the sperm (its vigor, motility, and morphology) is therefore of particular importance, and the composition of seminal fluid for sperm usages purposes. This social problem became very easy to be solved in medicine with the help of new technology even though these slightly new techniques assisted various infertile men to be a father, the long-standing dream that they had, several other males who were fertile and lost their probable fertility as a result of numerous causes for example exposure to chemical, physical, biological and additional ecological risk agents. Some men with low sperm counts are still fertile. Different types of sperm function tests can be performed in specialized laboratories. However, they do not add much to the treatment options. Etiology: toxic materials harm the testes as well, such as insecticides, fungicides, heavy metals, cottonseed oil, and chemical agents used during wars, which may create negatively affected the germ cells. Leydig cells are also less susceptible than Sertoli or germ cells to the majority of chemotherapeutic medication. However, serum testosterone stages are usually used in exposed men despite their infertility. However, exposure to these agents is not the only aspect that affects fertility. Suppose it is at work and to chemical agents. In that case, it will cause continuous exposure more likely; even people who live in contaminated zones could have infertility because the protection of humankind is a serious environmental crisis that can disturb this process (fertility). Such a problem cannot be terminated unless international cooperation and interference regarding this problem will be planned. Chemical Conflict Agents, used in wars between Iraq and Iran (S/16433, United Nations, 1986). For example, Sulfur Mustard is a lipophilic alkylating chemical agent used as a primary chemical warfare weapon. Most of the patients have been exposed to the chemical warfare agents, which existing a single contact with Sulfur - mustard gas. Nonetheless, both concentration and duration of the breath of mustard diverse. As long as we were investigating the effects of chemical warfare agents and organization over the histories of people who had been exposed, we comprised people with a single exposure. Terrorists could find agents problematic to employ as weapons of mass destruction for several similar reasons that apply to choking agents. The effects can enter the body in various ways like breath or interaction with the eyes or the skin. Some agents can infiltrate by regular clothing materials, triggering blisters even in parts with a cloth covering. Whereas agents that cause blisters can respond rapidly through contact with the skin, their symptoms could be overdue. In the agent mustard, impairment happens within 1 to 2 minutes during the exposure time, choking agents are mostly gases, have noticeable smells, and the color
maysurround the air. Investigation detecting chemical agents after chemical weapon exposure is the part of the concern for soldierly developers, although some of these agents need detection proficiencies at first responders qualified to handle risky materials, whereas, some of the armed units have suitable equipment for chemical weapon detection, civilian people who are the first responders, they use a diversity of profitable equipment to spot and recognize an inclusive variety of chemicals, mostly in a dangerous material background.12,13

**Material and Methods**

**Patient and sampling**

A descriptive cohort study is performed. In this study, we examined 63 exposed Peshmerga, 58 of them included and taken as a sample group. In contrast, 22 participants were taken from the same military unit as the control group (who are not exposed). During the study, initial abnormal results from both groups are excluded (exclusion criteria). The data collected difficulty, and it is not far from the examiner’s exposure to the remnant of chemical weapon products carried by the participants. However, data collection and analysis were performed in the forensic medical institute in Arbil city from 2016 till May 2018. All victims were transferred to the local military health care unit and then transferred to the emergency hospital at the Erbil west emergency hospital. They have both signs and symptoms of dyspnea, lacrimation, cough, skin sores, pruritus, and erythema. Samples were transferred to the forensic medicine institute, then to a specific international laboratory and exposed confirmed through examples taken from clothes blood, urine, hair, saliva, and semen fluid sample-exposed documented by international labs and through the help of some humanitarian organization. Inclusion criteria detailed chemical exposure. A single exposure to a different chemical agent is defined that can cause acute signs and symptoms exclusion (criteria 1) none exposed and non-documented exposure (criteria 2) history of primary infertility. All patients had the same clinical severity; that is why no categorization was performed for dividing exposed military units. However, the sample was taken as the case group, and an equal number of military units taken as the control group concerning age and location. Both groups are examined periodically by re-taking history and re-examination with repeating semen fluid analysis (with three-day abstinence from sex before examination) semen fluid analysis performed in labs of the forensic medical Institute of Arbil city and patient-guided by expert nurses with analysis of the sample according to international guideline and standard laboratory instruments and equipment. After the case group was exposed to the chemical warfare agent, the study group and the control group were followed up on during the study. The members of the control group had never been exposed to somewhat of chemical war agents. Both groups were nominated from a similar military service location and were told similar conditions apart from the chemical agent.

**Procedure**

After 30 minutes as the time of liquefaction, the sample (ejaculated semen) collected in a container-specific sterile tube, immediately taken to the lab, with labeled data, date, and time besides the name of the patient recorded on both container and visitors of the lab, for analysis, quantitative and qualitative study for semen fluid performed by an expert analyzer. Then all results were recorded and saved on the lab system for further evaluation. The method of Estimating the activity of the Superoxide Dismutase (SOD) enzyme depends on the SOD’s ability to prevent epinephrine from being autooxidized into adrenochrome, the reaction occurs at 30 °C, pH = 10.2, and SOD measured at wavelength 480nm, absorbance was measured using Spectrophotometer (G10S UV-VIS, Thermo scientific, USA). Thiobarbituric acid (TBA) reacts with Malondialdehyde (MDA), the lipid peroxidation process’s end product, at 100 °C and acidic medium produce a pinkish complex product. The product is measured at 532nm. Absorbance was measured using Spectrophotometer (G10S UV-VIS, Thermo scientific, USA).
**Statistical Analysis**

New WHO guidelines have been regarded in all aspects of the infertility questionnaire for data collection from the case and control group during the study period. All data were entered into a computer by using Excel program. All statistics analyzed using SPSS (Version 21) and a significant P-value of less than \( (P \leq 0.05) \) with 95 % confidence intervals.

**Results**

The average age in this study of the participants is 32.5 in the case group while 35.5 in the control group, with a standard deviation of 5 years in both groups. All participants are male, and no gender effect be seen. All chronic illnesses have been excluded from the study. The mean body mass index is 22.4, with a standard deviation of about 3.1 kg/m; nearly all exposed peoples are smokers, and all are married. Table 1 shows the effect of chemical exposure on semen fluid volume (semen volume of fewer than 2 ml is regarded as hypospernia), the incidence of hypospernia compared in both groups compared (relative risk). Hypospermia is increased in the exposed group by about 3.2 times than in the non-exposed group. The lower limit range of hypospermia recorded is 0.5 in three participants in the exposed group, with an average semen volume of about one milliliter in the exposed group with hypospermia. In contrast, the lower limit of hypospermia is 1 milliliter in the non-exposed group.

<table>
<thead>
<tr>
<th>ExposureStatus</th>
<th>SemenVolume</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Hypospernia</td>
</tr>
<tr>
<td>Non exposed</td>
<td>20 (90.9%)</td>
<td>2 (9.1%)</td>
</tr>
<tr>
<td>Exposed</td>
<td>40 (75.5%)</td>
<td>13 (24.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>60 (80%)</td>
<td>15 (20%)</td>
</tr>
</tbody>
</table>

Table 2 shows the effect of chemical exposure on sperm count, a prominent decrease in sperm count in the exposed group on with relative increase risk of oligospermia by about 2.6 times than in the non-exposed group. The minimum sperm count is 1 million seen in two participants in the exposed group, with an average sperm count of about 6 million and a standard deviation of about 3 million.

<table>
<thead>
<tr>
<th>ExposureStatus</th>
<th>SpermCount</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Oligospernia</td>
</tr>
<tr>
<td>Nonexposed</td>
<td>17 (89.5%)</td>
<td>2(10.5%)</td>
</tr>
<tr>
<td>Exposed</td>
<td>39 (76.5%)</td>
<td>12 (23.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>56 (80%)</td>
<td>14 (20%)</td>
</tr>
</tbody>
</table>
On further evaluation, the motility also decreased in the exposed group by 2.1 times than those not exposed (Table 3). On further analysis of the exposed group of sperm motility, the effect ranges from sluggish to extreme immobility, with nearly 70% of immobility in more than half of the exposed participants. However, the ratio is more extreme, reaching 90% of immobility in the exposed group but only reaches 65% in the non-exposed group with significantly fewer immortality cases.

### Table 3: Association between exposure status and sperm motility status

<table>
<thead>
<tr>
<th>ExposureStatus</th>
<th>MotilityStatus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Non expose</td>
<td>15(88.2%)</td>
<td>17(100%)</td>
</tr>
<tr>
<td>Exposed</td>
<td>32(78%)</td>
<td>41(100%)</td>
</tr>
<tr>
<td>Total</td>
<td>47(81%)</td>
<td>58(100%)</td>
</tr>
<tr>
<td></td>
<td>Immotile</td>
<td></td>
</tr>
<tr>
<td>Non expose</td>
<td>2(11.8%)</td>
<td></td>
</tr>
<tr>
<td>Exposed</td>
<td>9(22%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11(19%)</td>
<td></td>
</tr>
</tbody>
</table>

On the other hand, no effect of chemical exposure is seen according to this study on sperm morphology. The relative risk between the incidence of exposure to the non-exposed group is about equal (relative risk =1) (Table 4). The accepted lower limit of 5% normal morphology apparent in nearly all participants in both groups. Even those with abnormal morphology are a total of three participants, and one of them in the non-exposed group and has an abnormal morphology of about 4.5 %. The exposed group, on the other hand, displayed normal morphology in only two participants.

### Table 4: Association between exposure status and sperm morphology

<table>
<thead>
<tr>
<th>ExposureStatus</th>
<th>Morphology</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Non exposed</td>
<td>16(94.1%)</td>
<td>17(100%)</td>
</tr>
<tr>
<td>Exposed</td>
<td>46(95.8%)</td>
<td>48(100%)</td>
</tr>
<tr>
<td>Total</td>
<td>62 (95.4%)</td>
<td>65(100%)</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td></td>
</tr>
<tr>
<td>Non exposed</td>
<td>1(5.9%)</td>
<td></td>
</tr>
<tr>
<td>Exposed</td>
<td>2(4.2%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3 (4.6%)</td>
<td></td>
</tr>
</tbody>
</table>

In contrast, Table 5 shows the results of MDA and SOD levels for patients and controls. It shows that patients’ MDA levels have increased significantly (0.03558 ± 0.02012 µmol/l) compared to controls (0.006 ± 0.00285 µmol/l), at a probability (P ≤ 0.05), respectively. It also shows showed a significant increase in SOD values of patients (0.601 ± 0.052 µmol/min/ml) compared to controls (0.924 ± 0.066 µmol/min/ml) respectively.
Table 5: MDA and SOD levels

<table>
<thead>
<tr>
<th>Variants</th>
<th>Mean ± SD</th>
<th>T-test</th>
<th>P ≤ 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>MDA µmol/L</td>
<td>0.03558 ± 0.02012</td>
<td>0.006 ± 0.003</td>
<td>7.29</td>
</tr>
<tr>
<td>SOD µmol/min/ml</td>
<td>0.924 ± 0.066</td>
<td>0.601 ± 0.052</td>
<td>8.84</td>
</tr>
</tbody>
</table>

Discussion

Ghanei and Allameh, from Iran study, chemical warfare agents' effect on fertility in the case-control pattern through a questionnaire-based method to detect infertility in the exposed group versus the non-exposed group. This is in comparison to our study. We concentrate on the result of semen fluid analysis only; the comparison of each survey is to single exposure to chemical weapons with follow up in one year. The chemical materials used in the war on Iraq-Iran were archived in the United Nations S/16433, 1986. While our article is not documented by the United Nations but supported by several humanitarian organizations, Ghanei and Allameh, 2500 male study has participated, but in our research, there are 58 males, which is a relatively smaller sample. In both mentioned studies, the presence of a prior history of infertility is an absolute criterion for excluding the exposed person or participant in the control group. The research group's average age was 28.5 +/- 4 in the Ghanei, and Allameh, study versus 32.5 +/- 3 in our study, when compared to the control group, the mean age of participants was 35.4 +/- 5, while 38.5 +/- 3 in the current study, which indicates no so much difference in the age groups in both studies, making an age effect of being not significant. At the end of the study of Ghanei and Allameh, concluded no impact of a chemical weapon on male fertility while the results on semen fluid analysis were not declared. Still, in our research, it is finely analyzed the effects on semen fluid analysis, making a question for future study and the possibility of gene error detection as a plan for a prospective study. On the other hand, and in a study performed in Iran in 2005, there was no infertility registry available among the soldiers who had been exposed to chemical war agent throughout the first Gulf War. Male infertility was not proven, and there was no noticeable significant difference between the exposed and non-exposed soldiers. In these two contrary studies, we can cross to an idea in which the functional assessment may give the exact explanation for semen fluid. However, none of these studies, including the current research, did perform this step in his article during his phases of medical works. Many chemicals in use in industry and are over the earth and within the water of use, seems to be reaching the semen fluid of the infertile men, reports by a Queen's university research scientist, which may make the isolated effect of these chemical weapons of concomitant harm in both control and patient's groups, that is to say, the environmental impact of these chemicals to be of note. In patients with normal semen fluid parameters besides blood count and 24-hour urine analysis of most sensitive biologic index to think about psychiatric complain in the exposed group, and may which may give an excuse for further functional testing in current and future study. In this study also, a significant increase in MDA levels was detected in the patients; this elevation in MDA levels as a result of an overabundance of reactive oxygen species (ROS) contributing to an excess of oxidative damage in these patients. In turn, these oxygen types can oxidize various other vital biomolecules, including the membrane of lipids. This rise in MDA levels corresponds to a study that found an increase in MDA levels in patients suffering from reproductive problems.
in SOD activity stages may be due to excessive ROS generated through oxidative stress (OS). Also, the increase in SOD activity levels could be due to the rise in OS patients with dismutation of superoxide results in the development of \( \text{H}_2\text{O}_2 \). A study indicated that the levels of SOD activity were observed to be higher in the blood serum of male patients with reproductive problems.\(^{18}\)

**Conclusion**

In the current study, we can conclude that chemical weapons can significantly affect semen fluid analysis and interpretation through quantitative disturbances in the volume and account of sperm. In contrast, the percentage of abnormal sperm count has markedly increased in people exposed to chemical weapons. However, the morphology of semen fluid did not change to the same degree, and its function cannot be estimated. Even when functioning, the genetic errors on future generations may need further testing.

**Conflict of Interests:** None.

**Source of Funding:** Self.

**Ethical Clearance:** The study was undertaken after gaining the Medical Research Center/ Hawler Medical University’s ethics committee’s approval.

**References**


A Comparison Study of Adding Magnesium Sulfate to Local Anesthetics During Spinal Anesthesia for Cesarean Section

Yasir Fadhil Alkhazraji¹, Haitham AbdulSattar Sahib², Mukhallad Mahdi Saleh³

¹Lecturer, MBChB, FICMS-A & IC. Iraqi fellowship of interventional pain Management IBMS/IFIPM. College of Medicine/Al-Nahrain University, ²Researcher. MBChB, FICMS/CABA&IC. Baghdad Teaching Hospital, ³Researcher. MBChB, FICMS. Ghazi Al Harreri Hospital

Abstract

Background: Regional anesthesia is frequently used in obstetric, orthopedic, and urological procedures, commonly intrathecal and epidural local anesthetics are combined with opioids to prolong analgesia; however, these do not prolong the motor block time and can attenuate the response to surgical stress, in addition to being associated with side effects such as respiratory depression, urinary retention, pruritus, hemodynamic instability, nausea, and emesis. Objective: to investigate the effect of adding 75 mg of magnesium sulfate on the duration of sensory block and duration of motor block

Patients and Methods: In a prospective randomized study, ASA I or II, 128 (64 control and 64 experimental groups) pregnant women (at term) who were candidate for cesarean section with spinal anesthesia, were recruited in this study. They were collected from “Baghdad Teaching Hospital” at the period from (September 2020 to Feb 2021).

Each experimental woman received 12.5 mg (2.5 ml) of hyperbaric bupivacaine (0.5%) and 0.5 ml (75 mg) magnesium sulfate (15%), while controls received same does of hyperbaric bupivacaine and 0.5 ml of distilled water.

Results: The duration of analgesia (sensory blockade) and the duration of motor blockade manifested a statistically significant increase in experimental as compared to their controls (control = 116.41 ± 12.47, experimental = 159.75 ± 10.56, control = 180.76 ± 11.83, experimental = 240 ± 9.46 minutes respectively).

Conclusion: Significantly increased the duration of postoperative analgesia and prolonged the sensory and motor blockade without significant apparent maternal or fetal side effects.

Key words: Spinal anesthesia, Bupivacaine, Magnesium sulfate, Cesarean

Introduction

Regional anesthesia is frequently used in obstetric, orthopedic, and urological procedures, commonly intrathecal and epidural local anesthetics are combined with opioids to prolong analgesia; however, these do not prolong the motor block time and can attenuate the response to surgical stress, in addition to being associated with side effects such as respiratory depression, urinary retention, pruritus, hemodynamic instability, nausea, and emesis. There are other drugs that can enhance antinociception, including epinephrine, clonidine, ketamine, and neostigmine.

Spinal anesthesia, is a form of regional anesthesia involving injection of a local anesthetic into the
subarachnoid space, generally via a fine needle through intervertebral foramen (L4 –L5).\(^{(1)}\)

Cesarean section is one of the most frequent surgeries in the world, and surely the most frequent obstetric surgery, with an incidence that varies between countries, hospitals and even between obstetric groups 1 out of 23 \(\%\) and more than 50\(\%\) of pregnancies.\(^{2}\)

The type of anesthesia used depends on multiple factors, among others: availability of anesthesiologist, urgency of the procedure, maternal preoperative status, indication for cesarean section, personal preference, etc.

Currently, the type of anesthesia most used for this type of procedure is neuraxial regional anesthesia (epidural and / or subarachnoid), which offers the advantages of keeping the mother awake, allowing early contact with the newborn; minimize the potential risk of gastric content aspiration; avoid neonatal depression due to general anesthesia drugs 2 and be associated with a 16 times lower risk of maternal mortality due to anesthetic causes, when compared to that of general anesthesia \(^{3}\).\(^{\(\text{6}\)}\)

Magnesium sulfate was added to local anesthetics too for the same purpose. In experimental studies, spinal injection of magnesium sulfate reduces the response to painful stimulus in rats.\(^{(7)}\) On the other hand, magnesium sulfate potentiates morphine anti-nociception at the spinal level.\(^{(8)}\)

### Complications of Spinal Anesthesia

The real incidence of neurological complications after neuroaxis blockade is difficult to obtain due to its low frequency, its lack of registration and communication, and the legal implications that its declaration entails. Furthermore, the different definitions of complication and the heterogeneity of the groups studied make their measurement even more difficult. Overall, we can say that the incidence of neurological complications related to central blocks is less than 4 / 10,000 patients, with 0-4.2 / 10,000 and 0-7.6 / 10,000 being permanent after spinal and epidural anesthesia, respectively.\(^{(7)}\)

The incidence of post-dural puncture headache (CPPD) has now decreased greatly due to the use of small gauge “pencil point” needles (25G and 27G) (0.002-1.2\%) \(^{8},\ ^{9}\), designed to minimize the loss of cerebrospinal fluid when puncturing the dura mater.

### Aim of the Study

To investigate the effect of adding 75 mg of magnesium sulfate (to hyperbaric bupivacaine) on the duration of sensory and motor blockade.

### Patients and Methods

This study was performed after the approval of the Iraqi Council for Medical Specialization in anesthesia and intensive care, and after obtaining them consents from the patients.

In a prospective randomized study, ASA I or II, 128 (64 control and 64 experimental groups) pregnant women (at term) who were candidate for cesarean section with spinal anesthesia, were recruited in this study. They were collected from “Baghdad Teaching Hospital” at the period from (September 2020 to Feb 2021).

Each experimental woman received 12.5 mg (2.5 ml) of hyperbaric bupivacaine (0.5\%) and 0.5 ml (75 mg) magnesium sulfate (15\%), while controls received same does of hyperbaric bupivacaine and 0.5 ml of distilled water.

Patients were randomly chosen and divided into two groups: control group (64 patients) and experimental group (64 patients).

The inclusion criteria were:

1. Age (17-40) years
2. Height (151 - 170) cm
3. Weight (57 - 78) kg
4. ASA class I or II.

The exclusion criteria were:

1. Regional anesthesia refusal
2. Absolute contraindication of spinal anesthesia

3. Complicated pregnancy

Dosing:

After obtaining their consent and explaining the full details of the study.

Each experimental woman received 12.5 mg (2.5 ml) of hyperbaric bupivacaine (0.5%) and 0.5 ml (75 mg) magnesium sulfate (15%), while controls received same does of bupivacaine and 0.5 ml of distilled water.

Each patient, from control and experimental groups, were monitored by recording:

1. The time of onset of anesthesia (TO)
2. The time of end of surgery (TES)
3. The time of analgesia requirement (TAR)
4. The time of complete recovery (CR)

Statistical Analysis:

Data were analyzed using SPSS version 25. Descriptive statistics as mean ± standard deviation.

Student – T – test was employed for comparison between control and experimental groups.

All statistical analysis level of significance was set at P value equal or less than 0.05 to be considered as significant difference.

Results

General Notes

The side effects during anesthesia and in recovery, such as nausea, vomiting, headache and dyspnea and side effects during surgery such as hypotension were relatively lower in experimental group than controls although not statistically significant.

Age, height and weight:

The age, height and weight of control and experimental patients (the 128 patients who were included in this study) are summarized in table (1), (2) and (3) respectively.

No significant difference was found in the studied groups regarding to age, height and weight.

| Table (1); the age of the patients involved in this study |
|----------------------------------|----------------|----------------|
| Range   | Mean ± SD    | No. of patients |
| Control | 17 – 39    | 26.47 ± 5.65  | 64          |
| Experimental | 20 – 35 | 26.16 ± 4.43 | 64          |
| Total   |             | 128           |

P value = 0.237
Table (2): the height of control and experimental patients

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean ± Sd</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60 – 87</td>
<td>69.69 ± 3.99</td>
<td>64</td>
</tr>
<tr>
<td>Experimental</td>
<td>57 - 84</td>
<td>68.97 ± 5.98</td>
<td>64</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>128</strong></td>
</tr>
</tbody>
</table>

P value = 0.523

Duration of Surgery

The range and mean of time of surgery is summarized in table (Table 3). With respect to time of surgery, there was no significant difference between control and experimental groups.

Table (3): duration of surgery in control and experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Mean (minutes) ± SD</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>58.15 ± 13.10</td>
<td>64</td>
</tr>
<tr>
<td>Experimental group</td>
<td>59.10 ± 18.51</td>
<td>64</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>128</strong></td>
</tr>
</tbody>
</table>

Duration of analgesia (duration of sensoryblockade)

The range of duration of analgesia, in controls, was (80 – 145) minutes and its mean was 116.41, while the range in the experimental group was (115 – 190) with mean of 159.75 (Table 4). The mean duration of experimental group revealed a statistically significant increase when compared with that of controls (P value =0.041543).

Table (4): the duration of analgesia in control and experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Mean (minutes) ± SD</th>
<th>SE</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>116.41 ± 9.47</td>
<td>3.51</td>
<td>64</td>
</tr>
<tr>
<td>Experimental group</td>
<td>159.75 ± 7.56</td>
<td>4.29</td>
<td>64</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>128</strong></td>
</tr>
</tbody>
</table>
Figure (1): Bar diagram showing the time of analgesia in control and experimental groups

Duration of Complete recovery (time of motor blockade)

The range of the time of complete recovery was (130 – 230), while its mean was 180.76. On the other hand, the range the experimental group was (165 – 275) and its mean was 240 (Table 5). The increase of the time of recovery was statistically significant (P value=0.0315).

| Table (5): Duration of complete recovery in control and experimental groups |
|---------------------------------|--------|--------|------------------|
|                                 | Mean ± SD | SE    | No. of patients |
| Control group                   | 180.76 ± 11.83 | 4.60  | 64               |
| Experimental group              | 240 ± 9.46   | 3.68  | 64               |
| Total                           |          |       | 128              |

Discussion

Duration of analgesia (Duration of sensory block)

Few trials were done to increase the time of analgesia and anesthesia by adding different doses of magnesium sulfate to the local anesthetics. Shoeibi and his coworkers, recorded an increase in duration of analgesia after adding 50 mg magnesium sulfate to lidocaine spinal anesthesia. Moreover, Manjula and colleagues disclosed that addition of 50mg of magnesium sulfate to 2.4 ml of 0.75% of isobaric rupivacaine spinal anesthesia prolongs the duration of sensory and motor blockade. Furthermore, Buvanendran and colleagues found that intrathecal magnesium prolonged (20 µ) fentanyl analgesia. However, Mitra and Sayed found that adding 100mg of magnesium sulfate to hyperbaric bupivacaine prolongs the duration of analgesia, while 50 mg of magnesium sulfate didn’t do it. On the
other hand, Khalili and his coworkers arrayed that in patients undergoing lower extremity surgery with spinal anesthesia, the addition of 100 mg magnesium sulfate to 15 mg bupivacaione without opioid supplement, prolonged the duration of the sensory block, decreased postoperative analgesic consumption, and significantly prolonged the onset of spinal anesthesia. (13) All the above mentioned investigations agreed that magnesium sulfate revealed a statistically significant increase in the duration of anesthesia, and this coincides with the outcome of this study. However, the dose of magnesium sulfate that causes this statistically significant prolongation of the analgesia was controversial. All agreed that adding 100 mg of magnesium sulfate leads to a significant prolongation of sensory blockade (12,13), yet 75 mg and 50 mg disclosed significant and nonsignificant outcomes.(9,10,12)

Duration of anesthesia (duration of motorblock)

The significant prolongation of motor blockade that is elicited in this investigation coincides with previous studies. (10,12) However, other studies found that adding magnesium sulfate to bupivacaine and fentanyl was not effective. (14,15)

Conclusions and Recommendations

The addition of 75 mg of magnesium sulfate to hyperbaric bupivacaine in spinal anesthesia for cesarean section had significantly increased the duration of postoperative analgesia and prolonged the sensory and motor blockade without significant apparent maternal or fetal side effects.

No Conflicts of Interest

Self-Funding Source

Ethical Clearance: from the Ministry of health and Environment/ scientific committee

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Acute Pyrethroids Insecticide Ingestion in An Elderly Patient

Yhan Batista1, Novira Widajanti2
1Senior Resident, 2Geriatrician, Division of Geriatric Medicine, Department of Internal Medicine, Universitas Airlangga – Dr. Soetomo General Hospital Surabaya, Indonesia

Abstract

Insecticide ingestion is not an uncommon phenomenon found in daily practices. An elderly woman, 69-year-old, was rushed to emergency room due to household cleaning liquid ingestion. There was a history of previous suicide attempt and severe depressive episode before. Since there was no sign of severe intoxication, patient received symptomatic treatment. Patient was discharged with symptomatic treatment and antidepressants.

Keywords: pyrethroids, elderly, chemical ingestion, intoxication, depression

Introduction

Chemicals and chemical products stored in homes are the source of many accidental or intentional exposures that can be found in different ages. Elderly people are vulnerable to intoxication since they are more fragile than younger age groups so poisoning leads to severe complications.1 Pyrethroids have been developed for the control of household and agricultural insects, and human lice. Pyrethroids have extremely high selective toxicity for insects compared to mammals, due to higher insect nerve sensitivity, lower mammalian skin absorption and more efficient mammalian hepatic metabolism.2

Patients with significant pyrethroid ingestion can present with severe symptoms and signs, from nausea, headache to seizure and coma which would constitute a medical emergency and should be immediately referred to hospital for proper medical management.2

Case Illustration

Mrs. L, 69-year-old, a widow, was brought by her son to emergency room after ingesting household insecticide liquid 12 hours prior to admission. She complained nausea and vomiting after she ingested approximately 250 milliliters of the liquid 12 hours prior to admission. She vomited 3 – 4 times with food contents and accompanied with abdominal pain and soft stool defecation once. There was no trouble in swallowing meal or liquid. Excessive salivation, cough, lightheadedness, and seizure were denied. There was no problem in urinating. She intentionally ingested the household cleaning liquid to end her life. There was decreasing appetite in a month and weight loss 3 kilograms in one month. There was a history of diabetes mellitus for one year, controlled with metformin 500 milligram three times daily. She had controlled hypertension for two years with amlodipine 10 milligram daily. There was a history of being admitted in Psychiatric ward one month ago for four days, diagnosed with severe depression episode and suicide attempt. She received clobazam 5 milligram twice daily and sertraline 25 milligram once daily. History of recreational drugs abuse was denied. There is no psychosis history in her family. She is a

Corresponding author:
Yhan Batista
Department of Internal Medicine, Faculty of Medicine, Airlangga University, Jl. Mayjen Prof. Dr. Moestopo 47, Surabaya, East Java, Indonesia Phone: +6231-5020251
E-mail: yhanbatista.md@gmail.com
ORCID ID: 0000-0003-1068-9668
widow with four children.

From physical examination, it was found weak condition with GCS 456. Blood pressure was 130/80 mmHg, respiratory rate was 20 times per minute, heart rate was 88 beats per minute and axillary temperature 36.8° Celsius. From head and neck examination, no miosis pupils and no hypersalivation were found. No abnormality found in chest examination. From abdominal examination, epigastric tenderness was found. No abnormality found in extremities examination.

Laboratory results showed hemoglobin level 13.1 g/dL, hematocrit level 38.3%, leucocyte 11300/ mm³, platelet 387000 mm³, serum glutamic oxaloacetic transferase level 27 IU/L, serum glutamic pyruvate transfease level 34.4 IU/L, blood urea nitrogen 15 mg/dL, creatinine level 1.01mg/ dL, serum sodium level 141 mmol/L, serum potassium level 3.9 mmol/L, serum chloride level 98 mmol/L, random blood sugar level 132 mg/dL, Blood gas analysis showed pH 7.38, pCO2 43 mmHg, pO2 69 mmHg, BE 0.3 mmol/L, HCO3 25.4 mmol/L and SpO2 98% in room oxygen level. Chest x ray showed no abnormality with cardiothoracic ratio 54% and electrocardiography showed normal sinus rhythm with 92 beats per minute. Patient was consulted to Psychiatry Department in Emergency Room and assessed severe depressive episode without psychotic symptoms. Advises were delaying psycho-pharmacy with tight observation on organic conditions, placing at near nurse station, putting away dangerous substances or tools that patient could use as suicide media and psychotherapy intervention for patients and family.

Based on history taking, physical examination and laboratory findings, patient was assessed chemical ingestion, suicide attempt, severe depressive episode without psychotic symptoms, diabetes mellitus and hypertension. Diagnostic plans were fasting blood glucose, 2-hour post prandial glucose and HbA1c. Early management was intravenous line insertion with Ringer Dextrose 5% 1000 milliliters per day and intravenous omeprazole 40 milligram twice daily. Monitor plans were vital signs, complains and suicidal ideation.

On second day of admission, patient felt nauseous decreased, vomiting decreased and abdominal pain decreased. Vital signs were stable. No abnormalities were found in the physical examination.

On third day of admission, patient felt no nausea, no vomiting, and no abdominal pain. She could sleep soundly in the night. She still felt regret of what her doing. Vital signs were stable. Fasting blood glucose was 95 mg/dL, 2-hour post-prandial glucose was 123 mg/dL and HbA1c 6.2%. Therapy continued as previous day. On fourth day of admission, patient had no complains. Vital signs were stable. Patient was discharged with amlodipine 5 milligram peroral daily omeprazole 20 milligram peroral daily, sertraline 25 milligram peroral daily, clobazam 5 milligram peroral daily, and aripiprazole 5 milligram daily.

**Discussion**

HIT 0.26 AE mosquito repellent spray contains active ingredients of prallethrin 0.120%, cypermethrin 0.100%, and dimefluthrin 0.040%. Prallethrin, cypermethrin, and dimefluthrin are classified into pyrethroid group. Pyrethroids are ion channel toxins which interfere with nervous system function. They modify the gating characteristics of neuronal voltage-sensitive sodium channels by delaying the closure, thereby extending neuronal excitation. The toxic effects of pyrethroids result from this neuronal excitation, including a wide spectrum of signs and symptoms from mild to severe. Allergic reactions, including contact dermatitis or asthma, are rarely reported.

Pyrethroids are ion channel toxins which interfere with nervous system function. They modify the gating characteristics of neuronal voltage-sensitive sodium channels by delaying the closure, thereby extending neuronal excitation. The toxic effects of pyrethroids result from this neuronal excitation, including a wide spectrum of signs and symptoms from mild to severe. Type I pyrethroids (allethrin, permethrin) have a basic cyclopropane carboxylic ester structure and produce reflex hyperexcitability and fine tremor (T syndrome). Type II pyrethroids (cypermethrin, deltamethrin, fenvalerate) have a cyano-group and they cause salivation, hyperexcitability, choreoathetosis and
seizures (CS syndrome). Both types produce sympathetic activation. Dermal exposure causes paresthesia of the exposed skin while ingestion results in gastrointestinal irritation. Life threatening toxicities could be resulted from pyrethroid poisoning.6,7

The symptoms of acute pyrethroid poisoning in humans are supposed to be subdivided into two classes. However, since most of the reports on human poisoning are related to type II pyrethroids, it is not yet known whether the T-Syndrome applies also to humans.4,5,7

Acute pyrethroid poisoning may be confused with organophosphorus intoxication. There may be difficulty in making the diagnosis of pyrethroid poisoning since similar features are also found in severe organophosphorus pesticide poisoning. Pyrethroid ingestion causes sore throat, nausea, vomiting and abdominal pain within minutes. As systemic toxicity due to pyrethroid exposure is rare, most patients exposed to pyrethroids require only skin or eye decontamination and symptomatic and supportive measures. Measurement of the red cell cholinesterase activity which is reduced in acute organophosphorus poisoning but not in pyrethroid intoxication allows clarification.6,8

Following ingestion, gastric lavage is probably best avoided since solvents present in many formulations may increase the risk of aspiration pneumonia. Alternatively, although there is only limited experimental evidence that pyrethroid insecticides are adsorbed to charcoal, the administration of active charcoal 50 – 100 grams to an adult may be considered if a potentially toxic amount has been ingested within 1 hour. Isolated brief convulsions do not require treatment but intravenous diazepam 5–10 milligram should be given if seizures are prolonged. Rarely, it may be necessary to paralyze and ventilate the patient. Hypersalivation and pulmonary oedema are known to develop in cases of severe pyrethroid poisoning. Intravenous atropine 0.6 – 1.2 milligram may be useful in controlling excess salivation.8,9

Most patients with pyrethroid poisoning recover within 6 days and fatality is rare. There were seven fatalities among 573 cases in one series and one among 48 in another. Out of the 573 cases of acute pyrethroid poisoning reported by He et al., four of seven fatalities were due to convulsions and one was due to pulmonary edema.10,11

Although pyrethroids are considered less toxic than organophosphates, poisoning with large doses could be life threatening. Since its presentation mimics organophosphates, thorough history taking, and examinations are needed to manage the intoxication properly.6

**Conclusion**

Pyrethroids ingestion could be found in daily practice case since pyrethroids products are easy to access. We reported a mild case of intoxication which successfully treated symptomatically. Predisposing factors of ingestion such as depression should be investigated and treated to prevent repeated incidence.

**Conflict of Interests:** The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Funding:** None

**Ethical Clearance:** Not required for a case report

**Acknowledgement:** The authors would like to thank to patient, patient’s family, dr Soetomo General Hospital and Faculty of Medicine Airlangga University, Surabaya, Indonesia.

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The Development of a Community-Based Model as an Assisting Approach in the Prevention of Pregnancy and Labor Complications in Pandeglang Regency, Banten, Indonesia

Yudhia Fratidhina¹, Nina Herlina², Hamidahs¹, Sri Mulyati¹

¹Lecturer, Department of Health Science, Faculty of Midwifery, Politeknik Kesehatan Kementrian Kesehatan Jakarta III. Address: Jl. Melati 2 No.15, RT.001/RW.009, Jatiwarna, Kec. Pd. Melati, Kota Bks, Jawa Barat 17415, ²Head of the Midwifery Undergraduate Program, Faculty of Public Health, Gunadarma University, Jakarta. Address: Jl. Margonda N.100 Pondok Cina Depok, Jawa Barat

Abstract

Most of the direct causes of maternal death is obstetrical complications. Some programs have been done to reduce the incidence of complications on pregnancy and childbirth complications unfortunately the implementation of the program has not been maximized. Community empowerment is required to ensure the sustainability of the program. This research was conducted to develop community-based Model in preventing pregnancy and labor complications based on asset and community participatory. This research was conducted with a mixed method approach. On the stage of model development is conducted qualitatively through Focus Group Discussion and in-depth interview with 4 key informants and 13 support informants. Furthermore, model is developed quantitatively by using cross sectional study design on 80 respondents. The analysis that used in this research is SEM PLS. Once the model is formed, the evaluation was conducted on 83 respondents by using pre and post-test design. Application of community-based Model in the society is done by mentoring models using Module of asset and participatory-based prevention of pregnancy and labor complication through community-based Model. The results show that community-based model can be achieved through community participation, community assets development, knowledge, attitudes and behavior. The results also reveal that the community-based model is statistically proven to increase knowledge (p 0.000), attitude (p 0.000) and the behavior of expectant mother (p. 0.000). Community-based Model consists of five variables and it was proved to be effective in improving the knowledge, attitude and behavior of expecting mothers on the prevention of pregnancy complication and childbirth.

Keywords: Community-based Model, Pregnancy Complications, Community’s participation, Labor Complication, Maternal Mortality.

Introduction

Maternal Mortality Rate (MMR) is one of the important indicators of public health in Indonesia. The maternal mortality rate describes the number of women who die from a cause of death related to pregnancy disorders or their handling, excluding accident or incidental cases, during pregnancy, childbirth and postpartum 42 days after delivery, regardless of the length of pregnancy per 100,000 live births. Maternal mortality rates can be used in monitoring deaths related to pregnancy. This indicator is influenced by health status in general, education and services during pregnancy and childbirth. Based on the 2012 Indonesian Demographic and Health Survey (IDHS), MMR in Indonesia is still lower than the expected value.
high at 359 per 100,000 live births. This figure increased compared to AKI in 2007 which was equal to 228. Meanwhile, the fifth global Millennium Development Goals target was to reduce MMR to 102 per 100,000 live births in 2015.

The biggest cause of maternal mortality during the period 2010-2013 was bleeding, which was consecutively from 2010 amounting to 35.1%, in 2011 amounting to 31.9%, in 2012 amounting to 30.1% and in 2013 amounting to 30.3%. While prolonged delivery is a contributor to the lowest maternal mortality. While other causes also play a significant role in causing maternal deaths. Other causes are indirect causes of maternal death, such as cancer, kidney, heart disease, tuberculosis or other illnesses suffered by the mother. The direct cause of maternal mortality of more than 90% is a result of obstetric complications, especially labor complications. Complication is a complication, a condition that aggravates an illness. Pregnancy complications are obstetric emergencies that can cause death in the mother and foetus. Pregnancy complications include hypertension and preeclampsia, anaemia, placenta previa, and diabetes. While complications of labor are difficult labor (dystocia) that causes a disease. Complications of labor include premature rupture of membranes, preterm labor, foetal position abnormalities, and others.

Other factors that determine the regularity of pregnant women in their pregnancy checks by health workers, early detection of high risks and complications of pregnancy and childbirth, and family support in obtaining services for pregnancy and childbirth and emergency referrals. Early detection of high risks and complications of pregnancy and childbirth needs to be done as an effort to reduce the risk of maternal death. The introduction or socialization of the causes of pregnancy complications must be early and handled with the right standards. Every pregnancy hazard if not found early and handled to the correct standard will cause further pregnancy complications and will have an impact on maternal and infant mortality. These deaths are the effects of pregnancy complications, namely bleeding, infection, hypertension. Antenatal care plays an important role to be able to recognize risk factors in pregnancy as soon as possible so that deaths or unnecessary diseases in the mother and baby can be avoided. There are still many mothers who are less aware of the importance of prenatal care, which does not detect high risk factors that may be experienced by them. Antenatal examinations are related to the availability of health care facilities. There is a relationship between the distance of the house to the health service place and antenatal use.

The Government has carried out the Program for Maternity Planning and Complication Prevention (MPCP) as one of the efforts to accelerate the reduction of MMR. Maternity Planning and Complication Prevention is an activity facilitated by midwives in the village in the context of the active role of husbands, families and communities in planning safe deliveries and preparation for complications for pregnant women, including planning for the use of postpartum birth control, using stickers as a media notification target in order to improve coverage and quality of health services for mothers and new-borns. The program has been launched by the government since 2007. Through MPCP, it is expected that every pregnant woman will be properly recorded and monitored. The technical form of MPCP is that every pregnant woman gets stickers so that this sticker can be attached to every home that has a pregnant woman.

The target to be achieved in MPCP begins with monitoring of targets with high, medium, low risk directly in the hope that complications can be prevented and addressed early. In fact, the MPCP has not been implemented optimally, only around 55%. This is due to the lack of understanding of pregnant women to carry out high-risk early detection, lack of participation of cadres and health workers (midwives) in disseminating MPCP and unclear information delivery to pregnant women so that they do not understand the benefits and objectives of MPCP get support from husband and family. Based on the explanation by Prasetyawati (2012), it was stated that the health problems of
pregnant women and infants were not only focused on health workers but also needed community-based partnerships. MPCP is a program whose sustainability is highly dependent on government policies and budgets. Given the efforts to prevent these complications are important. So the sustainability of these efforts requires the active role of the community.

One strategic step is to increase the knowledge, awareness and motivation of pregnant women, husbands, families and communities towards improving behavior towards efforts to prevent complications of pregnancy and childbirth. This can be done through empowering and participating families or communities. Participation is the voluntary involvement of the community in self-determined changes, can also be interpreted as community involvement in the development of themselves, their lives and their environment. Community empowerment is intended so that the community can determine practices/actions to solve problems faced and manage planned activities, both individual capacity building, increased efforts to control, institutional improvement and environmental improvement. The purpose of empowerment is to increase community capacity and capability so that able to recognize the problem at hand, able to explore and utilize available resources, and be able to express themselves clearly.

This community empowerment approach has become the government’s concern. This can be seen from the formation of government programs that empower the community. One example is the Smart Healthy Generation Program, which is part of the government’s efforts to improve the quality of basic social services, especially in the fields of basic education and maternal and child health through a community empowerment approach, especially rural communities. Empowering rural communities in reducing maternal mortality is one of the important things, considering that maternal mortality rates are more prevalent in rural areas. These efforts are in line with the mandate of Law No. 6/2014 on villages, that villages have authority, based on village-based origin and local authority, to realize village independence as a strong foundation in implementing governance and development towards a just, prosperous society and prosperous. The development paradigm in the health sector as stated in Law No. 36/2009 on Health aims to increase awareness, willingness and ability to live a healthy life for everyone in order to realize the highest degree of public health as an investment for the development of human resources. This is in line with one of the strategic programs in the health sector, which is the development of Healthy Village Houses (HVH) mandated by the enactment of Law No. 6/2014 on villages that strengthens village autonomy in determining its development priorities and strategies.

A healthy village house is a facilitation to accelerate the improvement of the quality of basic health services in the village community which was initiated and facilitated by the government and implemented and managed by the village community in order to reduce the maternal mortality rate. Empowerment of village communities is used to increase public awareness and participation so that the potential/assets of the community in its implementation can be in accordance with the principle of village sovereignty. One approach is to increase community participation through the Participatory Action Research (PAR) approach. Research on the development and application of the mentoring model which is a combination of the theory of PAR and ABCD by including variables of knowledge, attitudes and behavior, such as those used in the community-based model has never been reported. The successful implementation of the community-based model as a mentoring approach to prevention of complications of pregnancy and asset-based childbirth and community participation in Banten Province is expected to be developed to be applied in other regions that have problems with high maternal mortality rates.

The concept of assistance in the community-based model that combines community empowerment with the use of community assets in order to reduce the complication rate of pregnancy and childbirth in line with the village development program as stated in Law No. 6/2014 on Villages. Village development, especially health development, is realized in the program of
Healthy Village Houses and Healthy Smart Generation (HSG). One of the implications of HVH’s achievements in achieving indicators of changes in community behavior includes the prevention of complications of pregnancy and childbirth through public health education, information communication and education and community-based health efforts. Meanwhile HSG put forward the basics of community development and mentoring that combined aspects of critical awareness, capacity building and strengthening of local institutions towards the integration of village development (health). With the existence of HSG, it is expected to facilitate access to maternal and child health services, especially for the First 1000 Days of Life intervention, especially for the poor and marginalized groups.

This study resulted in a community-based model that is a participatory mentoring model which is a model of assistance to prevent pregnancy-based complications of asset-based labor and community participation developed based on a comprehensive approach, namely community participation approaches, asset development approaches and behavioral change theories where communities utilize the potential that exists in society to solve problems, namely the incidence of complications of pregnancy and childbirth. This model is a new approach used in dealing with the problem of complications of pregnancy and childbirth. The use of this model approach involves developing assets that exist in the community with community participation. The dimensions of each variable were obtained from the development of the theory which was constructed and tested statistically. The results obtained are that in a homogeneous population, social dimensions can be excluded from community assets. The approach to participation and development of community assets, simultaneously able to increase knowledge, attitudes and ultimately behavior related to prevention of complications of pregnancy and childbirth. This community-based model can be adaptively used integrated in supporting and achieving existing programs such as MPCP, Desa Siaga, Healthy Indonesia Program with Family Approach and Healthy Village House Program.

Research Method

The study approach used in this study is mixed method with a sequential exploratory approach strategy. The strategy of this approach is the collection and analysis of qualitative data carried out in the first stage then followed by the collection and analysis of quantitative data in the second stage which is built on the initial qualitative results. More weight / priority is given to qualitative. This sequential exploratory design is usually used to prove the findings of the first (qualitative) results with the results of the next (quantitative) analysis. So that with this approach the direction of theory formation is inductive. Qualitative data were collected through focused group discussion, In-depth Interviews and observations. To guarantee the validation of qualitative results, triangulation is carried out, so as to increase confidence in the answers given. This research was carried out in all villages in the District of Cimanuk, Pandeglang Regency. Cimanuk District consists of 11 villages. Based on the preliminary study conducted by the author, it is known that of the 35 sub-districts in Pandeglang Regency, the highest proportion of groups of pregnant women at high risk in the District of Cimanuk. Research groups were built from the grass root level. The groups built consisted of groups of mothers and cadres, husbands, paraji. The aim of building this group is to increase knowledge and insight along with efforts to strengthen resilience, self-confidence, and community responsibility. The total population in this study was as many as 800 pregnant women who lived in the working area of the Cimanuk District Health Center. The minimum number of samples taken by researchers directly through the object as many as 80 respondents with the following criteria:

a. Inclusion Criteria: Second and third trimester pregnant women with healthy conditions, domiciled in the working area of Cimanuk Sub-District Health Center, high-risk pregnant women, mothers and husbands agree to become respondents.

b. Exclusion Criteria: Pregnant women who have experienced complications, abnormal pregnancies,
respondents moved to domicile.

Samples were taken in accordance with the rules of the number of samples in the PLS (Partial Least Squares) guideline where the sample size is 5 to 10 multiples of the number of indicators to be examined. In this study there were 16 indicators used to observe five variables, so in this case the sample size taken ideally was between 80 and 150. Sampling was done by simple random sampling (SRS). SRS is a sampling technique where each individual has the same opportunity to be selected as a sample. Since the population in this study is pregnant women, each pregnant mother who is in accordance with the inclusion and exclusion criteria has the same opportunity to become a sample.

Results and Discussion

Complications of pregnancy/Labor

Complications of pregnancy/Labor are estimated to occur in 15-20% of pregnant women, not all of which have been detected early. While those detected, not all have been handled in a timely and adequate manner. Delay in the detection and management of labor complications can threaten the mother and foetus. Labor complications include bleeding (25%), infection (14%), abnormalities of hypertension in pregnancy (13%), complications of unsafe abortion (13%) and the consequences of prolonged labor (7%). Bleeding is the main cause of death, which is largely due to retention of the placenta. As a result of the infection caused is an indicator that shows the poor efforts to prevent and treat infections in pregnancy and childbirth.

Research from the Ministry of Health of the Republic of Indonesia in 2013 mentions factors related to complications of prolonged labor delivery (15.4%), 27.5% of respondents aged <20 years and ≥ 35 years experienced labor complications, 27.8% of respondents lived in rural areas, 23.9% parity ≥ 4 and 16.7% were never ANC (antenatal care), and 17.4% never received Fe tablets, 24.6% had no time to deliver at health facilities, 35.6% experienced complications during pregnancy. Analysis of the factors causing pregnancy and childbirth complications is caused by distant determinants and determinants between. Both of these determinants have the opportunity to cause complications for pregnant women and childbirth which can ultimately lead to maternal death. Determinant variable factors include health status of pregnant women, reproductive status, access to health services and behavior / use of health services, while far determinants include maternal status in family and society, family status in society and community status.

Labor complications are difficult to predict and difficult to diagnose, so the treatment is difficult to be applied with certainty. Several factors can cause high-risk labor including maternal age, maternal education level, maternal occupation, family income, knowledge of ANC examination, maternal attitudes toward ANC examination, practice of mothers in ANC examination, parity, pregnancy distance, medical history, obstetric history and quality of ANC services.

Citizens’ Participation

The Focus Group Discussion results indicate community participation in efforts to prevent complications of pregnancy and childbirth is in the form of mutual cooperation, regular meetings of pregnant women and socialization of pregnancy myths. According to Laksana (2013) the participation of the community in Cimanuk Village was included in the form of ideas and social participation. Community empowerment and community involvement in reducing maternal mortality are also carried out by many other studies. A study conducted in Malawi in 2007 involved 729 women assisted by 81 voluntary facilitators to mobilize communities related to maternal and child health. Two years later, half of the group received a maternal and child health development program (part of the maternal and child health task force) which had a main focus to increase the coverage of ANC examinations, knowledge of maternal and child health and delivery in health services. In addition the group also identified high-risk pregnant women, promoted delivery in health services, ANC and PNC services, and conducted health education.
Community involvement in improving health status is a contribution of the community to the health of community members through giving responsibilities related to certain issues. Of course, this responsibility is also flexible in accordance with the characteristics of the community and region. In some conditions, sometimes communities only carry out social responsibility, such as making conditioning in the community that is able to support the implementation of health programs. But in other conditions, in addition to carrying out social responsibility, the community also carries out technical responsibilities. In general, the realization of the responsibilities carried out by the community towards the condition of public health can be through:

a. The adoption of behaviors to prevent health problems;

b. Effective participation in disease control activities;

c. Contribute to the design, implementation and monitoring of health programs;

d. Providing health resources.

In an effort to reduce the risk of complications that can cause maternal mortality, community participation can increase the coverage of the program. Participation can be in the form of community involvement and presence during health promotion, so that when participation increases, the information/knowledge delivered in the program is conveyed to more targets. As was done in Bangladesh\(^{[19]}\) that communities form groups that actively hold meetings every month that discuss maternal, infant, and toddler health. The result is that there are significant differences related to maternal mortality in areas that have active community participation compared to areas that do not have community participation. Community participation also means that people get additional learning about issues that are being worked on and at the same time take concrete actions. By attending the monthly meeting, the community will gain knowledge about ways to recognize pregnancy complications, ANC examinations and access to health facilities in the face of labor complications.

Impact of the Community-Based Model

Community-based model is carried out by involving health workers (midwives), families, cadres, and the community. All elements that play a role need to get training to better understand the concepts and roles in implementing this model. In addition, training is also given to equalize common goals, namely the formation of preventive behaviors for complications of pregnancy and childbirth which can ultimately prevent complications of pregnancy and childbirth. The involvement of cadres and communities in this model is very appropriate. Based on the results of a systematic review, cadres were proven to be able to convey a message of intervention to prevent maternal and child health problems in developing and poor countries. Because cadres are able to convey messages directly to the target, namely the mother, by using cultural and customary approaches that apply in the community\(^{[20]}\).

The success of the community-based model in increasing knowledge, attitudes, and behaviors about preventing complications of pregnancy and childbirth is a very good outcome in the effort to prevent complications of pregnancy and childbirth in particular, as well as increasing village health development in general. Substantially, the researchers have confidence that the community-based model can be applied and generalized to other villages. This is due to the relatively similar characteristics of villages in Indonesia, which are mostly self-help villages. The community-based model can be applied to support the Healthy Village and Healthy Smart Generation program launched by the Ministry of Village, Development of Disadvantaged Villages and Transmigration. This model comprehensively involves community participation and the development of community assets as carried out by the two programs. Healthy village houses are focused on the development and utilization of health assets, while the healthy smart generation focuses on community empowerment to raise awareness about health issues, one of which is the issue of maternal and child health. In addition, this model also
emphasizes community participation. This is consistent with the findings of Sulaeman (2012) suggesting that community participation has a contribution of 51.69% to solve health problems.\cite{21} This is implemented by participating in village meetings that determine village development plans. Communities can participate in the form of conveying problems around them for later analysis and problem solving solutions. This is in line with what is applied in the community-based model.

The effectiveness of the community-based model in increasing the knowledge, attitudes and behaviors of prevention of pregnancy and childbirth complications at the time of this study needs to be maintained. Maintaining the sustainability of this program is a challenge in itself. One possible way to do this is to integrate this model into established programs in the community such as alert villages, MPCP, HVH and HSG and through family empowerment and women’s empowerment. However, considering the issues that are of concern in the community-based model are specific issues related to complications of pregnancy and childbirth, it is necessary to refresh the material for the community-based model. Refreshing this material can be done every six months or once a year in a special activity, or also in a routine annual meeting of cadres in Cimanuk District. The results of this study indicate that there are assets that play a major role in preventing complications of pregnancy and childbirth. These assets need to be mapped in detail to continue to maintain their function in preventing complications of pregnancy and childbirth. With the complete asset mapping, even though there has been a change in leadership in the village, the focus on the use of assets remains unchanged, and may even develop. Community-based model significantly changes the level of knowledge of mothers about complications of pregnancy and childbirth. Knowledge. This result is in line with studies conducted in the United Kingdom, that significantly increased midwife knowledge in midwives who received obstetric emergency training.\cite{22}

When the pre-test was conducted, the respondents’ average knowledge score was only 41.01. This score falls into the category of low knowledge, meaning that less than 50% of knowledge about complications of pregnancy and childbirth is known by respondents. Knowledge about bleeding is the thing that most respondents know. The study conducted in Uganda showed results that were not much different. Only one third of respondents knew at least three danger signs during the pregnancy, childbirth and postpartum phases. Among them most of the respondents answered that bleeding during pregnancy and swelling of the hands and face were danger signs during pregnancy.\cite{23} Knowing the signs and symptoms of pregnancy and childbirth complications will make respondents anticipate and prevent more to mitigate the impact of complications of pregnancy and childbirth by reducing the first three late and late third if the health facility is ready to deal with complications.\cite{23}

The introduction of obstetric danger signs is the main key in finding health services for obstetric emergencies and in seeking prevention or promotion efforts during pregnancy and childbirth. So that the lack of awareness of danger signs will be related to the lack of preparation for normal delivery and preparedness in the face of complications.\cite{24} Assuming that all pregnancies are at risk, the mother must be aware of the danger signs of complications of pregnancy, childbirth and postpartum. Efforts to increase this knowledge can be done through health promotion, both verbally, written and audio. One of the most recent approaches to increasing knowledge and delivery outcomes is Centering Parenting.\cite{25} Centering Parenting is a model that involves groups of 6-7 mothers and babies together to get care for the first year. Through nine sessions, medical staff provide care and also provide information about health, infant development and issues of maternal and infant safety. This program integrates three main components of care, namely health, education and support in groups.\cite{26}

**Conclusion**

The construction results of the community-based model state that variables of community participation, community assets, knowledge, attitudes and behaviors to prevent pregnancy and childbirth complications
are issues / discourses obtained based on qualitative findings. This result is in line with the basic concept of this research. The variable forming construct on the model is in accordance with the theory that applies to the present. The construction of the community-based model consists of community participation which consists of knowledge about prevention of complications of pregnancy and childbirth, attitudes about prevention of complications of pregnancy and childbirth, and behaviors about preventing complications of pregnancy and childbirth. The results of the development of the community-based model show that all variables have a significant relationship with the prevention behavior of complications of pregnancy and childbirth. At the time of validity testing, it is known that social assets are invalid in forming community assets. Likewise for the right construct is invalid in forming behavioral variables preventing complications of pregnancy and childbirth. The community-based model is applied through the process of mentoring pregnant women by cadres. This assistance is carried out starting from the second or third trimester of pregnancy until the mother gives birth. As a tool to assist pregnant women, cadres are equipped with a mentoring module to prevent the complications of asset-based pregnancy and childbirth and community participation with the community-based model approach. The community-based model was significantly able to increase the score of knowledge, attitude and behavior to prevent complications of pregnancy and childbirth. This model is the novelty obtained from this study.

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Funding Source: Self-Funding.

Ethical Clearance: Taken from the Research Ethics Committee of the Faculty of Faculty of Midwifery, Politeknik Kesehatan Kementrian Kesehatan Jakarta III, Indonesia.

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Evaluation of the PaO$_2$/FiO$_2$ Ratio as a Risk Factor for Hypoxemia against Septicemia Mortality in Children who Treated at Dr. Soetomo General Hospital

Yuli Astika$^1$, Arina Setyaningtyas$^2$, Dominicus Husada$^2$, Retno Asih Setyoningrum$^2$

$^1$Pediatric Resident, $^2$Pediatrician and Researcher, Department of Pediatrics, Faculty of Medicine Universitas Airlangga - Dr. Soetomo General Hospital Surabaya, Indonesia

Abstract

**Background:** Severe septicemia can result in impaired oxygen perfusion to the tissues. The PaO$_2$/FiO$_2$ ratio (P/F ratio) is one of the measurement parameters for hypoxemia. This study aims to evaluate the P/F ratio as a risk factor for hypoxemia against septicemia mortality in children.

**Method:** An observational with a prospective cohort design was conducted. Thirty-six patients with septicemia, consisting of 18 patients who living (survivor) and 18 patients who died (non-survivor). The P/F ratio is obtained based on blood gas analysis carried out in the first 24 hours of treatment recorded on medical records and calculated manually. The P/F ratio has an area under the curve (AUC) of 0.83 (95% CI 0.71–0.95) with a cut-off of 226. Septicemia children with P/F ratio < 226 who have a mortality risk of up to 6.9 times (RR 6.9; 95% CI 1.719-27.957; $p=0.005$) with sensitivity 72.70%; specificity 72.20%; PPV 76.19%; NPV 68.42%. The mean P/F ratio in the non-survivor group was significantly lower than the survivor group 161.60 (95% CI 1.05) compared to 391.09 (95% CI 2.13); $p=0.005$).

**Conclusion:** The P/F ratio can be a risk factor for hypoxemia on septicemia mortality.

**Keywords:** Septicemia; P/F ratio; children; hypoxemia

Introduction

Septicemia is a complex and multifactorial syndrome that occurs due to the body’s response to infections that tend to be harmful, dangerous, or destructive. Untreated septicemia will progress to severe septicemia.$^1$ Currently, septicemia is associated with high morbidity, mortality, and care costs, and is the leading cause of infant and child mortality worldwide.$^2$ The high mortality in septicemia, often caused by delay in diagnosis and treatment.$^3$ In severe septicemia or septic shock, there is impaired oxygen perfusion to the tissues. The result of hypoxemia will affect outcomes such as length of stay, mortality, and morbidity. Based on the medical records at the Dr. Soetomo General Hospital (2015), septicemia in children was recorded as 124 cases, while in 2016 and 2017 there were 52 and 109 cases.$^4$ The number of deaths in PICU Dr. Soetomo General Hospital in 2018 with 23 cases of severe septicemia (35%) and 16 cases of septic shock (25%).$^5$ Therefore, it is necessary to recognize signs of hypoxemia both clinically and in laboratories examination. The diagnostic criteria for acute respiratory failure in children are varied and inconsistent. The PaO$_2$/FiO$_2$ ratio (P/F ratio) is one of the measurement parameters for hypoxemia.$^6$ This study has validated the cut-off value for the P/F ratio which can be
used as a predictor of septicemia mortality. Until now, research on the P/F ratio as a risk factor for hypoxemia on septicemia mortality has not been widely used. The examination of the P/F ratio is considered to be more accurate and easily available, allowing the application of appropriate therapy. This study was conducted to evaluate the P/F ratio as a risk factor for hypoxemia against septicemia mortality in children.

**Materials and Methods**

This study was an analytical observational study with a prospective cohort study design to analyze the role of the PaO2/FiO2 ratio (P/F ratio), as a risk factor for hypoxemia on septicemia mortality in children treated at Dr. Soetomo General Hospital, Surabaya. The sample of the study were pediatric patients with septicemia who were treated in the pediatric intensive care unit (PICU), emergency department, and pediatric ward at Dr. Soetomo General Hospital Surabaya in the period time from March 1st – September 30th 2020. Thirty-six patients met the inclusion and exclusion criteria with living (survivor) septicemia and 18 patients have died (non-survivor). We used a primary data based on the supporting examinations carried out during the treatment at Dr. Soetomo General Hospital Surabaya. The data collection instrument used in this study was the data collection sheet (DCS). The data from each examination result is confirmed to be complete and relevant first before further processing. The examination was carried out on incomplete and less relevant data. The demographic characteristics of participants were analyzed using T independent test. The correlation test between variables presented in cross-tabulation was analyzed using the Chi-Square test. The cut-off value is obtained using the receiver operating characteristic (ROC) curve. Statistical analysis using Chi-Square test, the significance value \( p < 0.05 \). The collected data were analyzed using the IBM SPSS Statistic program.

**Results and Discussion**

The basic characteristics of the participants were presented based on the basic characteristics of gender, age, Pelod II score. Meanwhile, for laboratory characteristics, various parameters were assessed from the results of blood gas analysis and complete blood counts such as PCO2, PaO2, SO2, FiO2, AaDO2, Hb, and leukocytes. The basic characteristics of the groups are listed in Table 1 and Table 2.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Survivor (N = 18)</th>
<th>Non-survivor (N = 18)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>10 (47.6)</td>
<td>11 (52.4)</td>
<td>0.726</td>
</tr>
<tr>
<td>Female (%)</td>
<td>8 (53.3)</td>
<td>7 (46.7)</td>
<td></td>
</tr>
<tr>
<td>Age of months (mean ± SD)</td>
<td>58.5 ± 55.1</td>
<td>87.4 ± 80.51</td>
<td>0.258</td>
</tr>
<tr>
<td>Pelod II Score (mean ± SD)</td>
<td>10.83 ± 2.149</td>
<td>12.55 ± 1.969</td>
<td>0.021*</td>
</tr>
</tbody>
</table>

*a p-value < 0.05 was statistically significant.*
Table 2. Laboratory characteristics of participants in the septicemia group survivor and non-survivor

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Survivor (N = 18)</th>
<th>Non-survivor (N = 18)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>PaO2</td>
<td>121.67 ± 75.179</td>
<td></td>
<td>89.14± 53.311</td>
</tr>
<tr>
<td>FiO2</td>
<td>26.00 ± 10.765</td>
<td></td>
<td>41.91± 22.320</td>
</tr>
<tr>
<td>PaCO2</td>
<td>48.83 ± 26.206</td>
<td></td>
<td>50.95± 31.029</td>
</tr>
<tr>
<td>SO2</td>
<td>91.83 ± 11.242</td>
<td></td>
<td>87.18± 11.603</td>
</tr>
<tr>
<td>AaDO2</td>
<td>90.72 ± 53.685</td>
<td></td>
<td>139.55± 75.503</td>
</tr>
<tr>
<td>Hb</td>
<td>10.37 ± 3.182</td>
<td></td>
<td>10.20± 2.835</td>
</tr>
<tr>
<td>Leukocyte</td>
<td>15993.89 ± 8410.961</td>
<td></td>
<td>16971.82 ± 9777.629</td>
</tr>
</tbody>
</table>

*a p-value < 0.05 was statistically significant.

The highest gender in the survivor and non-survivor septicemia group was male. The mean age in the survival septicemia group was 58.5 months while in the non-survivor group was 87.4 months. The mean Pelod II score was higher in the non-survivor group with p-value < 0.05. The number of AaDO2 and FiO2 in septicemia patients who non-survivor was significantly higher than in survivors with p-value < 0.05. The number of FiO2 (41.91 vs 26.00) had a relevant and significantly higher increase found in the septicemia patients who non-survivor with a p-value of 0.020. Meanwhile, for the values of PaO2, PaCO2, SO2, Hb, and leukocytes, there were no significant differences were found in non-survivor and survivor. A total of 36 patients with septicemia divided into 18 survivors and 18 non-survivors with the P/F ratio was calculated as shown in Table 3.

Table 3. P/F Ratio value of survivor and non-survivor septicemia group

<table>
<thead>
<tr>
<th>P/F Ratio</th>
<th>Outcome</th>
<th>p</th>
<th>RR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt; 226)</td>
<td>Survivor N (%)</td>
<td>Non-survivor N (%)</td>
<td>0.005*</td>
</tr>
<tr>
<td>High (&gt; 226)</td>
<td>13 (60)</td>
<td>6 (40)</td>
<td>(1.719 -27.957)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>391.09 ± 2.13</td>
<td>161.60 ± 1.05</td>
<td></td>
</tr>
</tbody>
</table>

*a p-value < 0.05 was statistically significant.

The results of this study showed a significant difference in the value of the P/F ratio (161.60 ± 1.05 vs. 391.09 ± 2.13; p = 0.001) between survivor and non-survivor septicemia patients as shown in Table 3. In patients with septicemia, a low P/F ratio value is significant for the occurrence of death, meaning that the lower the P/F ratio value, the risk of death is 6.9 times higher than those with a high P/F ratio value as shown in Table 3.
P/F ratio in septicemia has an area under the curve (area under curve / AUC) of 0.83 (95% CI 0.71-0.95). The cut-off reference value P/F ratio of 226 has a sensitivity of 72.70%, specificity of 72.20%, a positive predictive value of 76.19%, and a negative predictive value of 68.42% (Figure 1 and Figure 2).

Figure 1. Area under curve P/F ratio to mortality in septicemia patients

Figure 2. The cut off P/F ratio in septicemia
It was found that the number of males (52.5%) was greater than that of females (47.5%) in both the survivor and non-survivor septicemia group. Following the study of Zhou J et al. (2014) in China regarding the features and prognosis of severe septicemia and septic shock in the ICU saying that 60.4% of the study subjects were male. This is influenced by the female hormone estrogen which has a protective effect against infection, septicemia, and trauma. A study in Bandung found that in the case group, 50 (51%) subjects were male and 48 (49%) female subjects. Meanwhile, in the control group, 66 (66.7%) were male and 34 (33.3%) were female. There was no significant difference between the two sex groups.8

Many studies show that gender has a role in infectious diseases and septicemia. The female sex has shown to be protective against septicemia, whereas the male sex, on the other hand, interferes with cell-mediated immune responses and cardiovascular function. Male sex hormones, namely androgens, have been shown to suppress cell-mediated immunity. In contrast, female sex hormones exhibit a protective effect that may contribute to naturally benefiting women in septic conditions. The hormone estrogen affects increasing the immune system by increasing the production of IL-4 and IL-10 so that antibodies increase.9

A study in China on 461 children reported that the age of less than 1 year had the greatest percentage of 50.5% who were admitted to the PICU.10 This occurs because the responses of macrophages and other innate immune systems such as mucosal cells, skin cells, cilia, and acute-phase protein formation have decreased and the number of T lymphocytes is decreased due to thymus reabsorption. The median ages of cases and controls were 12 and 24 months.11

The number of AaDO2 and FiO2 in septic patients who died was significantly higher than in living septicemia patients with p-value <0.05. The number of FiO2 (41.91 vs 26.00) had a relevant and significantly higher increase found in the septic patients who died with a p-value of 0.020. Meanwhile, for the values of PaO2, PaCO2, SO2, Hb, and leukocytes, there were no significant differences were found in non-survivor and survivor septicemia patients.

The results of this study indicate a decrease in the P/F ratio value in patients with non-survivor septicemia compared to living septicemia. The mean PaO2/FiO2 ratio in this study was 161.60 in non-survivor septicemia and 391.09 in survivor septicemia. The decrease in the P/F ratio occurs due to a mechanism of impaired oxygen perfusion to the tissue. Hypoxia tissue condition will cause metabolism to be ineffective due to anaerobic metabolism and accumulation of lactate resulting in cell dysfunction and destruction which leads to multi-organ failure and death.12,13

The above studies show that the excessive release of proinflammatory cytokines triggers the release of vasoactive amines and chemokines as well as activation of the complement system, coagulation, and release of reactive oxygen species (ROS). These mediators are responsible for increased vascular permeability, hypotension, and septic shock. In an advanced stage, the release of a mediator such as high protein group box 1 (HMGB1), which allows the inflammatory reaction to being continued. Since O2 is the final receptor for electrons in the electron transport chain, measuring oxygen consumption is a good choice for assessing mitochondrial function. The peripheral blood of septic patients shows normal PO2 levels although the oxygen consumption by the cells may be reduced, which can lead to tissue hypoxemia followed by tissue damage and ultimately organ system failure. Multi-organ damage is the cause of death.14,15

P/F ratio is the partial pressure of arterial oxygen (PaO2 in mmHg) to fractional inspired oxygen (FiO2 is expressed as a fraction, not %) so that PaO2/FiO2 is commonly known by the abbreviation P/F Ratio. P/F Ratio is used to determine hypoxemia, although there are still several things that need to be reviewed regarding its function as a basis for diagnosis.16 The PaO2/FiO2 ratio (P/F ratio) is a commonly used oxygenation measurement tool. The normal P/F ratio is 300-500
mmHg, with values less than 300 mmHg indicating abnormal gas exchange and values less than 200 mmHg indicating severe hypoxemia.\textsuperscript{17}

The severity of hypoxemia classified according to the Berlin criteria was associated with an increase in mortality in adult patients, namely 27\% (95\% CI 24-30\%) in mild hypoxemia, 32\% (95\% CI 29-34\%) in moderate hypoxemia and 45\% (95\% CI 42-48\%) for severe hypoxemia with \textit{p}-value <0.001. The severity of hypoxemia was also associated with an increase in the mean duration of mechanical ventilation in the survivor group, namely 5 days (interquartile range/ IQR 2-11) in mild hypoxemia, 7 days (IQR 4-14) in moderate hypoxemia, and 9 days (IQR 5-17) in severe hypoxemia with \textit{p}-value <0.001. The value of the PaO$_{2}$/FiO$_{2}$ ratio has an area under the curve (area under curve/ AUC) of 0.577 (95\% CI 0.561-0.593) as a predictor of mortality.\textsuperscript{18,19}

A study by Rice et al in patients with acute respiratory distress syndrome (ARDS) has the most common causes of being septicemia, pneumonia, and trauma, found a mortality rate of 53.0\% at a PaO$_{2}$/FiO$_{2}$ ratio <100, a mortality rate of 39.8\% at 100<PaO$_{2}$ <200, the mortality rate was 39.8\% at 200<PaO$_{2}$/FiO$_{2}$<300, and the mortality rate was 16.75\% at PaO$_{2}$/FiO$_{2}$ >300 \textit{(p = 0.064)}. The results of the insignificant relationship between the PaO$_{2}$/FiO$_{2}$ ratio and the incidence of death can be due to the PaO$_{2}$/FiO$_{2}$ ratio data obtained from this study all above 100 mmHg /%, while according to Viviani et al, the PaO$_{2}$/FiO$_{2}$ ratio can only be used as a predictor of mortality if it is below that 100 mmHg /%.\textsuperscript{7,20}

Hypoxemia is defined as a decrease in the partial pressure of oxygen in the blood. Hypoxemia does not necessarily indicate tissue hypoxia. This can be caused by hypoventilation, ventilation-perfusion mismatch, right-to-left shunt, diffusion disturbance, or reduced inspired oxygen pressure.\textsuperscript{21} Arterial (PaCO) and alveolar (PACO) carbon dioxide pressure increase during hypoventilation, which causes alveolar oxygen pressure (PAO) to decrease. As a result, the diffusion of oxygen from the alveoli to the pulmonary capillaries is decreased, resulting in hypoxemia and hypercapnia. The results of the P/F ratio prognostic test in this study also showed that the cut-off P/F Ratio reference value of 226 had a sensitivity of 72.70\%, a specificity of 72.20\%, a positive predictive value of 76.19\%, and a negative predictive value of 68.42\%. In this study, the increase in mortality rate was inversely proportional to the degree of hypoxemia based on the PaO$_{2}$/FiO$_{2}$ ratio in this study, namely the lower the PaO$_{2}$/FiO$_{2}$ ratio, the higher the mortality rate.\textsuperscript{22}

Rice et al reported the cut-off ratio of PaO$_{2}$/FiO$_{2}$ 300 AUC 0.878 with a specificity of 56% with positive predictive value and negative predictive value were 2.06 (95\% CI 1.64-2.76) and 0.16 (95\% CI 0.12-0.21). PaO$_{2}$/FiO$_{2}$ ratio of 200 specificity 85\% with positive predictive value and negative predictive value of 5.64 (95\% CI 4.69-7.08) and 0.17 (95\% CI 0.15-0.20).\textsuperscript{7}

This study has validated the cut-off point for the SaO$_{2}$/FiO$_{2}$ ratio which can be used as a substitute for a prognostic predictor of severe septicemia when the PaO$_{2}$/FiO$_{2}$ ratio is not available.\textsuperscript{7} Perez et al investigated that the S/F ratio can be used in the assessment of respiratory distress and as a predictor of mortality in patients with septicemia in the ICU and has a correlation with the P/F ratio.\textsuperscript{6} Laila et al found that a low S/F ratio is associated with mortality and has a good correlation with the P/F ratio. The cut-off of S/F ratio <300 indicates high specificity of mortality prognosis.\textsuperscript{23}

Data on the first-day in septicemia patients who are non-survivor showed a low P/F ratio. Meanwhile, survivor septicemia patients showed a higher P/F ratio, but we still need further study, because the patient’s condition is certainly supported by many factors. In this study, the relationship between comorbid patients and the changes in the value of P/F ratio was not examined. However, the data showed that comorbid patients compared to a lower P/F ratio than non-comorbid patients, so indirectly there is a possibility that the patient’s comorbidities can influence the decrease in P/F ratio.

This study is the first study to investigate the P/F ratio as a risk factor for hypoxemia to septicemia.
mortality in children at Dr. Soetomo General Hospital. While the weakness of this study is the blood test. The blood gas analysis was done once when the patient was first diagnosed with septicemia (blood retrieval was only one observation). In this study, first-day data in septicemia patients who died showed a low P/F ratio. While in septicemia life shows a higher P/F ratio value, but still needs further evaluation, because of the patient’s condition is certainly supported by many factors. Many things can affect the level of P/F ratio in a person, be it heart, lung, and other abnormalities. We are aware that this study did not assess some aspects regarding the relationship between the comorbidity of the patient and the changes in the value of the P/F ratio. But, we believe that this study provides valuable information which help the health personnel treating the septicemia in children.

Conclusion

The cut-off value of the PaO₂/FiO₂ (P/F ratio) as a risk factor for hypoxemia on mortality in children with septicemia was 226. The lower the P/F ratio, the relative risk of death in children with sepsis is 6.9 times higher than children with sepsis who have higher P/F ratio.

Acknowledgment: We would like to thank our teacher Prof. I Dewa Gede Ugrasena with his permission we were able to carry out this research properly. We also appreciate the help of nurses and residents who give the support and warm welcome to the authors.

Ethical Clearance: We obtained an approval of whole project from Ethical Committee Review Board of Dr. Soetomo General Hospital Surabaya. The Ethical Clearance has issued by the Clinical Research Unit of Dr. Soetomo General Hospital Surabaya, Indonesia number 1852/KEPK/III/2020.

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References


Tuberculous Pneumonia as a Primary Cause of Acute Respiratory Distress Syndrome: A Case Report

Yuliza¹, Helmia Hasan²

¹Resident, ²Lecturer, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

Abstract

Background: Tuberculosis is an uncommon and very rare cause of Acute Respiratory Distress Syndrome. Moreover, it has been associated with poor outcomes with a very high mortality rate (40-80%). The clinical symptoms and radiological findings of tuberculous pneumonia that resemble bacterial pneumonia result in difficulties to decide definitive diagnosis. Case Presentation: A 35-year-old male patient was admitted to hospital with shortness of breath, fever, cough without expectoration. The patient had history of asthma since childhood. Chest physical examination showed rhonchi and wheezing on both lungs. Initial laboratory findings were leucocytosis, granulocytosis, eosinopenia, increased ALT and ALP levels, blood gas analysis revealed acute respiratory failure. PaO2/FiO2 was 49 mmHg. ECG showed sinus tachycardia and chest X-ray showed consolidation on both lungs. The patient was initially managed for asthma exacerbation and bilateral pneumonia with ARDS. The result of microbiological findings showed acid fast bacilli (AFB) positive. He had been treated with anti-TB first category. Clinical improvement was achieved after 1 week of anti-TB treatment. Summary: Tuberculous pneumonia with ARDS is a rare case of pulmonary tuberculosis. The diagnosis is established by a prompt and complete examination that lead to optimal treatment with appropriate anti-TB.

Keywords: tuberculous pneumonia, tuberculosis with acute respiratory failure, tuberculosis with ARDS

Introduction

Tuberculosis (TB) is one of the major health problems in the world, especially in developing countries¹. The disease usually has a slow onset and progression. Tuberculosis patients usually experience coughing, weight loss, anorexia, night sweats, and malaise, which occur for several weeks before getting sick. Tuberculosis with pneumonia and acute respiratory distress syndrome (ARDS) is very rare². Data on TB patients with pneumonia was 10% of community-acquired pneumonia (CAP) cases in Asia³. Meanwhile, TB with ARDS was reported to account for 1.5-5% of all pulmonary TB cases⁴. Based on the description above, we would like to report a case of a 35-year-old man who was diagnosed with TB with symptoms of pneumonia and ARDS.

Case Presentation

A 35-year-old man with main complaint of shortness of breath for the past 3 days before being admitted to hospital. Shortness of breath occurred in the early hours of the morning and decreased slightly after using fenoterol and salbutamol sprays. The patient also complained of fever for the past 1 day and cough
with phlegm since 1 week before being admitted to the hospital. There was no weight loss and night sweats. The patient had a history of asthma since the age of 10 and he had never received TB treatment before.

The results of physical examination showed 124 pulse per minute, the respiratory rate was 34 beats per minute, the oxygen saturation was 92% using a non-rebreathing mask of 10 lpm oxygen, and an axillary temperature of 39.1°C. Chest physical examination showed vocal fremitus increased on 2/3 upper of both lung, percussion was dull in 2/3 upper of both lung, bronchovesicular sound in 2/3 upper of both lung, rhonchi in 1/3 lower of right lung and 2/3 lower of left lung, and wheezing in 1/3 upper of both lung. The laboratory examination results showed leukocytosis (14.11/mm³), Granulocytosis (94.3%), decreased lymphocytes (3.7%), decreased eosinophils (0.4%), increased SGOT (181 U/L), increased SGPT (155 U/L), hypoalbuminemia. (3.02 g/dL), negative HIV test. The results of blood gas analysis showed respiratory failure with PaO2/FiO2 49 mmHg. Echocardiography results showed neither volume overload nor acute heart failure. Chest X-ray revealed extensive consolidation in both lung fields (Figure 1A).

The patient was diagnosed with severe pneumonia, severe attacks acute exacerbation of moderate persistent bronchial asthma with ARDS with a differential diagnosis of Allergic Bronchopulmonary Aspergillosis (ABPA) and planned to install a ventilator but the care giver refused. The patient was given nebulized therapy for asthma and empiric antibiotics in the form of Ceftazidine 1000 mg intravenously every 8 hours and Levofoxacine 750 mg intravenously every 24 hours. The day-6 evaluation showed leukocytosis (16.06/mm³), granulocytosis (90.7%), lymphocytopenia (5.4%), eosinopenia (0.4%), anemia (9.0 gr/dL), procalcitonin 4.49 ng/mL. The results of blood gas analysis showed respiratory failure with PaO2/FiO2 49 mmHg. Echocardiography results showed neither volume overload nor acute heart failure. Chest X-ray revealed extensive consolidation in both lung fields (Figure 1A).

The patient was subjected to a chest CT scan with contrast on the 7th day of treatment. The results showed a tree in bud pattern accompanied by consolidation with air bronchogram in the right and left lung. These findings suggested a picture of pulmonary tuberculosis that was still possibly accompanied by secondary infection with multiple bullae in the superior lobe of the left lung and the superior lobe of the right lung. Multiple nodules in the inferior lobe of the right and left lung, with the largest size of +/- 0.9 cm in the posterobasal of the inferior lobe of the right lung, could represent a tuberculoma (Figure 2). On the 8th day, the patient’s clinical condition had not improved. The results of the blood gas analysis still showed respiratory failure with PaO2/FiO2 139 mmHg. Antibiotic therapy was changed to Meropenem 1000 mg intravenously every 8 hours and Levofoxacine 750 mg intravenously every 24 hours. The patient was also given adjuvant therapy of methylprednisolone 62.5 mg through intravenous injection every 8 hours. Sputum induction was conducted on the 9th day, with Gram stain examination, BTA examination and Xpert MTB/RIF sputum. On the 13th day, the results of morning scanty sputum BTA, with the result of Xpert MTB/RIF MTB detected very low Rifampin resistant not detected. The patient was then treated with a special liver anti-TB regimen Streptomycin 750 mg through intramuscular injection, Levofoxacine 750 mg through intravenous injection, and Etambutol 1000 mg orally on day 13 of treatment because there was still an increase in ALP (115 U/L) and ALT (230 U/L). On the 20th day of treatment, the patient began to show clinical improvement with a significantly reduced breathlessness. The patient was given 3 lpm nasal cannula oxygen therapy, showing moderate hypoxemic blood gas analysis results. The results of chest X-ray on the day 24 (day 11 using OAT
therapy) (Figure 3) showed improvement in the patient’s condition.

The patient was then subjected to a bronchoscopy to take sample of Broncho Alveolar Lavage (BAL) on the 29th day of treatment, with the result of M. tuberculosis. The patient was discharged from the hospital on the 31st day with 100 mg of INH-sensitized therapy, 750 mg of Levofloxacine and 1000 mg of Etambutol orally.

Figure 1. (a) Chest x-ray on the first day and (b) the third day

Figure 2. Thoracic CT-scan on day 7
Discussion

Several cases of tuberculous pneumonia reported that cough with phlegm occurred prior to respiration and other systemic complaints. The resulting acute respiratory failure also confuses the likelihood of TB because the incidence is very low among hospitalized pulmonary TB patients\(^\text{(5)}\). The diagnosis of tuberculous pneumonia can be made should the patient has a high fever with signs of severe toxicity, thoracic physical examination showing signs of consolidation, the presence of consolidation of a minimum area in one lobe, and microbiological examination of sputum revealing tuberculous puncture\(^\text{(6)}\). ARDS in TB patients is an extremely rare occurrence\(^\text{(7)}\). ARDS criteria include onset within 1 week of known clinical insult, or deteriorating or recent respiratory system, SpO\(_2\)/FiO\(_2\) ≤315 with SpO\(_2\) ≤97\%, features of bilateral opacity evaluated by ultrasonography or chest X-ray should available, respiratory failure unrelated to heart failure or fluid overload, the need of objective assessment such as echocardiography to rule out hydrostatic edema in the absence of risk factors\(^\text{(8, 9)}\).

Anti-TB drugs are therapy given to TB patients with a combination of at least 4 types of drugs at a dose in accordance with the patient’s body weight. Treatment requires a minimum of 6 months in category 1, given in 2 stages, namely the intensive stage (2 months HRZE) and the advanced stage (4 months HR) to prevent recurrence and resistance\(^\text{(10)}\). Patients with a threefold increase in liver enzymes before starting anti-TB therapy can be given a special liver regimen according to WHO guidelines (2HRES/6HR or 2SHE/10HE). Tuberculosis is rarely considered a diagnosis should the patient has features of acute pneumonia and respiratory failure. This can lead to the use of antibiotics that have anti-TB therapeutic effects such as fluoroquinolones which temporarily improve clinical conditions, increase the resistance of fluoroquinolones used as monotherapy, thereby accelerating the occurrence of XDR and pre-XDR cases\(^\text{(2)}\).

Tuberculous pneumonia therapy is similar to TB therapy in general. Corticosteroids are used as adjuvant therapy in TB treatment to overcome this inflammatory reaction \(^\text{(11)}\). Corticosteroids work by inhibiting the release of cytokines that play a role in constitutional symptoms and tissue damage. In addition, corticosteroids make it easier for anti-TB drugs to enter the granuloma and destroy mycobacterium tuberculosis. The use of corticosteroids can make patients more susceptible to

![Figure 3. Chest x-rays on day 24 and day 11 using anti-TB drugs show improvement.](image)
other infections\(^{(3, 11, 12)}\). The benefits of corticosteroids in tuberculous pneumonia with acute respiratory failure have been reported in several studies. Tuberculous pneumonia patients with acute respiratory failure who received corticosteroid therapy showed lower mortality than those who did not receive corticosteroid therapy (56.7% vs 77.8%)\(^{(9)}\). The prognosis of tuberculous pneumonia patients has improved with the use of corticosteroids, in which corticosteroids are a favorable prognostic factor for tuberculous pneumonia patients\(^{(10, 13)}\). Corticosteroids play a role in improving the clinical situation in TB patients when used as adjunctive therapy\(^{(14, 15)}\).

In conclusion, a 35-year-old man was reported having symptoms of acute pneumonia with ARDS. The 2-week pneumonia therapy showed no improvement. Moreover, the results of chest CT scan with contrast and Xpert MTB/RIF examination of sputum induction detected MTB. The patient was given anti-TB therapy and experienced improvement both clinically and radiologically.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** None.

**Statement of Ethics**

The present case report adhered to the Declaration of Helsinki. Informed consent for publication was obtained from the patient.

**References**


A Comprehensive Study on Homicidal Deaths in a Tertiary
Health Care Institution in Nellore, Andhra Pradesh

Z. Sashikanth
Associate Professor, Department of Forensic Medicine, ACSR Medical College

Abstract

Homicide is a serious crime committed against humans and its detection and solution is important to the entire society. Homicide may be the result of accidental, reckless or negligent acts even if there is no intent to cause harm. The present study was taken up to know the incidence of homicides in the city of Nellore and to determine the trends in committing homicides.

Aim: To study the pattern of homicides and to elucidate the different aspects of homicidal deaths.

Methodology: This was a retrospective study done on alleged homicide cases autopsies done in Forensic Department of ACSR Medical College from March 2019 to February 2020 (1 year). Data was collected from 39 reports with an alleged history of homicide. All the homicide cases for which autopsies were done except for homicide cases which involved death due to rash negligent act. Data was collected from the Postmortem reports of victims of homicide, police inquest reports, medical records and relevant history collected from family members, relatives and friends was studied. Data was entered into MS excel and expressed as frequencies and percentages.

Results: Total autopsies done during the study period from March 2019 to February 2020 (1 year) were 902, of which 39 were alleged homicide autopsies. Majority of the victims belonged to 21-30 age group. Majority of the homicide cases were males. Majority of the deaths were caused by blunt objects (48%). In about 67% of the cases the assailant was a known person. Most of the homicides were family centered and due to quarrels. Early detection and prevention is the need of the hour.

Key Words: Homicide, Autopsy, Blunt and Sharp weapon, negligent act

Introduction

Homicide is the act of one human killing another. A homicide requires only a volitional act by another person that results in death, and thus a homicide may result from accidental, reckless, or negligent acts even if there is no intent to cause harm.

“Homicide is defined as the killing of one human being by the act, procurement or omission of another and the term applies to all such killings whether criminal or not.”

Homicide is prevalent widely almost all over the world. Murder of an opponent for various reasons is one of the oldest tools of power struggles from Stone Age.

Homicide is a large killer globally – in some countries it’s one of the leading cause of death. According to the Global Burden of Disease study, just over 400,000 (405,000) people died from homicide in 2017. The scale of homicide is even more pronounced among younger adults aged 15 to 49 years old.

We see that homicide ranks within the top ten causes of death globally. Globally, 0.7% deaths in 2017 were the result of homicide. Homicide rate is measured as the number of homicide deaths per 100,000 people. It was estimated that around 3.9 deaths occur/100,000 South east Asia according to 2017 statistics. These statistics vary from country to country and region to region.
consistent uptrend both in absolute number of homicides and in homicide rates reflects the general increase in all crimes of violence i.e., homicide, rape, armed robbery, aggravated assault etc.\textsuperscript{7} Explanations for increase in the Incidence of homicides probably due to rapid rise in population, industrialization, internationalization, growing unemployment, stressful life, depression in day-to-day life, lack of harmony in family members, drug addiction and socio-political factors.

The grave implications of homicide make its detection, solution and adjudication, a matter of vital importance to the entire society. The perpetrators’ of a homicide should be promptly identified and apprehended for the good of the society and for doing justice.

Sometimes it may not be possible to find the perpetrators due to complexity of the circumstances & motives surrounding the fatal incident. Unlike other serious criminal offences, homicide is to a large extent an intimate, personal crime where the crime is committed by relatives, friends or enemies. A minor number of homicides are “felony – homicides”.\textsuperscript{8} Therefore, homicide should be taken as a public health issue and emphasis has to be laid on reliable data and surveillance mechanisms, so that we can bring a practical and simple approach to homicide prevention. The pattern of homicide may be a useful indicator of the social stresses in a community and may also provide useful information for the law enforcement agencies. Several workers have tried to elucidate the different aspects of homicidal deaths in various parts of the world and in India also. As there is no previous literature or study on the mortality profile of homicidal cases from this region, the present study was undertaken to study these aspects.

Aim: To study the pattern of homicides and to elucidate the different aspects of homicidal deaths.

Material and Methods

Study design: Retrospective study done for a period of 1 year from March 2019 to February 2020 based on records.

Study setting: Department of Forensic Medicine, ACSR Medical College, Nellore.

Sample size: Data was collected from 39 reports with an alleged history of homicide

Inclusion Criteria: All alleged case of homicides irrespective of age and both genders among whom autopsies were done from March 2019 to February 2020 (1 year).

Exclusion criteria: deaths occurred due to rash and negligent act were excluded.

Data Collection: Postmortem reports of victims of homicide, police inquest reports, medical records if available, crime scene photographs and history collected from family members, relatives and friends were studied to analyze the factors such as Age and Sex of victims, Method of homicide, Weapons used, Motive for such acts, Place of occurrence, Time of occurrence, etc. involved with the homicide. All the homicide cases for which autopsies were done for a period of 1 year from March 2019 to December 2019.B.G. Prasad Socio-economic status classification 2020 was used.

Statistical Analysis

Data was collected from the Postmortem reports of victims of homicide, police inquest reports, medical records. Data was entered into MS excel and expressed as frequencies and percentages.

Permissions were obtained from the Institutional Head (Principal, ACSR Medical College, Nellore) and Institutional Ethics Committee, ACSR Medical College, Nellore for the conduct of the study.

Results: Of the total autopsies done from March 2019 to February 2020 were 902, of which 39 were alleged homicide autopsies (4.3%).

Sex of the Victim: Of the total 39 alleged homicide cases, in 23 (58.9%) cases were males and rest of the cases were female 16(41.1%),(Table 1)

Male-to-female ratio was 1.4:1.
IDENTITY OF VICTIM: The identity of homicide victims was known in 36(92.3%) of cases and in 3(7.7%) of cases the identity was not known. All the 3(100%) unidentified or unknown homicide victims were male.

SOCIOECONOMIC STATUS: With regard to socioeconomic status, majority of homicide victims were from lower and lower middle socioeconomic strata 21(53.8%) followed by middle class 8(20.5%). Only few belongs to upper and upper middle class 7(18%). (Table 1)

MARITAL STATUS: Majority of homicide victims were married 25(64.1%) and 11 (28.2%) were unmarried. In 3(7.7%) of cases the marital status was not known. Among 25 married, 13 were males and 12 were females. Among 11 unmarried victims, 7 were males while 4 were females.

LITERACY: Majority 23(59%) of homicide victims were illiterate whereas 13(33.3%) were literates and in 3(7.7%) the literacy status was not known. Around 10(77) % of literates were males and the rest3(23%) were females. (TABLE: 1)

PLACE OF CRIME: in the present study the most common places of occurrences of crime were deserted and secluded places like roadside, bridge underpass, agricultural fields (43.5%) followed by at house of the victim in 30% of the cases. About 25.8% of the victims were attacked their work place. Majority of the females are victims of homicides occurring at house.

TIME OF CRIME: Most of the crimes took place during the night time 28(71.8%) while 11(28.2%) of the crimes happened at morning time.

IDENTITY OF THE ASSAILANT :(Table 1) In about 29(74.3%) of the total homicides, the assailant was a family member of victim. In 7(18%) of cases, the assailant was a known person/neighbour/friend. In 3(7.7%) of cases the assailant was not related to victim. (Table 1)

DEATH ON SPOT OR HOSPITAL: most of the victims 28(71.8%) died on the spot of the incident due to fatal injuries while rest 11(28.2%) dies while receiving treatment at a hospital.

MOTIVE OF CRIME: The motive of crime was revenge in 2(5.1%), extramarital affairs 5(12.8%), love affair 1(2.5%), financial issues 4(10.2%), property dispute 7(18%), quarrels 14(35.8%) and 3 (7.8%) of crimes were related to dowry in which victims were only females. In 3(7.8%) of cases the motive of crime was not known.

In majority cases 14(35.8%) quarrel was found to be the reason for homicide. Among homicides committed because of quarrel 10 (71.5%) were males and 4 (28.5%) were females. (TABLE:1)

WEAPON USED FOR CRIME:In majority of homicides, the inflicting injuries were caused by blunt weapons 11(28.2%), followed by sharp weapon 9(23.1%).

INJURIES: In majority of homicide victims, the cause of death was found to be head injury 11(28.2%) followed by stab injuries 9(23.1%). Poisoning as cause of homicide death was found in only 6(15.4%) victims and 7(18%) burns and 6(15.4%) are strangulation. (TABLE:1)

AGE OF THE VICTIM: Majority of the homicide victims belonged to 21-30 age group 15(38.5%) followed by 31-40 years 13(33.3%). Figure 1 shows 82% of homicides victims were less than 40 years of age.
TABLE: Different Parameters taken in the Study (Proportions/Percentage)

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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Literate</td>
<td>13(33.3%)</td>
</tr>
<tr>
<td></td>
<td>Illiterate</td>
<td>23(59%)</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>3(7.7%)</td>
</tr>
<tr>
<td></td>
<td>Place of crime</td>
<td></td>
</tr>
<tr>
<td></td>
<td>House</td>
<td>12(30.7%)</td>
</tr>
<tr>
<td></td>
<td>Agricultural fields, roadsides, bridge underpass</td>
<td>17(43.5%)</td>
</tr>
<tr>
<td></td>
<td>Work place</td>
<td>10(25.8%)</td>
</tr>
<tr>
<td></td>
<td>Time of occurrence of crime</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Morning</td>
<td>11(28.2%)</td>
</tr>
<tr>
<td></td>
<td>Night</td>
<td>28(71.8%)</td>
</tr>
<tr>
<td></td>
<td>Evening</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Not known</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Identity of assailant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Family member</td>
<td>29(74.3%)</td>
</tr>
<tr>
<td></td>
<td>Neighbor/friend</td>
<td>7(18%)</td>
</tr>
<tr>
<td></td>
<td>Not related</td>
<td>3(7.7%)</td>
</tr>
<tr>
<td></td>
<td>Place of death</td>
<td></td>
</tr>
<tr>
<td></td>
<td>On the spot</td>
<td>28(71.8%)</td>
</tr>
<tr>
<td></td>
<td>At hospital</td>
<td>11(28.2%)</td>
</tr>
</tbody>
</table>
Continued... TABLE 1: Different Parameters taken in the Study (Proportions/Percentage)

<table>
<thead>
<tr>
<th>Motive of crime</th>
<th>Revenge</th>
<th>2(5.1%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Extramarital affair</td>
<td>5(12.8%)</td>
</tr>
<tr>
<td></td>
<td>Love affair</td>
<td>1(2.5%)</td>
</tr>
<tr>
<td></td>
<td>Financial issues</td>
<td>4(10.2%)</td>
</tr>
<tr>
<td></td>
<td>Property dispute</td>
<td>7(18%)</td>
</tr>
<tr>
<td></td>
<td>Quarrels</td>
<td>14(35.8%)</td>
</tr>
<tr>
<td></td>
<td>Theft</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dowry related</td>
<td>3(7.8%)</td>
</tr>
<tr>
<td></td>
<td>Not known</td>
<td>3(7.8%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Injuries</th>
<th>Head injury</th>
<th>11(28.2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stab injuries</td>
<td>9(23.1%)</td>
</tr>
<tr>
<td></td>
<td>Poisoning</td>
<td>6(15.4%)</td>
</tr>
<tr>
<td></td>
<td>Burns</td>
<td>7(18%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weapon used</th>
<th>Blunt trauma</th>
<th>11(28.2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sharp trauma</td>
<td>9(23.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>0-10</th>
<th>0(0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11-20</td>
<td>4(10.2%)</td>
</tr>
<tr>
<td></td>
<td>21-30</td>
<td>15(38.5%)</td>
</tr>
<tr>
<td></td>
<td>31-40</td>
<td>13(33.3%)</td>
</tr>
<tr>
<td></td>
<td>41-50</td>
<td>5(12.8%)</td>
</tr>
<tr>
<td></td>
<td>51-60</td>
<td>1(2.6%)</td>
</tr>
<tr>
<td></td>
<td>&gt;61</td>
<td>1(2.6%)</td>
</tr>
</tbody>
</table>

Figure 1: Distribution of homicides according to age of the victim
Figure 2: Demographic Profile of Homicide cases

Figure 3: Distribution of Assailant-Victim Relationship
Figure: 4 Distribution of Homicidal cases based on Motive of crime

Figure: 5 Distribution of Homicidal cases based on Nature of Injuries
Discussion

In the present study, out of 902 medico-legal cases autopsied at ACSR Medical College, Nellore 39 cases (4.3%) were reported to be alleged homicidal deaths. Whereas in a study done by PrashanthMada et al.9 the percentage of alleged homicide autopsies was 3.24.

The incidence of homicide in the present study was lower when compared to those observed by Murthy et al10 15.1%, Gupta et al.11 7.5%, Sinha et al12 5.9%, Dhaval13 9.13% and B.C. Shiva Kumar et al. (14) 4.76%, whereas it was consistent with studies of Prajapati et al.15 4.12% and O. Gambhir16 2.89%.

In the present study 58.9% of the cases are males and 41.1% are females. In the present study, M:F ratio was 1.4:1. Almost similar findings were also observed in various other studies like Murthy et al10, Prajapati et al15. This is because males are more aggressive, rash and earning members, they have more opportunity for interpersonal interactions. Generally, women and children are spared off even in case of revenge, whereas males are the main target.

In the present study out of 39 victims, 64.1% were married and marital status was not known in 7.7%. The findings are consistent with the studies done by Dhaval13 (69.62%) and O. Gambhir16. In the present study homicides were commonly seen in 21-30 years age group 38.5% followed by 31-40 years of age (33.3%) 41-50 years age group (12.8%). This observation goes in favour that homicides involve the age group, which is in most active and productive period of life. People of 21-30 years age group are violent and more vulnerable to fast changing social trends, ending up in misunderstanding, frustration, hatred, etc. Studies done by Rekhi et al17 and Wahlsten et al.18 reported 31-40 years as the most commonly involved age group.

In this study personal quarrels, extramarital affairs and love affairs, property disputes came out as the major motives behind the act which indicates that the victims were mostly married well into the lives or were getting into the love affairs because of this age. Family disputes, financial burdens, lack of understanding between the spouses and other family members having extramarital affairs are the reasons for very high incidence of homicide among married people when compared to unmarried persons. In the present study majority of the cases (71.8%) occurred during night time. These findings were consistent with those of Dhaval13, CT and Sinha et al.12, whereas Bhupinder S et al.19 and B.C. Shiva Kumar.14 reported the incidence to be more between 6 AM-12 PM. As darkness of night provides favourable conditions and easy execution and escape for the accused, it is more preferred.

Quarrels were the main reason behind majority the homicidal act (35.8%) followed by property disputes (18%), extramarital affairs (12.8%), financial matters (10.2%), Dowry related issues (7.8%) and revenge (5.1%). In 7.8% cases motive was not known.

Contrary to our findings, revenge as a major reason for homicide by Dhaval. CT13 (29.4%), B. C. Shiva Kumar et al.14 (32.5%). In the present study financial matters contributed to only 10.2% of cases whereas financial matters were reported as a major reason for homicide by Sinha et al.12. In the present study, majority of homicides, the inflicting injuries were caused by blunt weapons 11(28.2%), followed by sharp weapon 9(23.1%). The findings are consistent with the studies of Dhaval13 (32.5%) and Prajapati et al15 where blunt weapon was commonest but contradict with the studies of B. C. Shiva Kumar et al14 (50%) and Vij et al20 (49.4%), where sharp weapon was commonest. Easy availability of hard and blunt objects.

In the present study place of occurrence of crime was at deserted and secluded places like roadsides, bridge underpass, agricultural fields, (43.5%) followed by at house in 30% cases. About 25.8% of the victims were attacked at their work place. Similar findings were observed in studies done by Dhaval13 and B. C. Shiva Kumar14 where outdoors was the major site of homicidal act. In the present study most of the victims died on the spot (71.8%). While 28.2% died while receiving treatment at hospital. Higher rate of death on the spot
could be due to occurrence of crime during night time and delay in approaching hospital as most of the crimes happened in secluded places and help was not readily available.

**Conclusion**

Out of 39 homicidal death cases, age group of 21-40 years was mostly affected with a male-to-female ratio of 1.4:1. About 64.1% of the victims were married and most cases of homicide took place during night hours. Quarrel was the most common reason for committing homicide, followed by extramarital affair in second place. Blunt weapon was the commonest weapon used with most of the homicides occurring at places other than the house of victim. Homicide is one of the worst forms of crime as no one has the right to take the life of an individual. Socioeconomic wellbeing, removal of poverty and enhanced employment opportunities will help to check the incidence of homicides. Importance of ethical and moral values should be taught to the children in schools itself, which will help in strengthening of human relationships. Overall, law and order of the state has to be strong quick and rigorous punishment will deter people from committing heinous crimes in future.

**Ethical Clearance:** Taken from Institutional Ethics Committee, ACSR Medical College, Nellore.

**Source of Finding:** Nil

**Conflict of Interest:** Nil

**References**


Aadhar Arogya: Integrated Electronic Health Record as an Aid in Bridging the Gap between Individual Health and Public Health in India – A Review

Haritha R.K1, Jagannatha G.V2, Nagaland T3, Cyril Benedict3

1Dentist, 2Head of the Department, 3Senior Lecturer, Department of Public Health Dentistry, Chettinad Dental College and Research Institute, Kelambakkam, Tamilnadu

Abstract

Aadhar card is an Indian Government ID card that is issued to all Indian residents. It is one of the largest biometric databases in the world with over 1.28 billion enrollments thereby constituting the most ubiquitous ID card in India. With the additional features of QR code and biometric scanning, it forms an ideal database to be linked with the electronic health record. The health records of an individual currently in India is being maintained by the individuals. But most often they are misplaced, disorganized, incomplete or lost. This would force the clinician to assume or ascertain the current medical status by subjecting the individual to tests. Improving the quality of the medical and dental services rendered paves way for an integrated electronic health record. The common concerns arising from such a linkage is data management and data security. Big data management provides a systematic approach to maintaining such large data. The data security concerns can be addressed by employing a data controller in every hospital and a cell to inform the security breach, who can further take up legal penalties. Pseudonymisation, cryptography, audit trial, firewall, antivirus software, and cloud computing are some techniques that can be incorporated.

Keywords: Electronic health record, Aadhar card, Data security

Introduction

Patients seeking dental care often visit a clinic with a disorganized or incomplete set of medical records and sometimes without any past records. Most often the dental clinicians rely on patients’ recall to reveal their past medical illness, which often incomplete. This could result in the clinician taking one of the two possible approaches. Firstly, he/she could assume the medical history given by the patient as complete and true and initiate treatment. Secondly, the clinician could subject the patient to the required tests to ascertain the existing medical condition, which sometimes could be repetition as the patient could not produce the recent reports of the tests, he/she underwent. Moreover, most often these conditions are misreported and this significantly increases with an increase in age [1] and some individuals who don’t deem their medical history important either due to a lack of awareness or just negligence might not report accurately.

Migrant workers and people from low socioeconomic backgrounds face practical difficulty in maintaining such records. Most often they do not seek treatment and if they do seek treatment it is for acute health issues.
Integrated medical and dental records can help close these misreporting gaps and improve the quality of dental services rendered especially to individuals with underlying medical conditions minimize the duplication of resources. The word ‘Aadhar’ means foundation or base while ‘Arogya’ means health. Why the concept of ‘Aadhar Arogya’ could be a one-stop solution to finally bridging the gap between public health and individual health is the objective of this review.

Why the Aadhar Card?

Aadhar card is the Indian Government-issued ID and is fast becoming the Central and State Government’s base for public welfare and citizen services. It assigns a unique 12 digit number to all the Indian residents. It is invariable i.e., it doesn’t change in the lifetime of the individual. It is one of the largest biometric databases in the world with over 1.28 billion enrollments. Linking the medical records with the Aadhar card could improve the quality, accuracy, efficiency of diagnosis & treatment planning and reduce ambiguity in the process. Unique patient identification is a technique for linking patients to their electronic medical records that exist globally in a domain.

1. UNIQUE PATIENT IDENTIFIER (UPI)

The American Society for Testing and Materials (ASTM, 2000) Standard Guide lists desirable attributes of a UPI: unique, non-disclosing, invariable, canonical, and ubiquitous. Unique i.e., one UPI for one individual which cannot be shared, non-disclosing such that it maintains the confidentiality of the individual, and the number does not indicate aspects like name, address or phone number. Invariable as it doesn’t change in the lifetime of the individual; Canonical i.e., an individual is entitled to only one UPI; Ubiquitous i.e., all the individuals have one. Out of all the Indian government-issued IDs, a passport complies with all the desirable attributes except being ubiquitous, since it is voluntary. The other government-issued ID cards like Driver’s license, Voter’s ID require the individual to have a certain set of skills or be of a certain age. The Aadhar card additionally also has biometric and QR scanning options making it an ideal option for linking with the electronic health record.

PAST EXPERIENCES

Since 2013, Aadhar has been linked to several health-related schemes like Janani Suraksha Yojna wherein pregnant women are encouraged to undergo institutional deliveries for a direct cash transfer benefit. Linking of Aadhar card ensures that the right person is benefited from it.

Revised National Tuberculosis Control Programme mandated those who are eligible for financial benefits under the scheme to get their Aadhar authenticated or furnish proof of Aadhar. This is done to ensure validated services via Aadhar enabled direct benefit transfer and improve the scope of surveillance. However, the remaining elements of the scheme like free diagnosis and treatment would continue without interruption. Aadhar linked smart cards are assigned to all the tuberculosis patients undergoing treatment. It acts as a single unique identifier for the patients availing various public & private sector services, social, state & central services to which the patient is entitled during treatment.

Other National health programs, such as the National AIDS Control Program, have started linking their databases with Aadhar as a pilot project in Delhi Reference. This would allow people living with HIV (PLHIV) to have access to various state, central and social schemes ensuing financial benefits transferred to their accounts obviating the need to produce life certificates periodically. However, the intended benefit wasn’t realized as the patients quit the treatment program fearing privacy issues. Recently, the CoWIN app for COVID-19 vaccine registration has started linking the Aadhar card for its registration process. Beneficiary registration, beneficiary verification, Aadhar authentication, vaccination status and reporting of adverse events following vaccination can be carried out with the aid of the app, which helps to keep track without concerns of duplication.
WHAT CAN BE EXPECTED FROM LINKING THE HEALTH RECORD WITH THE AADHAR CARD

It aids in easier delivery & monitoring of primary health care services. Once a patient visits a PHC or any screening camps his/her Aadhar no. can be noted and relevant medical and dental history could be uploaded to the database. The patient can then be counselled & monitored to see if he/she has undergone treatments further - which provides a valuable tool to assess the patient’s health-seeking behaviour and serves as reliable feedback for the services rendered. Hence it bridges the gap between public health & individual health. It can aid in fulfilling the three core functions of public health that is the assessment, policy development and assurance. Public health surveillance is the data collection, analysis and dissemination of data at the appropriate time for preventing and controlling the disease or condition. Establishing public health surveillance classically involves 6 steps that are to establish goals, develop case definition, select appropriate personnel, acquire tools and clearances for collection, analysis and dissemination, implement surveillance system, and evaluate surveillance activities. It can also aid in the development of evidence-based medicine and dentistry which is the intersection of individual clinical expertise, external evidence and value to the patient.

CLINICAL IMPLICATIONS

During times like road traffic accidents & other emergencies wherein the patient is not in a position to relay the medical history, the clinician can gain access to unambiguous & reliable information by scanning the patient’s retina or fingerprint. Inter department coordination could be strengthened, for example, cleft palate surgery in childhood requiring further orthodontic treatment. Tailored diagnosis & treatment planning based on the treatment outcomes, existing health conditions etc. and lifelong monitoring could be made possible.

India is a diverse country with an internal migration of 450 million as per the 2011 census. There is a lack of continuity of treatment, repetition of treatment to be sure, higher recurrence rate due to missed appointments, language & literacy level barriers. All this hinder the migrant population’s wellbeing, who form a significant portion of the population who are often overlooked by state government schemes.

HOW CAN THIS BE ACHIEVED?

1. PILOT QUESTIONNAIRE SURVEY

A pilot questionnaire survey in the maintenance of health record showed that 80% of people have misplaced some or all of their records previously and 70% of people have been asked to repeat a test which was taken elsewhere. This clearly shows that there is a fragmentation of manual health record and an array of scope for Aadhar Arogya to bloom. Furthermore, the questionnaire was framed to assess the willingness to accept a digitalized health record. It showed that only 40% of people were willing to link their medical record to the Aadhar card and the common concerns expressed were issues of security.

2. BIG DATA MANAGEMENT

In India, health data are not digitalized and when digitalized they are not standardized. Big data management offers a systematic approach to this. Big data analysis challenges include capturing data, data storage, data analysis search, sharing, transfer, visualization, querying, updating, information privacy & data source. The data lifecycle involves – plan, collect, assure, describe, preserve, discover, integrate, analyze. It is used in healthcare to deal effectively with a large volume of data both from internal and external sources i.e., from the providers, payers, researchers, consumers & marketers, government and developers. Big data management tools and technologies are either available as open-source or commercial products. Some of the open-source products are the Apache Hadoop framework, NoSQL databases etc. The commercial products include the Hadoop distributed file system.
simultaneously. This is known as parallel processing. [21]

Big data analytics in healthcare are of three types, they are predictive, descriptive and prescriptive. Predictive analytics is used to predict treatment outcomes, descriptive analytics deals with current and past data to make evidence-based decisions. Prescriptive analytics is used to showcase to the policymakers the things to be acted upon. [17]

3. DATA SECURITY

To address the data security concerns of the general public the centralized electronic health record maintenance security regulations of developed nations were considered. Based on these and other electronic health record maintenance suggests that - proper informed consent be taken explaining in simple language regarding the whereabouts of the data shared and for what purpose. [24] A data controller should be assigned to every 250 bedded institutions, who are governed by a data supervisor. Whenever there is a data breach it needs to be informed by the data controller to the supervisor and/ or the legal panel within 72 hours. Based on the scale of breach legal penalties are sentenced. Access to all the sensitive information should be on a need to know basis.

Pseudonymisation or partial anonymization can be done so that with the data as a sole reference one cannot be able to identify a patient. It can be achieved in two ways i.e., encryption and tokenization. Encryption involves the need for a decryption key to access the information. While tokenization is the replacement of sensitive information with non-sensitive substitutes. [22] The GDPR also keeps track of the records of processing activities.

The Health Insurance Portability and Accountability Act 1996 (HIPAA) suggests the use of access control to the patients like passwords and PINs. An individual can ask for an audit trail at any point in time, wherein who, when and what information is accessed can be checked. [25]

The Personal Information Protection and Electronic Documents Act (PIPEDA) uses technological tools which are constantly changed to be up to date like firewalls and security patches. Organizational controls like security clearances, limiting access, staff training and agreements are ensured. Sensitive information can only be accessed on a need to know basis. [23]

Cryptography involves techniques like passwords and digital signature. Other simple techniques involve cloud computing and antivirus software. It is cheap and effective, additionally, cloud computing reduces the burden of maintenance.

Conclusion

The current review suggests that linking the Aadhar card with an electronic health record has the potential to improve the quality and continuity of the treatment. The potential of the Aadhar card has been realized in the recent past and has been linked with various National-level health-related schemes. To expand this comprehensively to cover all the health data under one roof, the current review suggests the need to emphasize data safety laws and regulation before they can be implemented on a large scale. A brief look at the data safety regulation in developed nations suggests many techniques like pseudonymisation, cryptography, audit trial, firewalls, cloud computing, antivirus software and legal penalties. Further research on the implementation in the Indian scenario and addressing problems therein are suggested.

Ethical Clearance: Taken from CARE IHEC, Tamilnadu

Conflicts of Interest: There is no conflicts of interest

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COVID war or KARMA war: Who Deserves Homecoming??
Are We Taught Anonymously the Forgotten Lesson of Bhagavad Gita?

Ashok Kumar Jena¹, Venkatachalapathy Anusuya², Jitendra Sharan³
¹Additional Professor, ²Senior Resident, ³Assistant Professor, Department of Dentistry, All India Institute of Medical Sciences, Sijua, Dumduma, Bhubaneswar, Odisha, India

Abstract

History repeats itself until you learn and correct the mistakes. We all tend to create the future with the same past experiences. Nothing new than the forgotten old. The great Kurukshetra war of Indian Hindu epic the Mahabharata and the Lord’s unsung song Bhagavad Gita from it remind us the same. Although centuries had crossed, the lessons to be learned, and learning from the Gita are the same. Amidst the COVID-19 pandemic, the entire world is battling against the unseen enemy. We are fighting physically, mentally, economically, and socially. Each and everyone is contributing to the maximum to win the battle. But, the invisible enemy, coronavirus is seemingly stronger than the advanced technologies and growth of humanity. This short write up is in search of the true etiology of COVID-19 that came up in the curious mind of a medical researcher who is amazed in the bizarre situation and uncontrolled fight against this unseen enemy.

Key words: Death, COVID-19, KARMA, SARS-CoV-2 virus

Introduction

Only one person had known the “truth”, the truth of actual scenarios in the greatest Kurukshetrawar of Indian Hindu epic the Mahabharata. He was the only one who was given the chance to see the complete incidences of eighteen days war with unbiased eyes; was the one who told very clearly that who killed whom during the entire war; how Pandavasarose to the victory with a homecoming to the golden land (Hastinapur). It was none another but the Barbarika.¹ ²

The battle was between the dharma and adharma i.e. righteous and iniquity. The war was getting ready between the loved ones Pandavas and Kauravas. Even the incredible minds were oscillating to choose the side to fight against, as it was within the family and each one was equally important. Barbarika was not an exception, the grandson of Bhima (second of the Pandava brothers) choose to join the weaker side if need urges. He was famously known as “bearer of three arrows”, as he was blessed with three infallible arrows in a boon.²

Krishna, the descendant of Lord Vishnu stood the side of Pandavas charioting for Arjuna(third of the Pandava brothers). Before the beginning of the war, Krishna asked the warriors that how long will take for them to complete the war individually and Barbarika told that he could finish the entire war in a minute. As he had three arrows, he would use the first arrow to fix whom to be destroyed, the second one to fix whom to be saved, and then third to destroy all the objects marked by

Corresponding Author:
Dr. Ashok Kumar Jena,
Additional Professor, Department of Dentistry
All India Institute of Medical Sciences, Sijua,
Dumduma, Bhubaneswar, Odisha, India.
Mob:+91-9438884077
Email- dent_ashok@aiimsbhubaneswar.edu.in
the first arrow or destroy all the objects not marked by the second arrow. And at the end of this, all the arrows would return to the quiver.1,2

The Krishna was astonished by the infallible power of three arrows. Hence, he asked the Barbarika’s head as a charity, so that the strongest Kshatriya warrior’s sacrifice would help in beginning the war. By doing so, Krishna did not allow the Barbarika to participate in the war; Accepted Barbarika asked Krishna that his last wish was to watch the great battle, so Krishna let his head to watch the whole battle from a mountain top nearby the battleground.2

At the end of the battle, Pandavas won and the time came to dedicate the victory to the responsible person. Each warrior was justifying their points and chaos started. When Barbarika was asked about his view regarding the victory of Pandavas, he explained that it was neither Kaurava’s weakness nor Pandava’s strength. All he could saw in the whole war was that the divine invisible chakra of Krishna, which was spinning all around the battlefield and killed all those who were not on the side of dharma.1,2

Amidst the COVID-19 pandemic, the entire world is battling against a known enemy with an unknown trait. The initial days of COVID-19 were like a bio-war with the highest threat to humanity.3 In Italy, healthcare workers were considered as God who decides who would survive and who would not. Geriatric populations were thought to have a poor prognosis and were triaged under the least preference category for intensive treatment.4 As days moving on, the entire world was infected and different countries came up with different traits of coronavirus.5 The virulence of the virus was changing in terms of mortality rate, and more asymptomatic cases were increased.

In many countries, aged geriatric patients were started to survive by winning the coronavirus infection but many healthy young patients and health care workers have succumbed to the same despite intensive treatment. Aged men were suggested to have high risk but adolescents have also lost their lives. Despite immunity, sex, age, co-morbidity, and many other factors as suggested, no peculiar factor was defined as a predictive factor for mortality, but, we all are losing precious lives. The so-called God, health care workers started failing to hold the lives back. Day by day, this bizarre scenario is moving towards the hype of uncertainty. Thus, the time calls to analyze what factor is responsible for this mortality in the COVID-19 pandemic. Though multifactorial, the uncertainty in the individual risk poses it very difficult for health care workers to predict the outcome.6 Thus; it was not the health care workers who are deciding the lives. It was not the pre-determined co-morbid, risk factors that are choosing the lives. If not them, then what is responsible to win or lose the COVID-19 battle???

Like in Bhagavad Gita, though the Pandavas won the battle, it was not them, who were responsible for the victory. They were mere instruments for the execution of Lord Vishnu’s actions. Pandavas were thinking that they killed the Kauravas. Like we think that the coronavirus is killing the lives. But the Barbarika who had watched the war from outside only knew the truth that it was the divine chakra of Krishna, not those who were participating in the battle. Likewise, being health care workers, government sectors, society, and victims of coronavirus infection, all are battling against COVID-19 selflessly and staying detached from personal emotions and attachments7, we all have succumbed to the thought that the coronavirus is killing the lives and threatening humanity. But who knows, this myth could just be the unproven scientific phenomenon and the karma of individuals that are risking their own lives in the form of COVID-19 infection. The good and bad deeds of individuals are called karma. Like the divine chakra of God, the individual’s karma might be spinning all around this COVID-19 battle choosing whom to survive and whom to leave this earth. The SARS-CoV-2 virus may just be a mere invisible instrument, masking the deeply lying truth. The time will unfold the answers; like the eyes of Barbarika, the eyes of researchers could only tell the truth in the future.
Conflicts of Interest: No

Source of Funding: Self

Ethics Clearance: Not required

References


Important of Morphological Examination of Bone Marrow for a Hematological and Non-Hematological Disorder

Safaa Jassim Mohammed
Researcher, Department of Hematology, Ibn Sina Teaching Hospital, Directorate of Ninava Health, Ministry of Health/Environment, Ninava, Iraq

Abstract

Background: The bone marrow examination is important for the investigation, diagnosis, and management of the blood, bone marrow and many systemic disorders. Morphological Bone marrow examination alone is sufficient to diagnose nutritional anemias and most acute leukemia.

This study aimed to determine the importance of morphological examination of bone marrow to diagnose hematological and non-hematological disorders like various systemic illnesses that affect the bone marrow.

Methods: A prospective study carried out for one year at the Hematology lab, Ninava Ibn Sena Teaching Hospital, A total of 201 cases were investigated. The bone marrow examination was taken from the posterior superior iliac spine.

Results: The age range was (15-87) years. The male-to-female ratio was 1:1.2. The most common disorder finding was acute leukemia 17% (include AML & ALL) and AML is more common than ALL, whereas lymphoproliferative disorders was second bone marrow finding fallowed by Megaloblastic anemia of Myeloid dysplasia syndrome (MDS) and Essential thrombocythemia (ET).

Conclusion: Morphological bone marrow examination is a useful procedure in ascertaining the diagnosis of several hematological and non-hematological disorders.

Keywords: AML (acute myeloid leukemia), ALL( acute lymphoid leukemia), MDS(Myeloid dysplasia syndrome), Essential thrombocythemia (ET).

Introduction

Diagnosis and management of many hematologic diseases depend on bone marrow evaluation. Bone marrow examination usually involves two separate, but interrelated, specimens. The first is a cytologic preparation of bone marrow cells. The second specimen is a needle biopsy of the bone and associated marrow (1). Marrow biopsy is useful for diagnosing and following the course of disorders such as megakaryoblastic leukemia, hairy cell leukemia, and chronic myeloproliferative neoplasms. In myelodysplastic syndromes, marrow biopsy is useful for evaluating abnormal localization of immature precursor cells and abnormal megakaryocytes (2, 3). Satisfactory samples of bone marrow can usually be aspirated from the sternum, iliac crest, or anterior or posterior iliac spines. In most circumstances, the posterior superior iliac spine is the preferred biopsy site and selection of this site has the advantage that, if no material is aspirated, a trephine biopsy can be performed immediately (4).

Corresponding:
Mosul, Ninava, Iraq
rawaqjasim@gmail.com
Zipcode: 41001
**Methods**

A cross-sectional study was conducted in the Hematology lab, Ninava Ibn-Sena Teaching Hospital, from January 2019 to December 2019. Clinical data concerning age, sex, dietary habits, mode of onset, history of drug intake or exposure to toxic chemical agents, H/O bone pains, hepatosplenomegaly, and lymphadenopathy respectively, were recorded. This study excluded patients who are present for assessment response of bone marrow to the treatment of previous diagnosis patients. Amongst all the subjects, peripheral smears, reticulocyte counts, and bone marrow aspiration and biopsy were obtained.

The procedure of BMA was done after giving local anesthesia from the posterior iliac spine. Bone marrow trephine biopsy was performed. Bone marrow examination was done on Leishman stained for smears and Hematoxylin-eosin-stained for trephine biopsy sections.

**Results**

The age group ranged from 15 years to 87 years. Most of the patients are belonged to the 51-60 years old group. 89 of 201 cases were male and 112 cases were female, and male-female-ratio is 1:1.2 (Table 1). The commonest mode of presentation was anemia; other main symptoms were pancytopenia, organomegaly, whereas fever was the rare presentation (Figure 1). Finding of Bone marrow aspiration normal active bone marrow was (29.3%) cases, AML (9%) cases, Megaloblastic anemia (7.4%) cases, Multiple myeloma (7%) cases, CML (5.4%) cases, secondary metastasis (4.4%) cases, erythroid hyperplasia (4%) cases, were plasma cell leukemia, ET and MDS (0.5%) cases for each of them (Table 2).

<table>
<thead>
<tr>
<th>Table 1. Patients features.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
</tr>
<tr>
<td>15-20</td>
</tr>
<tr>
<td>21-30</td>
</tr>
<tr>
<td>31-40</td>
</tr>
<tr>
<td>41-50</td>
</tr>
<tr>
<td>51-60</td>
</tr>
<tr>
<td>61-70</td>
</tr>
<tr>
<td>&gt; 70</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
</tbody>
</table>


Figure 1. Indication of bone marrow examination

Table 2. Bone marrow examination finding:

<table>
<thead>
<tr>
<th>BMA diagnosis</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>59</td>
<td>29.3</td>
</tr>
<tr>
<td>Blood tap</td>
<td>13</td>
<td>6.5</td>
</tr>
<tr>
<td>Lymph proliferative disorder</td>
<td>17</td>
<td>8.4</td>
</tr>
<tr>
<td>Acute leukemia including:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-AML</td>
<td>34</td>
<td>17</td>
</tr>
<tr>
<td>2- ALL</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>3- M3</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>4- M3V</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
<td>11</td>
<td>5.4</td>
</tr>
<tr>
<td>Essential thrombocytethemia</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Erythroid hyperplasia</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Multiple Myeloma</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Megaloblastic anemia</td>
<td>15</td>
<td>7.4</td>
</tr>
<tr>
<td>Hairy cell leukemia</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Secondary metastasis</td>
<td>9</td>
<td>4.4</td>
</tr>
<tr>
<td>Dry Tap ( primary myelofibrosis)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Aplastic Anemia</td>
<td>3</td>
<td>1.4</td>
</tr>
<tr>
<td>Plasma cell leukemia</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hemophagocytic syndrome</td>
<td>7</td>
<td>3.4</td>
</tr>
<tr>
<td>Myeloid dysplasia syndrome</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>201</td>
<td>100</td>
</tr>
</tbody>
</table>
Hematological disorders include a wide range of diseases ranging from reactive hyperplasia to hematological malignancies.

Male to female ratio was 1:1.2 which similar to the study done by Sreedevi et al (5) and Chand et al (6). The majority of the patients in the study belonged to (51-60) years old which similar to the studies done by Joshi et al (7) and Saeed et al (8). The commonest indication in our study was anemia (42%). Similar findings, anemia was the commonest indication in a studies done by Sreedevi et al (5), Saeed et al (8), Kumar et al (9), Timothy et al (10).

Bone marrow examination showed (29.3%) of patients with normal findings, which similar to the study done by Saeed et al (8), which reporting normal bone marrow 29.05% of biopsy. About (70.7%) of bone marrow was pathological and largest hematological malignancy is acute leukemia 17% (included AML, ALL, M3 and M3V) and AML is more common than ALL similar result reported by other studies (11-14).

Lymphoproliferative disorders are the second pathological bone marrow finding followed by Megaloblastic anemia which different from the percentage report by other studies (6, 8, 15), this difference may be due to the difference in sample sizes.

The lowest percentage (only one case) of myeloid dysplasia syndrome and Essential thrombocythemia was diagnosed in similar reported by the study done by Shah et al (8, 16, 17).

**Conclusion**

Morphological examination of bone marrow aspirate is important for the diagnosis of many diseases hematological disorders like leukemia, lymphoma, multiple myeloma, and myeloproliferative neoplasm, and non-hematological diseases like the cause of anemia, thrombocytopenia, and pancytopenia.

**Conflict of Interesting** - Nil

**Source of Funding** - Self

**Ethical Clearance** – Taken from Ninava Ibn-Sena Teaching Hospital committee.

**References**

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Statistic Assessment of Spreading Cases (Total Confirmed, Recovered, Death) From Different Areas Contain Novel Coronavirus (2019-nCov)

Huda Sahib Abdul Mohammed Al-Rawazq

Assistant Professor, Department of Anatomy-Biology Section, College of Medicine, University of Baghdad, Baghdad, Iraq

Abstract

Background: In Wuhan, Hubei Province, China 31, December 2019 were discovered several cases of pneumonia of unknown causation and correlated to Wholesale Market Huanan Seafood. And caused acute viral respiratory diseases called Coronavirus Disease 2019 (COVID-19).

Aim: The aim of this study is statistic assessment of cases (total confirmed, recovered, death) from different areas in world, Arab nation and in Iraq contain 2019-nCoV.

Methods: The first study include collected data from Center for System Science and Engineering (CSSE) at John’s Hopkins University and Iraqi Ministry of Health website daily reports from 29th February to 31th December 2020 of people infected with 2019-nCoV.

Results: From this statistics the prevalence of 2019-nCoV the spreading cases (83,561,252 total confirmed, 47,136,188 global recovered and 1,820,668 global death) from 191 countries in the world. The most distribution confirmed cases represent in (19,975,250 US, 10,286,709 India, 7,675,973 Brazil, 3,153,960 Russia, 2,677,806 France, 2,496,231 United Kingdom, 2,208,652 Turkey, 2,107,166 Italy). Also, global recovered represent in (9,883,461 India, 6,848,844 Brazil, 2,553,467 Russia, 2,100,650 Turkey, 1,508,419 Colombia, 1,463,111 Italy, 1,426,676 Argentina, 1,355,384 Germany). And global death represent in (345,844 US, 194,949 Brazil, 148,994 India, 125,807 Mexico, 74,159 Italy, 73,622 United Kingdom, 64,760 France, 56,798 Russia). From this result the Arab nation including 3,139,205 total confirmed cases, 2,783,448 recovered cases and 55,326 death cases from 21 countries infected with 2019-nCoV between this result the Iraqi infected patients represent 595,291 total confirmed cases, 537,841 recovered cases and 12,813 death cases.

Conclusions: In concluded, the percentage of 2010-nCoV increase in all the global confirmed cases in different areas of the world and become pandemic. In Iraq show the infection is mild to moderate in spite of no special antiviral and vaccine is present.

Key Word: 2019 Novel Coronavirus (2019 n-CoV), Cases (infection, recovered, death), different countries, Pandemic.

Introduction

In Wuhan, Hubei Province, China 31, December 2019 were discovered several cases of pneumonia of unknown causation and correlated to Wholesale Market Huanan Seafood1. And caused acute viral respiratory diseases called Coronavirus Disease 2019 (COVID-19)2. Began, in Wuhan and rapidly diffused into different area of China, many factors were accountable for this fast diffuse of the virus to various groups like; different route of infection, virulence of virus and peoples capability3. World health organization (WHO) were pronounced as a universal public health emergency because, the type of pneumonia caused by the COVID-19 is continued
outbreak and a highly contagious. Declared on March 11, 2020 as a cosmopolitan pandemic. In the past two decades that has emerged the third coronavirus causing multinational outbreaks and transferring substantial mortality and morbidity. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was named by International Committee on Taxonomy of Viruses (ICTV), which is a novel strain of human coronaviruses. Coronaviruses belong to the subfamily Coronavirinae, stranded RNA viruses are single positive, enveloped, the length of genome ranging from 26 to 32 kilo-bases, possibly known the largest viral RNA. The four genera of subfamily Coronavirinae consists of: Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus. The infectious only to mammals are Alphacoronaviruses and Betacoronaviruses genera while infectious to birds are Gammacoronaviruses and Deltacoronaviruses, but also some of them can infect mammals.

Also have been known in many avian hosts. Coronaviruses in various animals may cause infections in multiple systems, and in humans cause mainly respiratory tract infections. Even though most coronavirus infections in human are mild, the two betacoronaviruses epidemics, Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus (SARS-CoV), have caused in the past two decades accumulative cases more than 10,000, with 37% mortality ranges for MERS-CoV and 10% for SARS-CoV. At the same time, the transmission human-to-human was confirmed and staff caring for patients were reported infections with 2019-nCoV in hospital. And mainly spread through respiratory droplets or close contact, latest suggest possibility of oral-fecal route transmission. Transmission of droplet may occur out of tears, close contact and fomites around the infected person by immediate environment. The range of incubation period was 1 to 14 days with average five days and experience signs within 11 to 12 days approximately 95% of contact patients. During the incubation period when patients show without symptoms is believed the novel coronavirus may be infectious. The potential sources of SARS-CoV-2 infection is asymptomatic transporters. The most popular symptoms of the COVID-19 which are like to that of MERS-CoV and SARS-CoV infection: fever, dry cough, fatigue, lethargy and muscle pain. The clinical presentation range from mild to severe, but the high case-fatality rate show in elder patients or comorbidities patients, such as diabetes, hypertension, chronic renal failure, cardiomyopathy, decreased immunity and chronic obstructive pulmonary disease. WHO enabled various laboratories to produce reverse-transcription diagnostic polymerase chain reaction (RT-PCR) test especially for revelation of viral RNA, due to the genetic sequence of the 2019-nCoV was obtainable. CT findings mainly in the lower lung lobes consist of bilateral multipolar ground-glass obscurities, with marginal posterior distribution. When the RT-PCR screening test is negative, chest CT may prove to be a precious savings because it may display distinctive features of the disease. The own immune status becoming the majority definitive agents affecting disease progression and predication, due to the absence of vaccine and specific antiviral drugs. Clinically researched to discovery any thinkable treatment for COVID-19, by many antiviral complex that are applied to treat other infections.

Methods

The first study include collected data from Center for System Science and Engineering (CSSE) at John’s Hopkins University and Iraqi Ministry of Health website daily reports from 29th February to 31th December 2020 of people infected with 2019-nCoV.

Statistical Analysis

The next step of this study analyze the data calculated using (IBM SPSS statistics 20) they include the following:

Descriptive statistics: Statistical tables including observed frequencies with their percentages. Inferential statistics: for statistical hypotheses which maybe accept or reject, Persons Chi-Square test ($\chi^2$) at level of...
Result

From this statistics the prevalence of 2019-nCoV the spreading cases (83,561,252 total confirmed, 47,136,188 global recovered and 1,820,668 global death) from 191 countries in the world show in Figure (1). The most distribution confirmed cases represent in (19,975,250 US, 10,286,709 India, 7,675,973 Brazil, 3,153,960 Russia, 2,677,806 France, 2,496,231 United Kingdom, 2,208,652 Turkey, 2,107,166 Italy). Also global recovered represent in (9,883,461 India, 6,848,844 Brazil, 2,553,467 Russia, 2,100,650 Turkey, 1,508,419 Colombia, 1,463,111 Italy, 1,426,676 Argentina, 1,355,384 Germany). And global death represent in (345,844 US, 194,949 Brazil, 148,994 India, 125,807 Mexico, 74,159 Italy, 73,622 United Kingdom, 64,760 France, 56,798 Russia). From this result the Arab nation including 3,139,205 total confirmed cases, 2,783,448 recovered cases and 55,326 death cases from 21 countries infected with 2019-nCoV show in Figure (2) between this result the Iraqi infected patients represent 595,291 total confirmed cases, 537,841 recovered cases and 12,813 death cases show in Figure (3).

Figure (1): Spreading Cases (Total Confirmed, Global Recovered, Global Death) in the World.

Figure (2): Spreading Cases (Total Confirmed, Recovered, Death) in the Arab Nation
Table (1): Distribution of Cases (Infection, Recovered, Death) in Arab Nation from different Countries.

<table>
<thead>
<tr>
<th>Region</th>
<th>Cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infection</td>
<td>Recovered</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Iraq</td>
<td>595291 (51.9)</td>
<td>537841 (46.9)</td>
</tr>
<tr>
<td>Morocco</td>
<td>439193 (51.4)</td>
<td>407504 (47.7)</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>362741 (50.2)</td>
<td>353853 (49.0)</td>
</tr>
<tr>
<td>Jordan</td>
<td>294494 (51.8)</td>
<td>270551 (47.6)</td>
</tr>
<tr>
<td>United Arab Emirates</td>
<td>209678 (52.9)</td>
<td>186019 (46.9)</td>
</tr>
<tr>
<td>Lebanon</td>
<td>181503 (58.4)</td>
<td>127959 (41.2)</td>
</tr>
<tr>
<td>Kuwait</td>
<td>150584 (50.5)</td>
<td>146517 (49.2)</td>
</tr>
<tr>
<td>Qatar</td>
<td>144042 (50.4)</td>
<td>141556 (49.5)</td>
</tr>
<tr>
<td>Tunisia</td>
<td>139140 (55.8)</td>
<td>105364 (48.3)</td>
</tr>
<tr>
<td>Egypt</td>
<td>138062 (53.6)</td>
<td>112105 (43.5)</td>
</tr>
<tr>
<td>Oman</td>
<td>128867 (51.1)</td>
<td>121890 (48.3)</td>
</tr>
<tr>
<td>Libya</td>
<td>100744 (57.4)</td>
<td>73252 (41.7)</td>
</tr>
<tr>
<td>Algeria</td>
<td>99610 (58.8)</td>
<td>67127 (39.6)</td>
</tr>
<tr>
<td>Bahrain</td>
<td>92675 (50.6)</td>
<td>90217 (49.2)</td>
</tr>
<tr>
<td>Sudan</td>
<td>23316 (60.9)</td>
<td>13524 (35.3)</td>
</tr>
<tr>
<td>Mauritania</td>
<td>14364 (55.1)</td>
<td>11380 (43.6)</td>
</tr>
<tr>
<td>Syria</td>
<td>11434 (65.4)</td>
<td>5350 (30.6)</td>
</tr>
<tr>
<td>Djibouti</td>
<td>5831 (50.2)</td>
<td>5728 (49.3)</td>
</tr>
<tr>
<td>Somalia</td>
<td>4714 (55.7)</td>
<td>3612 (42.7)</td>
</tr>
<tr>
<td>Yemen</td>
<td>2099 (51.2)</td>
<td>1394 (34.0)</td>
</tr>
<tr>
<td>Comoros</td>
<td>823 (53.5)</td>
<td>705 (45.8)</td>
</tr>
<tr>
<td>Total</td>
<td>3139205 (52.5)</td>
<td>2783448 (46.6)</td>
</tr>
</tbody>
</table>

P-Value 0.000
In December 2019 were examined in Wuhan, China, to this moment I was writing the research, the numeral of confirmed cases raising dramatically in all the world and reach to (83,561,252 total confirmed, 47,136,188 global recovered and 1,820,668 global death) this spreading cases show in Figure (1). And refers for the most cases only [(19,975,250 US, 10,286,709 India, 7,675,973 Brazil) most global recovered is (9,883,461 India, 6,848,844 Brazil, 2,553,467 Russia) and the most global death is (345,844 US, 194,949 Brazil, 148,994 India)]. Due to the existing of infectious disease the characteristic of COVID-19 outbreak and control cannot
be used to observed the data and describe directly. The survey is making similar to other studies from 80 to 90% carrying out performed during the COVID-19 outbreak in China. Including the application of extraordinary public health measures throughout the world on traditional strategies of social distancing, quarantine, isolation and community containment. Also, took some aggressive measurement inclusive the shutdown of workplaces, schools, cancellation of public gatherings, roads and transit systems, compulsory quarantine of uninfected people without known disclosure to COVID-19. In Figure (2) show spreading cases (total confirmed 139,205, recovered 2,783,448, death 55,326) in the Arab Nation. The most cases show in Table (1) [Infection in Iraq 595,291 (51.9), Morocco 439,193 (51.4), Saudi Arabia 362,741 (50.2), Jordan 294,494 (51.8), most recovered in Iraq 537,841 (46.9), Morocco 407,504 (47.7), Saudi Arabia 353,853 (49.0), Jordan 270,551 (47.6) and most death in Iraq 12,813 (1.1), Egypt 76,31 (3.0), Morocco 73,888 (0.9), Saudi Arabia 62,223 (0.9)]. The p-value 0.000 in 2-sided represent non-significant result. Spreading cases (total confirmed 595,291, recovered 537,841, death 12,812) in the Iraq show in Figure (3). On 24 February 2020, the first assured case of COVID-19 has been recorded in Iraq from Najaf province to Iranian student came from Iran, followed on 25 February, in Kirkuk province from one family by four cases, they have as well travel date to Iran. On 27 February, An additional case was registered in Baghdad, for a patient who visited Iran lately. The distribution of cases (infection, recovered, death) in Iraq from different Months show in Table (2) which began the first weeks in 24th February to 31st December 2020 the p-value 0.000 in 2-sided represent non-significant result. During this time the infection is increase due to some people is close contact being within the room of a novel coronavirus case for a long period of time while not wearing bespoke personal covering equipment, living with, visiting, or input a health care suspense area or room with a novel coronavirus case or having direct contact with contagious excretion of a novel coronavirus case while not wearing counseled personal preservative tools. In Iraq the percentage of mild to moderate in severity until todays represent 80% and the percentage of chronic represent 20% in elder patients or comorbidities patients, such as diabetes, hypertension, chronic renal failure, cardiomyopathy, decreased immunity and chronic obstructive pulmonary disease this led to death.

Conclusion

In concluded, the percentage of 2010-n CoV increase in all the global confirmed cases in different areas of the world and become pandemic. In Iraq show the infection is mild to moderate in spite of no special antiviral and vaccine is present.

Ethical Clearance: Nil

Source of Funding: Self

Conflict of Interest: Nil

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Selective Caries Removal- A New Improvised Technique for Caries Management

Megha J Nair, Arathi Rao, Suprabha BS

Post Graduate Student, Professor, Professor & Head, Department of Pediatric and Preventive Dentistry, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education (MAHE) Manipal

Abstract

The strategies to manage deep caries are constantly evolving as evidence heaps for the use of minimal conservative techniques which would prevent pulpal exposure leading to preservation of pulp vitality, remineralisation of the carious dentin and arrest the progression of caries and offer a predictable success of the restoration. Changes in our understanding about the caries process, advent of new adhesive restorative materials and the possibilities of remineralization of demineralized dental tissues helps us to explore different techniques in caries management. Selective caries removal is now considered an efficient and evidence based approach in deep caries management and focuses on maximum retention of tooth material thus avoiding the probability of pulp exposure to provide a sound peripheral bondable surface for restoration and creating excellent hermetic seal.

Key Words: Caries, caries removal, pulp protection

Introduction

Dental caries result from an ecological shift in the dental biofilm from a healthy balanced state of microbial population to an unbalanced population of microbials dominated by acidogenic and aciduric bacteria, developed and maintained by the frequent consumption of fermentable carbohydrates, eventually leading to further imbalances in demineralization- remineralization cycle manifesting the carious lesion. This understanding of caries has changed the treatment strategies from a traditional “drill and fill” approach to a minimal invasive approach aiming at the control of biofilm activity.

Great many options are available for such a biofilm activity control in non cavitated lesions. Strategies for noncavitated lesions include diet control to manipulate the composition of biofilm, inactivation or removal of biofilm by mechanical and chemical means as well as use of fluoride agents to minimize the susceptibility of demineralization. Micro invasive methods like resin infiltration is recommended for early caries. Cavitated lesions warrants an conservative operative technique facilitating effective restoration of form and function.

With the changing of our understanding about the caries process, advent of new adhesive restorative materials and the possibilities of remineralization of demineralized dental tissues emphasis is on the selective removal of carious tissue to create a favorable surface for bonding as well as for supporting a restoration with good resistance and hermetic coronal seal.

This review explores this concept of selective caries removal, detailing the different techniques involved and the materials used for restoration following selective caries removal.

Evolution

More than a century ago, G V Black in his bookmentioned that an ideal cavity preparation needed removal of all the carious lesion. He believed that it was better to expose the pulp than leave it covered...
with a layer of soft dentin.\textsuperscript{7} There was a change from this belief when Fusyama et al\textsuperscript{8,9} identified two layers of carious dentin namely, inner demineralized dentin & outer microbial contaminated dentin and recommended that only the contaminated dentin needed to be removed and the inner layer (firm) would be remineralized. This marked the beginning of a minimally invasive treatment in cavity preparation and was termed as selective caries removal.

Massler et al coined the terms infected (previously termed outer contaminated) and affected (previously termed inner demineralized) dentin. Little was known that these terms would have a huge impact on the current concepts of caries management and how caries should be removed.\textsuperscript{10}

The techniques mentioned above have approximately 40\% (in permanent) and 53\% (in primary) risk of pulpal damage or exposure in cavitated moderate-deep lesions. In such a case, treatment modalities would include direct pulp capping, pulpotomy, pulpectomy/Root Canal Treatment or extraction of teeth. The success rate of pulp therapy in mechanical exposure of pulp is reported to be high especially in young permanent teeth with an open apex. However, a carious exposure significantly brings down the success rate to 33-37\% after 3 years to as low as 13\% after 10 years.\textsuperscript{11}

In International Caries Consensus Collaboration meeting in 2015, participated by 21 experts in Cariology from 12 countries assessed a compilation of evidences from various studies and discussed on different treatment strategies for caries management. This consensus elaborated on various methods and techniques of caries tissue removal and management with special focus of teeth that could be retained and with pulp with a potential to respond positively to pulp testing as well as with reversible pulpitis.\textsuperscript{6}

Objectives

The objectives of selective caries removal are to avoid exposure of the pulp during cavity preparation in an attempt to preserve the pulp vitality, manipulate the microbial population, provide a sound peripheral bondable surface for restoration creating excellent hermetic seal thereby cutting off the residual microbes of substrate/carbohydrates for survival subsequently preventing the progress of the carious lesion.\textsuperscript{6}

A shallow lesion which is not extending into the inner 1/3\textsuperscript{rd} to 1/4\textsuperscript{th} of the total dentin thickness, is at low risk of pulpal damage and therefore priority has to be given for restoration success than pulpal health. But, in deep carious lesions extending into the inner 1/3\textsuperscript{rd} to 1/4\textsuperscript{th} of dentin, priority should be for preservation of pulp vitality and reducing risk of pulpal damage which may lead to pulpal necrosis demanding invasive painful procedures later.\textsuperscript{10}

Procedure

Selective caries removal in shallow to moderate cavities:

This technique is employed in primary & permanent dentition with shallow and moderately deep cavities (radiographically extending less than the pulpal 1/3\textsuperscript{rd} or 1/4\textsuperscript{th} of dentin).\textsuperscript{2} The carious tissues are removed with the help of a spoon excavator from the base of the cavity until a resistance is felt. At this point, firm dentin is reached which is more often not discolored. The carious tissue from the walls of the cavity is excavated completely up to hard dentin which is “scratchy” when felt with a probe.\textsuperscript{10}

Selective caries removal in deep cavities:

Attempt is made to avoid the chances of pulpal exposure and preserve pulp vitality. A layer of ‘soft to touch’, ‘wet and moist’ dentin is left deliberately at the base of the cavity over the pulp. But a complete caries excavation on the walls is done to ensure a good coronal seal and a durable restoration.\textsuperscript{2} A provisional restoration which is durable up to a year is given keeping in mind the patient may not return to complete the treatment. This would also give time for dentinal changes to take place. During second visit, cavity is reentered, the formation of tertiary dentin confirmed by visual and tactile criteria and caries removal is continued up to leathery dentin after which a durable restoration is given. The reasoning for this method is that after the initial visit, the microbial diversity significantly reduces, remineralization of demineralized dentin and formation of tertiary dentin take place.\textsuperscript{12}

Methods of carious tissue removal

There is a wide array of criteria useful for evaluation of caries removal including tactile (hardness) and visual evaluation (dryness & color) of dentin, use of caries detector dyes.\textsuperscript{5}
Although there is no strong recommendations, hardness of dentin may be used as a primary criterion for assessing the effectiveness of carious dentin.  

Polymer burs work on the principle of differential hardness of the tooth tissues. The Knoop Hardness of a sound dentin is 66-80 while that of carious dentin is around 30. A polymer bur of Knoop Hardness Number 50 removes caries selectively.  

The erbium laser at 2940 nm is absorbed by water and hydroxyapatite crystals. This absorbed energy causes vaporization of the water making the area dry and results in micro-explosions and expulsion of the target material. This helps to remove the tooth structure rapidly especially having higher water content. This is of importance in cavity preparation since the carious tissue has more water content than healthy tissue which facilitates its easy and rapid removal. However, lasers are comparatively slow in the removal of carious lesions as compared to using a dental handpiece. 

Air abrasion makes use of the kinetic energy of aluminium oxide particle stream to remove carious lesion. They abrade the lesions without any vibration, sounds or heat production. Since the particles exist at the end of the tip, it is an end cutting instrument. There is evidence that the tooth preparation done using this technology results in rounder internal angles as compared to conventional cavity preparation, thus resulting in restorations with more longevity. 

Cavity disinfection following selective caries removal

Cavity disinfection following selective caries removal is a debated topic. It is said that microbial numbers play a limited role in the success of this treatment provided, a tight and good hermetic seal is achieved. Cavity disinfection with anti-microbial agents like 0.12% chlorhexidine with 35% phosphoric has been found to reduce the bacterial penetration into the dentinal tubules after selective caries removal. Phosphoric acid removed the smear layer which opens up the dentinal tubules and facilitates effective penetration of chlorhexidine. 

Use of Cavity liners

Cavity liners are routinely used in deep caries management in an attempt to keep the pulp viable, reducing the bacterial numbers, promote reactionary dentin formation, remineralization of demineralized dentin and to isolate the pulp from noxious stimuli. Cavity liners are beneficial while using resin-based cements as the final restoration after selective caries removal as it prevents the seeping of the monomers through the dentinal tubules. Also, the caries affected dentin has a low elastic modulus and poorly withstand the tensile forces which may lead to fracture lines in dentin during the polymerization shrinkage of resin-based cements due to which cavity liners are beneficial. 

Calcium hydroxide, used as a liner material reduces the number of microorganisms, promotes remineralization and aids in the formation of dentin bridge formation. However, its use as cavity liners following partially excavation of caries has shown unsatisfactory results. The high solubility of calcium hydroxide over time, tendency to cause internal resorption, tunnel defects in the dentin bridge formed makes it unfavorable for cavity lining after selective caries removal. 

Other bioactive materials that induce deposition of minerals in the dentin leading to the formation of apatite-like material protecting the underlying pulp are MTA, Biodentine, light cured calcium silicate base materials (TheraCal LC) . MTA is considered the most validated material for use as lining the base of the cavity. 

There is a lack of consensus in the literature about the ideal cavity liners to be used after selective caries removal. While some argue that liners don’t contribute to the success of selective caries removal if a good hermetic seal is achieved, some studies show that MTA be preferably used because of their high biocompatibility, osteoconductive properties as well as superior dentin bridge formation. It has been shown that MTA produces less pulpal inflammation and a thicker dentinal bridge with an odontoblastic layer formation. It maintains the vitality of the pulp and ensures a normal physiological pulpal function. A randomized clinical trial compared calcium hydroxide and MTA after selective caries removal in primary molars and reported no statistical differences after a 6 month and 1 year follow up. 

The key factors to be considered is the bond between the cavity liners and the residual dentin as well as the bond between the cavity liner and the final restoration. Even though its proved time and again that MTA and Biodentine may be used as reliable pulp capping agents, evidence suggest that MTA continues to mature a year after placement beyond the setting time. This would impact its mechanical integrity and bond strength.
Likewise, studies show that Biodentine matures up to 2 weeks after its placement.\textsuperscript{23}

Restorative materials used following selective caries removal

The primary objective of a restorative material is to promote the remineralization of carious left out dentin to restore the original mechanical properties and the choice is made based on the remaining coronal tooth structure, size of restoration, occlusal forces, caries risk of the patient, location of the carious lesion, esthetic needs of the patient and other related factors.\textsuperscript{24}

Amalgam and composite resin are globally accepted permanent restorations. Recent meta-analysis indicates that amalgam has better longevity as compared to composite restorations.\textsuperscript{25,26} In spite of good mechanical properties of amalgam, the poor esthetics and limitations of use of mercury containing materials and procedures,\textsuperscript{27} its use as a restorative material has drastically reduced globally.

Composite restorations form a tight micromechanical bond at the cavity margins by forming resin tags. But this bond with the carious dentin is very poor.\textsuperscript{28,29} Among the various systems, etch and rinse adhesive system yield a better bond to carious dentin as compared to self-etch systems.\textsuperscript{29} Some clinical available techniques to improve the bond strength is the pretreatment of cavity with chlorhexidine and use of Quaternary Ammonium Methacrylate containing products like 12-Methacryloyloxy-odecylpyridinium bromide (MDPB). They are polymerizable anti-microbial agents which inhibit collagenolytic enzymes that degrade the adhesive collagen, preventing cohesive failure.\textsuperscript{30}

Conventional Glass Ionomer Cements (GIC), Resin Modified Glass Ionomer Cements (RMGIC), comomers are cariostatic restorative materials due to their fluoride releasing properties and may be used as alternatives to composites. High viscosity GICs (HV GIC) are gaining increasing popularity as a restorative material after selective caries removal. Unlike the adhesive systems, HV GIC has similar bond strengths to both sound dentin and carious dentin. (31) In a study carried out in head and neck irradiated xerostomia patients providing ideal conditions for study of restorative material over a short period of time, HV GIC has been shown to have good longevity. Also further studies have proven that both Low Viscosity GIC (LV GIC) and HV GIC completely prevented the development of secondary caries even after the restoration was lost and in low fluoride compliant patients. Most notably, deteriorated restorations could be transformed into sandwich restorations.\textsuperscript{31-33}

Follow up

Any deep caries management places the patient under medium and high risk category which warrants the requirement of a follow up at 6 months. A positive pulpal response may be ensured by assessing the dentin beneath the restoration and non-progression of carious lesion compared to baseline.\textsuperscript{34}

Conclusion

The strategies to manage deep caries are constantly evolving as evidence heaps for the use of minimal conservative techniques which would prevent pulpal exposure leading to preservation of pulp vitality, remineralisation of the carious dentin and arrest the progression of caries and offer a predictable success of the restoration. Selective caries removal is now considered an efficient and evidence based approach in deep caries management. The decision to use cavity liners should be based on the need for additional pulp protection. The choice of final restoration should be based on clinical factors like size of cavity, type of carious dentin left behind, location of the cavity.

Ethical Clearance- Taken from Institutional Scientific Committee, MCODS Mangalore

Source of Funding- Self

Conflict of Interest - NIL

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Effectiveness of Educational Package Regarding Knowledge of Somatic Symptom Disorders among Adolescent Girls- A Pre-Experimental Study

Vishakha Rani¹, Raman Deep², Poonam Ahlawat¹

¹MSc Nursing, ²MSc Nursing & Associate Professor, SGT University, Gurugram, Haryana

Abstract

Objective: This study was undertaken to assess the effectiveness of educational package regarding knowledge of Somatic symptom disorders among adolescent girls.

Methods: 75 adolescent girls were selected by using non probability convenient sampling technique. The data was collected by using a reliable and validated tool to assess the knowledge regarding Somatic symptom disorders.

Results: The quantitative assessment of knowledge regarding Somatic Symptom Disorders revealed that after administration of educational package, 56 (74.67%) adolescent girls had average knowledge and 19 (25.33%) adolescent girls had adequate level of knowledge. There was significant difference between knowledge in pre test and post test after administration of educational package. Also, the post test knowledge was statistically associated with educational level of father of adolescent girls (0.043), educational level of mother of adolescent girls (0.046) and occupation of mother of adolescent girls (0.003). The study revealed that, the pretest mean score value of knowledge was 9.52 with standard deviation (SD) of 2.21 and the post-test mean score value of knowledge was 17.00 with SD of 4.01 with a mean difference of 7.48. The calculated-t value was 13.87. This indicated that there was a high statistical significant difference in the pre and post test knowledge level regarding Somatic symptom disorders among adolescent girls, p value 0.05.

Conclusion: Results of this research generates evidence that educational package regarding knowledge of Somatic symptom disorders among adolescent girls was effective.

Keywords: Assess, effectiveness, educational package, knowledge, Somatic symptom disorders and adolescent girls.

Introduction

“All emotions, even those that are spoken and unspoken, have physical effects. Primarily unspoken emotions tend to stay inside the body like tiny ticking time bombs, them are unit sicknesses in incubation.”

Marilyn Van M. Derbur, Miss America by Day

In today’s world of excellence, almost everyone experiences various degrees of stress in their daily lives. Even children aren’t an exception there too as they’re experiencing greater stress on account of rising expectations at both school and residential to perform beyond their aptitude. India accounts for 21% of adolescent population worldwide.

Literature shows that academic pressure causes various mental and behavioral disorders like depression, frustration, anxiety, helplessness, and suicidal behavior; these are more common emotional disorders in adolescents. [1]
Somatic symptom disorder Somatic comes from soma, the Greek word for body. Somatic symptom disorders are characterized by physical symptoms suggesting medical disease, but without demonstrable organic pathology or known pathophysiological mechanism to account for them.

The Somatic symptom related disorders include Somatic symptom disorder, factitious disorder, Hypochondriasis, conversion disorders, and psychological factors affecting other medical conditions. Additionally, DSM-5 has added new severity ratings for Somatic symptoms.

Invasive and costly diagnostic procedures are also done repeatedly in these cases. Hence, it’s vital to know the emotional factors that contribute to the event and maintenance of the functional Somatic symptoms and supply proper management at the earliest.

Somatic symptoms disorders, is characterized by 6 months of a general and non-delusional preoccupation with fears of getting, or the thought that one has, a significant disease supported the person’s misinterpretation of bodily symptoms. This preoccupation causes significant distress and impairment in one’s life; it’s not accounted for by another psychiatric or medical disorder; and a subset of people with Somatic symptoms disorder have poor insight about the presence of this disorder.

In many psychiatric disorders, including anxiety and depressive disorders, headache is usually prominently symptomatic. Children who undergo stress may present with complaints of nausea, vomiting, diarrhea, palpitation, headache, abdominal pain, limb pain, and pain over multiple sites, difficulty in breathing, hyperventilation, aphonia, giddiness, fatigue, weakness, non-epileptic seizures, tremors, abnormalities of gait and problems associated with micturition.

Sometimes psychological factors can cause unhealthiness without actually causing a disease. As results of unhappiness, anxiety or stress because of personal problems, physical symptoms may develop. We are all conversant in the headache that develops as a result of stress. Similarly, other physical symptoms can develop. These include nausea, abdominal pain and pain, breathlessness, diarrhea and giddiness and muscle pains.

Very few studies are conducted during this context, in India. Among these studies, varying prevalence of psychological state problems has been reported, from 9.5/1000 to 370/1000 population in India.

Somatic symptoms in adolescence are predicted as a severe adult mental disease as measured by hospital-based care also when controlled for important confounders. Adolescents with Somatic symptoms need early treatment and extended follow-up to treat these specific symptoms, no matter co-occurring depression and anxiety.

In general medical clinic populations, reported 6-month prevalence of Somatic symptom disorders are 4 to 6 percent, but it may be as high as 15 percent. It is more common in women than in men, in rural areas and less educated person (Black & Andresen). The disorder is chronic, with symptoms starting before age 30. Some evidence indicates that this diagnosis is more common among blacks than among whites.

Lifetime prevalence rate of conversion disorder vary widely. Statistics within the general population ranged from 5-30 percent. Somatic symptom disorders are more common in women than men and more frequently in adolescents and young adults than old age groups.

In India, the number of cases reported due to mental illness are 1-2% neurosis, somatic diseases 2-3%, mental retardation 0.5-1%, psychiatric disorders in children 1-2%, outpatient department attended in government hospital 3.63 million/year, outpatient department attended in private hospitals 2.63 million/year, 15 to 20% of all help seekers in general health services in both developed and developing countries.

Richa, Ghildiyal RG, Subramanyam A, Sharma P (2018) conducted a study which highlights the need for a joint effort by parents, pediatricians, psychiatrists
and teachers to help our children cope with the stress of today’s fast paced competitive world.\textsuperscript{[15]}

Somatic symptoms occur more commonly in females than males. Girls are reporting more symptoms as puberty and adolescence progresses (LeResche et al, 2005). In the majority of studies, girls have been found to report symptoms at increasing rates during adolescence, whereas reporting levels by boys of some Somatic symptoms (e.g., abdominal pain) fall during this time (LeResche et al, 2005).\textsuperscript{[16]} Worldwide, 20% of youngsters and adolescents suffer from a disabling mental disease. Therefore, knowledge of the prevalence of these disorders can be used to design intervention strategies.\textsuperscript{[17]}

The most common complaints are abdominal pain, headaches, and muscular or joint pains. Recurrent and troublesome Somatic symptoms occur in fewer than 10% of children and adolescents (Garralda, 2005; Rask et al, 2018).\textsuperscript{[18]}

Somatic symptom disorders are most common psychiatric disorder found in general practice. Somatic symptom disorders are very prevalent mental health problem among adolescents and they have lack of knowledge regarding Somatic symptom disorders. Somatic symptom disorders are more common among adolescent girls. That’s why researcher took this study to improve the knowledge regarding Somatic symptom disorders among adolescent girls with the help of educational package.

In the present study the investigator adopted a modified conceptual framework based on Ludwig Von Bertalanffy’s (1968) General System theory.

Ludwig general system theory model focuses on three areas, i.e., Input, throughput and output.

Beutel ME, Wiltink J, Kerahrodi JG, Tibubos AN, Brahler E, Schulz A, et. al (2019) conducted a study to assess the (1) prevalence of Somatic symptoms in men and women in the general population and (2) to identify the contributions of psychosocial factors and Somatic disease on symptom reporting. A total of 7,925 participants aged 40 to 80 years underwent medical and psychological assessments, based on the PHQ-15 (Patient Health Questionnaire). Pain complaints (arms, legs, joints, back pain) affected the majority of men and women, and Somatic symptom reporting increased with age. Findings highlight the complex psychosocial and Somatic contributions to Somatic symptom reporting.\textsuperscript{[19]}

Acheampong K, Baffour-Awuah D, Ganu D, Appiah S, Pan X, Kaminga A, et al. (2019) conducted a study to assess the predictors of dysmenorrhea, its impact, and methods for dealing with stress among adolescents in Shai Osudoku District, Ghana. Method: self-controlled survey to get information from adolescents elected to take an interest with in study. Conclusion: This examination uncovered that dysmenorrhea is high among young people in Shai Osudoku District which adversely influences the day by day action of greater part of them.\textsuperscript{[20]}

Richa, Ghildiyal RG, Subramanyam A, Sharma P (2018) conducted a study to evaluate the clinical profile of patients presenting with Somatic symptom and related issues. An open labeled, unidirectional and prospective study was conducted at a tertiary care hospital on 60 children in 5 to 16-year age group over a period of 18 months. The overall prevalence of Somatic symptom and related disorders was 60. The most common presenting symptom reported was generalized pain by 30 (50.0%) among Somatic symptoms and Pseudo seizures (33.3%) among conversion symptoms. Family issues (most common stressor) was found in 38 (63.3%) subjects. 30 (50.0%) patients had authoritarian parents. 46.7% of those counseled did not require any further intervention. In the present study, the most important areas in which stress was apparent were in school and family. So study highlights the need for a joint effort by parents, pediatricians, psychiatrists and teachers to help our children cope with the stress of today’s fast paced competitive world\textsuperscript{[15]}
Cerutti R, Spensieri V, Valastro C, Presaghi F, Canitano R, Guidetti V (2017) conducted a study to evaluate the pervasiveness and recurrence of Somatic symptoms additionally on explore the useful inability in children with high number of self-detailed substantial manifestations versus those with less Somatic symptoms. Also, the parental sentiment of their children’s Somatic symptoms and working was investigated. At long last, we assess the legitimately and by implication impacts of challenges in distinguishing sentiments in foreseeing Somatic symptoms and useful inability among school-aged children. Method: 356 Italian school-aged children and their moms partook during this examination. Results among children 66.3% didn’t pronounce physical manifestations and 33.7% detailed at least one Somatic symptom inside the most recent fourteen days. A positive connection rose among kids and moms. Determinations Findings uncovered that a more recurrence of substantial indications is related to useful inabilities and alexithymic features in school-matured kids. \[21\]

**Materials and Methods**

A pre-experimental research design was adopted for conducting the present study by using only manipulation. The research design selected for the present study is one group pre-test & post-test design.

**Table-1**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PRE-TEST (O1)</th>
<th>INTERVENTION (X)</th>
<th>POST-TEST (O2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>Pre-test</td>
<td>Implementation of Educational package on Somatic Symptom disorders</td>
<td>After 7 days post-test</td>
</tr>
</tbody>
</table>

The variables under study are: The independent variable of the study was educational package on Somatic symptom disorders. The dependent variable of this study was Knowledge regarding Somatic symptom disorders among adolescent girls. The extraneous variables of this study were age, educational level of adolescent girls, educational level of parents, occupation of parents, monthly income of family, type of family and exposure to knowledge regarding Somatic symptom disorders.

In the present study, the research setting was Govt. Senior Secondary School, Village Chhara, Jhajjar, Haryana, 75 adolescent girl students (age group 13-17 years) were selected. Sampling technique adopted for the study was non probability convenient sampling technique.

**Inclusion criteria:**

The study includes:

1. Adolescent girl students of age group 13-17 years.
2. Girl students who were present /available at time of data collection.
3. Girl students those who were willing to participate in the research study.

**Exclusion criteria:**

The study excludes:

1. Girl students who were below 13 year or more
than 17 years.

2. Girl students who were not willing to participate in the study.

3. Girls who were not available at the time of data collection.

The tool was constructed after extensive review of literature and discussion with experts, to collect the data. The tool to measures the knowledge of adolescent girls was self-structured questionnaire. A structured questionnaire was developed and the same tool was used for pre-test and post-test for collecting the data. It consists of two parts namely Section-1 and Section-2.

Table 2

<table>
<thead>
<tr>
<th>Interpretation of Score</th>
<th>Score Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor knowledge</td>
<td>0-8</td>
</tr>
<tr>
<td>Average knowledge</td>
<td>9-16</td>
</tr>
<tr>
<td>Adequate knowledge</td>
<td>17-25</td>
</tr>
</tbody>
</table>

Minimum score: 0    Maximum score: 25

Interventional Tool: Educational Package

Educational package aimed to improve knowledge of Somatic symptom disorder among adolescent girls. The educational package included

1. Video on Somatic symptom disorders

2. Lecture cum discussion on Somatic symptom disorders, power point presentation for a period of 45 min.

3. Blackboard, chart, flashcards and handouts were used for effective teaching and learning experience.

The reliability of the tool was assessed by doing pilot study. The reliability was assessed by using test retest method.

\[
r = \frac{\text{n} \text{ΣXY} - \text{(Σx)(Σy)}}{\sqrt{\text{n} \text{Σx}^2 - (\text{Σx})^2 } \text{n} \text{Σy}^2 - (\text{Σy})^2}}
\]

The reliability score obtained was 0.881 by test retest method. The r-value indicated positive correlation, which showed that the tool was reliable for the researcher for conducting the main study.

Ethical Considerations

The pilot study and main study was conducted after the approval from Departmental Research Committee (DRC) & Institutional Ethical Committee. Permission was obtained from concern head. After that the
researcher explained the study to the participants. An informed consent was obtained and assurance was given regarding maintenance of confidentiality and anonymity of the data collected.

**Pilot Study**

Pilot study was conducted for a period of one week from 7 February to 14 February, 2020, at Govt. Senior Secondary School, at Village Talao, Jhajjar, Haryana, after taking formal permission from Principal. After pre-test, interventions which include educational package regarding Somatic symptom disorders given to adolescent girls for a period of one hour. After 7 days the Post-test was conducted by using structured knowledge questionnaire devised by investigator.

Analysis of the findings depicts that the educational package had a significant effect on enhancing the level of knowledge regarding Somatic symptom disorder among adolescent girls. The result of the pilot study revealed the feasibility and practicability of the study.

**Procedure For Data Collection**

The pilot study and main study was conducted after the approval from department of research committee (DRC) & Ethical committee. Permission was obtained from concern head. Data collection procedure was done at Govt. Girl Sr. Sec. School, Jhajjar, Haryana. Formal administrative approval was obtained from the Dean, Faculty of nursing, SGT University, Gurugram, Haryana and the Principal, Government senior secondary schools. Data collection was carried out for a period of two weeks.

During the first week the researcher selected the adolescent girls based on sample selection criteria. There were totally 75 adolescent girls selected as samples. Samples were obtained by non-probability convenient sampling technique. A brief introduction about self and purpose of the study was given; consent was obtained from the adolescent girls. Confidentiality regarding the data was assured so as to get co-operation during the procedure of data collection. The school authority provided well ventilated separate class room for data collection. Data was collected by using structured questionnaire based on demographic variables and knowledge questions devised by the investigator.

After pre-test, intervention which included educational package regarding Somatic symptom disorders given to adolescent girls for a period of one hour. After 7 days (26 Feb. 2020) the post-test was conducted by using structured knowledge questionnaire devised by investigator.

The data analysis was done under the following headings by using both descriptive and inferential statistics.

- Frequency and percentage distribution were used to analyze the demographic variables of adolescent girls.
- Mean and standard deviation was used to analyze the pre and post-test level of knowledge regarding Somatic symptom disorder among adolescent girls.
- Paired ‘t’ test was used to compare the pre and post-test level of knowledge regarding Somatic symptom disorder among adolescent girls.
- Chi-square test was used to find out the association of Post-test knowledge level regarding Somatic symptom disorders with selected demographical variables of adolescent girls.
## Results

**TABLE 3: FREQUENCY AND PERCENTAGE OF ADOLESCENT GIRLS ACCORDING TO SOCIO-DEMOGRAPHIC VARIABLES**

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Demographic variables</th>
<th>Frequency (f)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age in years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>13-14 years</td>
<td>47</td>
<td>62.67%</td>
</tr>
<tr>
<td>b</td>
<td>15-16 years</td>
<td>21</td>
<td>28.00%</td>
</tr>
<tr>
<td>c</td>
<td>17-18 years</td>
<td>7</td>
<td>9.33%</td>
</tr>
<tr>
<td>2</td>
<td>Educational level of adolescent girls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>8th class</td>
<td>24</td>
<td>32.00%</td>
</tr>
<tr>
<td>b</td>
<td>9th class</td>
<td>30</td>
<td>40.00%</td>
</tr>
<tr>
<td>c</td>
<td>11th class</td>
<td>21</td>
<td>28.00%</td>
</tr>
<tr>
<td>3</td>
<td>Educational background of father</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>Illiterate</td>
<td>4</td>
<td>5.33%</td>
</tr>
<tr>
<td>b</td>
<td>1 - 5th class</td>
<td>19</td>
<td>25.33%</td>
</tr>
<tr>
<td>c</td>
<td>6- 10th class</td>
<td>25</td>
<td>33.33%</td>
</tr>
<tr>
<td>d</td>
<td>11- 12th class</td>
<td>22</td>
<td>29.33%</td>
</tr>
<tr>
<td>e</td>
<td>Graduation</td>
<td>5</td>
<td>6.67%</td>
</tr>
<tr>
<td>4</td>
<td>Educational Background of Mother</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>Illiterate</td>
<td>13</td>
<td>17.33%</td>
</tr>
<tr>
<td>b</td>
<td>1 - 5th class</td>
<td>18</td>
<td>24.00%</td>
</tr>
<tr>
<td>c</td>
<td>6- 10th class</td>
<td>29</td>
<td>38.67%</td>
</tr>
<tr>
<td>d</td>
<td>11- 12th class</td>
<td>10</td>
<td>13.33%</td>
</tr>
<tr>
<td>e</td>
<td>Graduation</td>
<td>5</td>
<td>6.67%</td>
</tr>
<tr>
<td>5</td>
<td>Occupation of father</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>Government Job</td>
<td>7</td>
<td>9.33%</td>
</tr>
<tr>
<td>b</td>
<td>Private Job</td>
<td>10</td>
<td>13.33%</td>
</tr>
<tr>
<td>c</td>
<td>Businessman</td>
<td>4</td>
<td>5.33%</td>
</tr>
<tr>
<td>d</td>
<td>Farming</td>
<td>29</td>
<td>38.67%</td>
</tr>
<tr>
<td>e</td>
<td>Other</td>
<td>25</td>
<td>33.33%</td>
</tr>
</tbody>
</table>
### TABLE 3: FREQUENCY AND PERCENTAGE OF ADOLESCENT GIRLS ACCORDING TO SOCIO-DEMOGRAPHIC VARIABLES

<table>
<thead>
<tr>
<th></th>
<th>Occupation of mother</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Government Job</td>
<td>1</td>
<td>1.33%</td>
</tr>
<tr>
<td>b</td>
<td>Private Job</td>
<td>7</td>
<td>9.33%</td>
</tr>
<tr>
<td>c</td>
<td>Businesswomen</td>
<td>3</td>
<td>4.00%</td>
</tr>
<tr>
<td>d</td>
<td>Daily Wages</td>
<td>4</td>
<td>5.33%</td>
</tr>
<tr>
<td>e</td>
<td>Housewife</td>
<td>60</td>
<td>80.00%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Monthly income of family (in rupees/month)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>&lt;=10000</td>
<td>57</td>
<td>76.00%</td>
</tr>
<tr>
<td>b</td>
<td>11,000-20,000</td>
<td>12</td>
<td>16.00%</td>
</tr>
<tr>
<td>c</td>
<td>21,000-30,000</td>
<td>2</td>
<td>2.67%</td>
</tr>
<tr>
<td>d</td>
<td>&gt;=31000</td>
<td>4</td>
<td>5.33%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Type of family</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>a</td>
<td>Nuclear family</td>
<td>28</td>
<td>37.33%</td>
</tr>
<tr>
<td>b</td>
<td>Joint family</td>
<td>18</td>
<td>24.00%</td>
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<tr>
<td>c</td>
<td>Extended Family</td>
<td>12</td>
<td>16.00%</td>
</tr>
<tr>
<td>d</td>
<td>Single parent family</td>
<td>17</td>
<td>22.67%</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Exposure to knowledge regarding Somatic symptom disorders</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Mass Media</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>b</td>
<td>Friends and relatives</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>c</td>
<td>Teachers and Parent meetings</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>d</td>
<td>Self Experience</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>e</td>
<td>Others</td>
<td>16</td>
<td>21.33%</td>
</tr>
<tr>
<td>f</td>
<td>None</td>
<td>59</td>
<td>78.67%</td>
</tr>
</tbody>
</table>

Assessment of pre-test and post-test level of knowledge regarding Somatic symptom disorders  N=75
Fig. 1  Bar graph showing frequency and percentage of pre-test and post-test level of knowledge regarding Somatic symptom disorders among adolescent girls

The data presented in figure: 12 depicts that in pre-test 26 (34.67%) adolescent girls had poor knowledge and 49 (65.33%) adolescent girls had average level of knowledge regarding Somatic symptom disorders, and out of 75 no adolescent girls had adequate knowledge regarding Somatic symptom disorders. After administration of educational package, the post-test findings revealed that 56 (74.67%) adolescent girls had average knowledge and 19 (25.33%) adolescent girls had adequate level of knowledge, and out of 75 no adolescent girls had poor knowledge regarding Somatic symptom disorders.

Fig. 2  Bar graph showing pre-test and post-test mean score value and standard deviation

<table>
<thead>
<tr>
<th>Poor knowledge (0-8)</th>
<th>Average knowledge (9-16)</th>
<th>Adequate knowledge (17-25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 (34.67%)</td>
<td>49 (65.33%)</td>
<td>19 (25.33%)</td>
</tr>
</tbody>
</table>

Pretest and Posttest mean score and Standard Deviation

<table>
<thead>
<tr>
<th>Pretest mean Score</th>
<th>9.52 ± 2.21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posttest mean Score</td>
<td>17.00 ± 4.01</td>
</tr>
</tbody>
</table>
The data presented in figure 13 reveals that pre-test mean score value and standard deviation score was 9.52 ± 2.21 and the post-test mean score value and standard deviation was 17.00 ± 4.01 and the calculated t-value was 13.87 which was statistically significant at p= 0.001. The above result reveal that there was a significant difference between pre-test and post-test knowledge levels among school going adolescent girls after implementation of educational package regarding Somatic symptom disorders.

**Analysis of effectiveness of educational package regarding knowledge on Somatic symptom disorders**

Table 4 Comparison table between pre-test and post-test level of knowledge regarding Somatic symptom disorders among adolescent girls

N=75

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Mean ± SD</th>
<th>Mean difference</th>
<th>Df</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-test</td>
<td>9.52 ± 2.21</td>
<td></td>
<td></td>
<td>74</td>
<td>13.87</td>
</tr>
<tr>
<td>Post-test</td>
<td>17.00 ± 4.01</td>
<td>7.48</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at 0.05 level of significance

DF (74) = 1.98 Significant at 0.05 level of significance

The data presented in table 5 explains that the pre-test mean score value and standard deviation score was 9.52 ± 2.21 and the post-test mean score value and standard deviation was 17.00 ± 4.01 and the calculated t-value was 13.87. Which was statistically significant at p= 0.001. Hence, it can be inferred that obtained mean difference 7.48 is not by chance but it is a true difference, thus research hypothesis was accepted. So, it can be concluded that there was a significant difference between pre-test and post-test knowledge levels among school going adolescent girls after implementation of educational package regarding Somatic symptom disorders.

Significant at the level of p<0.05 - significance

*S -Significant NS- Not Significant

[** values were calculated by Fisher’s exact test]

Table 5 depict that chi-square value obtained to find out the association between post-test knowledge level with demographic variables.

The data presented in table 6 reveals that there was statistically significant association between post-test knowledge and educational level of father of adolescent girls (0.043), educational level of mother of adolescent girls (0.046) and occupation of mother of adolescent girls (0.003). No significant association was found with post-test knowledge level and other demographic variables, such as age (0.375), educational level of adolescent girls (0.186), occupation of father (0.179), monthly income of family (in rupees/month) (0.724), type of family (0.506), and exposure to knowledge regarding Somatic symptom disorders (0.508)
Discussion

The pre-test knowledge level regarding Somatic symptom disorders among adolescent girls, out of 75 adolescent girls 26 were in poor knowledge category, 49 adolescent girls were in average knowledge category and 0 (no) adolescent girls were in adequate knowledge category. The study reveals that, the pretest mean score value of knowledge was 9.52 with standard deviation (SD) of 2.21. Mean difference was 7.48 the calculated-t value was 13.87.

There was a significant association to develop and administer educational package to enhance knowledge regarding Somatic symptom disorders. The findings of study were similar to the findings reported by Rota E, Evangelista A, Ciccone G, Ferrero L, Ugolini A, Milani C, et al (2011) in their study to assess the effectiveness of an educational and physical program in reducing accompanying symptoms in subjects with head and neck pain in 384 employees. The study findings shows that educational and physical program were effective in reducing the burden of behavioral or somatic symptoms along with headache, neck and shoulder pain in a working community.

The study reveals that, the pretest mean score value of knowledge was 9.52 with standard deviation (SD) of 2.21 and the post-test mean score value of knowledge was 17.00 with SD of 4.01. Mean difference was 7.48 the calculated-t value was 13.87. This indicated that there was a high statistically significant difference in the pre and post-test knowledge level regarding Somatic symptom disorders among adolescent girls, p value 0.05.

The effectiveness of educational package was analyzed using unpaired ‘t’ test. The calculated unpaired ‘t’ test value for knowledge was 13.87 which was greater than the table value and this indicated that there was statistically high significant difference in Post-test level of knowledge regarding Somatic symptom disorders among adolescent girls. It indicated that educational package was significantly effective in improvement of knowledge regarding Somatic symptom disorders among adolescent girls.

Hence, the null hypothesis $H_0$ stated earlier that “There is no significant difference between the mean pre-test and Post-test knowledge level regarding Somatic symptom disorders among adolescent girls at $p<0.05$ level of significance is rejected and $H_1$ is accepted.

There was a significant association between effectiveness of educational package on knowledge regarding Somatic symptom disorders which was found consistent with the study conducted by Manonmani C (2011) conducted a pre experimental study to determine the effectiveness of Information, education, communication package on knowledge and expressed practice regarding management of primary dysmenorrhea among adolescent girls at Girls Higher Secondary School in Srirangam. The study showed that IEC was effective in increasing the knowledge and expressed practice of adolescent girls regarding primary dysmenorrhea management.

The association of post-test knowledge regarding Somatic symptom disorders of adolescent girls with selected demographic variables (age, educational level of adolescent girls, educational level of father and mother, occupation of father and mother, monthly income of family, type of family, exposure to knowledge regarding Somatic symptom disorders) were analyzed by using chi-square & fisher’s exact test and found that there were statistically significant association between post-test knowledge and educational level of father of adolescent girls (0.043), educational level of mother of adolescent girls (0.046) and occupation of mother of adolescent girls (0.003). No significant association was found with Post-test knowledge level and other demographic variables, such as age (0.375), educational level of adolescent girls (0.186), occupation of father (0.179), monthly income of family (in rupees/month) (0.724), type of family (0.506), and exposure to knowledge regarding Somatic symptom disorders (0.508).

There was a significant association between Somatic symptoms and some of the selected socio demographic variables. The findings of study were in line with
the findings reported by Aswathy Raj, Saleem TK; Varsha Vidyadharan (2019). They conducted a study to assess the Somatic complaints and depressive symptoms of 250 adolescent girls and to identify the relationship between these variables. Association between Somatic complaints/depressive symptoms and selected socio personal variables was also analysed. The study found a significant positive correlation between Somatic complaints and depressive symptoms.

Conclusion

The study concluded that there is a significant difference between knowledge level before and after implementation of educational package regarding Somatic symptom disorders among adolescent girls at selected Govt. Sr. Sec. School, Jhajjar, Haryana. There is significant association between post-test knowledge level and selected demographic variables such as educational level of father and mother, and occupation of mother. The present study aimed at assessing the effectiveness of educational package on knowledge regarding Somatic symptom disorders among adolescent girls and the findings of the study showed that That the pre-test means value and standard deviation score was 9.52 ± 2.21 and the post-test mean value and standard deviation was 17.00 ± 4.01, and mean difference value was 7.48. The calculated t-value was 13.87. Which indicated that educational package was an effective intervention to improve the level of knowledge regarding Somatic symptom disorders among adolescent girls.

Recommendations

A study can be done to assess effectiveness of educational package on Somatic symptom disorders among adolescence including both boys and girls. A comparative study can be done to assess the prevalence of Somatic symptom disorders in different age groups. A descriptive study can be done to assess the prevalence and knowledge regarding Somatic symptom disorders with a large number of sample sizes. An experimental study can be done to assess the effect of cognitive behavior therapy and meditation, yoga on Somatic symptom disorders. An experimental study can be done to assess the prevalence, knowledge and attitude regarding Somatic symptom disorders.

Source of Funding: Self

Conflict of Interest – Nil.

References

4. Somatic Symptom Disorder DSM-5 300.82 (F45.1)
and mental health. 2018 Dec 1; 12(1):42.


Changes in the Cancer Antigen Markers in the Pleural Liquid During Chemotherapy among Ovarian Cancer Patients

Abbas Abdulmueed Mustafa Alani¹, Ihab Q. Ali², Yasir Khaleel Almusawi³

¹Lectural Doctor, Medical Laboratory Department, Pharmacy College, Uruk University, Iraq, ²Lecturer, ³Assistant Lecturer, Medical Laboratory Techniques Department, Al-Esraa University College, Iraq

Abstract

The silent killer or ovarian cancer (OC) is one of the major causes of cancer deaths among women in the modern age. In view of the therapeutic roles of platinum based chemotherapy among the ovarian cancer patients, objective of this report was to assess the effects of the first round of chemotherapy among women with ovarian cancer. Here, the levels of the pleural markers for cancer bio markers, before and after the chemotherapy were tested among ovarian cancer patients. The biochemical indices may be of use in deciphering the relation between cancer relapse and platinum retrieval. The pleural analyses pre and post platinum chemotherapy session demonstrated wide range of biochemical and protein marker changes among ovarian cancer patients. The various cancer antigens were variedly reduced post chemotherapy. Hence, it is proposed that biochemical markers in pleural liquid may serve as better indicators and early diagnostic tools for ovarian cancer.

Keywords: Ovarian cancer, chemotherapy, pleural liquid, cancer antigens.

Introduction

One of the major cancer deaths among women is caused by ovarian cancer (OC). There has been an immense development in the field of cancer therapy, the majority of patients experience disease recurrence and receive second-line and sometimes several lines of treatment (¹⁴). The progression-free survival among OC patients is fairly constant at about 1.5 years with respect to the modern diagnostic tools and treatment patterns. As high as 80 percent women with advanced stages of OC report tumor progression or recurrence. As high as 70% cases of OC are diagnosed at advanced stages leading to bad prognosis. Generally late-stage ovarian cancer is incurable in the majority of cases. The recent developments in surgical technology and contemporary regimes of systemic treatment, as well as some new drugs have turned the late-stage OC as a chronic condition by extending the survival days (³). The “silent killer” synonym for ovarian cancer is because the majority of patients are diagnosed in late stage, and that in early stages of the disease symptoms are not evident.

Pleural effusion is a common feature among women with OC and breast cancer. Pleural effusion is an abnormal accumulation of fluid in the pleural cavity which is the space between lungs and chest wall (⁴). The liquid starts accumulating the between the layers of the pleura. When pleural effusion is related to cancer or there are cancer cells in the fluid, it may be called malignant pleural effusion.

Approximately 20% of women with advanced-stage ovarian cancer survive beyond 12 years after treatment and are effectively cured. Initial therapy for ovarian cancer comprises surgery and chemotherapy, and is given with the goal of eradicating as many cancer cells as possible. Indeed, the three phases of therapy are as follows: debulking surgery to remove as much of the cancer as possible, preferably to a state of no visible residual disease; chemotherapy to eradicate any microscopic disease that remains present after surgery; and second-line or maintenance therapy, which is given
to delay disease progression among patients with tumour recurrence (2). Epithelial ovarian cancer has the highest mortality rate of all gynaecological malignancies. Most women present with advanced disease and develop a recurrence after radical surgery and chemotherapy. Improving the results of first- or subsequent-line chemotherapy has been slow, and novel approaches to systemic treatment are needed. Ovarian cancer is a heterogeneous disease with complex molecular and genetic changes. Understanding these better will provide information on the mechanisms of resistance and opportunities to target therapy more rationally, exploiting specific changes in the tumour (4,5).

Primary debulking surgery followed by platinum-based chemotherapy remains the standard treatment of patients with stage III-IV epithelial ovarian cancer. Neoadjuvant chemotherapy is an alternative treatment regimen that can be considered in selected patients. Complete cytoreduction, both through primary debulking surgery and interval debulking surgery, has a major positive effect on survival and should be the goal, even if this requires extensive surgery. When thorough assessment of tumor spread and performance status of the patient indicates that complete primary cytoreduction is not feasible without unacceptable morbidity, then alternative therapeutic strategies, such as neoadjuvant chemotherapy, must be considered (5,6,7).

Malignant pleural effusion is the most common site of stage IV ovarian cancer. A positive cytology is required for a stage IVA diagnosis. Unfortunately, the accuracy rate of pleural cytology remains low. A number of factors have been identified as prognostic for clinical outcomes in patients with epithelial ovarian cancer (7,8,9). The pleural cavity constitutes the most frequent extra-abdominal metastatic site in ovarian carcinoma (OC). In patients with OC and pleural effusions, a positive fluid cytology is required for a stage IV diagnosis. Unfortunately, about 30% of malignant pleural effusions exhibit false-negative cytological pleural fluid results. In those circumstances, exploratory video-assisted thoracoscopic surgery (VATS) serves as a diagnostic, staging and even therapeutic modality (8,9).

We hypothesize that the pleural liquid markers could serve as significant markers of disease progression and response to therapy among OC patients before and after one cycle of chemotherapy.

Materials and Methods

Participants: All participants (patients) were enrolled as part of clinical trial going on according to the IP and GCP/GLP guidelines at a reputed hospital. Patient/immediate relative consents were obtained for using the serum/plasma for research and data management.

Drug and dosage: PEGylated liposomal cis-platini is a formulation of cis-platini in polyethyleneglycol-coated liposomes with a prolonged circulation time. Cisplatin intercalates between base pairs in the DNA helix, thereby preventing DNA replication and ultimately inhibiting protein synthesis. Dosage is calculated by estimating Body Surface Area (BSA) and at 50mg/m² BSA is infused intravenously (2mg/ml).

\[
\text{BSA (m}^2) = \sqrt{\frac{\text{height (cm)} \times \text{weight (kg)}}{3600}}
\]

Collection of Pleural liquid: Patients having ovarian cancer were selected by the Medical Practitioner and suggested for the study (n=31). The pleural samples were collected before the initiation of chemotherapy (PEGylated cis-platin liposomes) and after three weeks of first chemotherapy session (PEG= poly ethylene glycol). The Pleural liquid of the patient was collected by the nursing professional and immediately introduced into collection bottles. Approximately 100ml aliquot from each patient was collected and immediately used for all the biomarkers and stored at -20°C.

Estimation of the biomarkers: Level of pleural markers were estimated by using ELISA kits according to manufacturer’s instructions.

Statistical Analysis

The data expressed as mean ± SE were analyzed by one-way ANOVA followed by a post hoc Tukey’s test to compare the control and treatment groups as well as
among the groups (p ≤ 0.05) using GraphPad Prism 5.0 software. Different alphabet letters indicate significance difference among the respective groups. In some assays, * indicates significance difference from control (p≤0.05).

**Results and Discussion**

Among the ovarian cancer patient volunteers who belonged to an age range of 43 to 65 years, nearly 45% of them complained of pain in the abdominal area while 35% of them presented nausea as their main symptom. In my previous report I demonstrated that the pleural thickening was significantly reduced among the ovarian cancer patients after first round of Platinum-based chemotherapy. The reduction in the pleural thickness is observed in most of the clinical trials. However, in this study, the reduction was not to a marked extent but significant change was recorded. The hexose levels were unaffected while the cholesterol was markedly increased among the patients. Anti-inflammatory markers also elevated among chemotherapy patients which was duly reported in my previous publication. As a proof of principle, the chemotherapy resulted in biochemical changes in the pleural liquid among ovarian cancer patients.

![Figure 1](image.png)

*Figure 1. Effect of chemotherapy on the Lactic dehydrogenase activity in the pleural liquid among patients of ovarian cancer before and after one cycle of chemotherapy (n=31). Different letters indicate significant difference between groups (p≤0.05).*

The enzymic levels of Lactic Dehydrogenase were decreased among post chemotherapy samples (Figure 1).
The cancer Biomarker: Carcinoembryonic antigen (CEA) levels were significantly reduced among post chemotherapeutic samples, however, the levels were not reaching the safe diagnosis (Figure 2).

The cancer Biomarker: Cancer antigen (CA 125) levels were significantly reduced among post chemotherapeutic samples (Figure 3). However, the levels were not reaching the safe diagnosis levels.
The cancer Biomarker: Cancer antigen (CA 27.29) levels were very marginally but significantly reduced among post chemotherapeutic samples, however, this data is suggestive that multiple sessions of chemotherapy could bring down the cancer marker levels (Figure 4).

**Conclusion**

Analysis of the pleural samples from ovarian cancer patients before and after one round of chemotherapy revealed that there were varied biochemical changes. In lines with my previous report, here additional data about cancer antigen markers are presented. There was a significant change among cancer antigens in pleural liquid after chemotherapy. Hence, it is proposed that biochemical markers in pleural liquid may serve as better indicators and early diagnostic tools for ovarian cancer.

**Conflict of Interest:** There are no conflicts of interests.

**Acknowledgement:** The author thanks the patients who were willing to participate in the study.

**Ethical Clearance:** This study regimen was approved by and followed the guidelines of the institutional ethical committee.

**Source of Funding:** This research work was conducted as a part of project work of the author Mr. Alimuneer Abdul Rahman with self-financing. No other government or private funding were used for the study.

**References**


An Analysis of the Implementation of Diversion in Efforts to Settle Medical Disputes in Indonesia

Arista Candra Irawati¹, Sarsintorini Putra², Retno Mawarini Sukmariningsih², Adhi Putra Satria³

¹Lecturer at the Faculty of Law of Ngudi Waluyo University and a Law Faculty Student of University of 17 Agustus 1945 Semarang, ²Lecturer at the Faculty of Law of University of 17 Agustus 1945 Semarang, ³Law Faculty Student of University of 17 Agustus 1945 Semarang

Abstract

Based on the legal construction contained in Article 29 of Law Number 36 the Year 2009 concerning Health, it has ordered that health workers suspected of neglecting in carrying out their profession. There is a need for a settlement preceded by the mediation stage or what is known as the settlement of disputes out of the court. The facts in the field show that the handling of medical disputes, especially for doctors who commit negligence, has not been fully implemented according to the provisions of Article 29 of the Health Law, meaning that the handling is not carried out through the first mediation stage, as in the case experienced by dr. Ayu et al. They had to be brought to court accused of committing medical malpractice because they failed to save the patient during a cito-cesarean operation. Dr. Ayu et al. at that time did not fulfill their rights to get mediation. This is because there has been no further regulation governing the procedures, procedures, and rules for implementing mediation, especially health services. Due to this fact, it becomes an obstacle to the consistency of mediation efforts as a necessity in solving medical disputes. Law No. 29 of 2004 concerning Medical Practice and Regulation of the Medical Council Number 32 of 2015 concerning procedures for handling suspected disciplinary violations of Doctors and Dentists do not regulate mediation efforts, so the implementation of mediation efforts in health services is not optimal.

Kata Kunci: Diversion; Mediation; Medical Disputes;

Introduction

Conflicts in the health care profession that culminate in the court for health professionals are indeed a lot related to ethical problems that have the potential to cause medical disputes by placing everyone entitled to claim compensation for health workers and/or health providers who cause losses due to errors or negligence in the health services they receive.[1] This is due to the characteristics of the legal relationship between patient and doctor in health care, based on a therapeutic contract and the relationship due to laws and regulations.[2] In the first relationship, it begins with an agreement (unwritten) so that the will of the two parties is assumed to be accommodated when the agreement is reached. The reached agreement, among others, is in the form of approval of medical action or even rejection of a medical action plan.[3] While the second relates to relationships because laws and regulations usually arise because of the obligations imposed on doctors because of their profession without the need for patient consent.

These two relationships give birth to legal responsibility, professional responsibility, and ethical responsibility. Violation of doctor or dentist discipline can be prosecuted in several courts. For example, there are civil courts, criminal courts, and administrative courts in the legal field.[4] In addition, doctors or dentists can also be brought before the Ethics Court in professional organizations and the Professional Discipline Court by (MKDKI). The basis for a doctor’s obligation is a professional contractual relationship between medical personnel and their patients, which creates general obligations and professional obligations for the medical
personnel. Professional commitments are described in the professional oath, ethical rules, various service standards, and various operational procedures.

The enactment of Law Number 36 of 2009 concerning Health is considered flexible because it can keep up with developments in science and technology in the medical field. This is based on the consideration that there are 5 (five) reasons for considering why it is necessary to establish a health law quo. The five primary considerations as intended include: first; health is a fundamental right and an element of well-being. Second; principles of non-discriminatory, participatory, and sustainable health activities. Third; health is an investment. Fourth; health development is the responsibility of the government and society, and fifth is that Health Law No. 23/1992 is no longer in line with developments, demands, and legal needs in society.[5]

A step forward in the provisions of Article 29 of Law Number 36 the Year 2009 concerning Health states that “If a health worker is suspected of negligence in carrying out his profession, the negligence must first be resolved through mediation”. Referring to the article in question, the necessity to take mediation efforts is highly expected/recommended or even obligatory if a medical dispute occurs between a patient or his family and a health worker or between a patient and a hospital/health facility in Indonesia.[6]

However, the obligation to exercise discretion as mandated in Article 29 of the Health Law has never been achieved in practice. This is following the case that happened to dr. Ayu, which occurred in April 2010. Dr. Ayu and her colleagues, namely dr. Hendry Simanjuntak, and dr. Hendy Siagian are working with Puskesmas referral patients in the Manado area. Due to the urgent situation, dr. Ayu performed a cito-cesarean surgery. But those measures failed to save the patient. The patient’s family reported having operated without permission. At the Manado District Court (PN) trial, dr. Ayu et al. were demanded 10 (ten) months in prison. At the level of Appeal, it is decided to be Free. The Public Prosecutor (JPU) who handled the case filed a cassation, and the Supreme Court granted a decision issued on November 18, 2012. In a legal reconsideration effort through a decision in February 2014, dr. Ayu et al. were acquitted because they were not proven to have committed malpractice. The basis for the consideration of granting the PK was that the convicts did not violate the SOP in the handling of the Sesco Ciceasria operation so that the judex facti considerations at the Manado District Court were correct.

Medical dispute complaints to the police at Polsek, Polres, and Polda levels are received and processed like a criminal case. Shifting civil cases to the realm of crime, inconsistent use of articles, difficulties in proving legal facts, and limited understanding of medical insights by law enforcers at almost every level make medical disputes threatened with criminal disparities.

**Aims and Objective**

With the regulation of the necessity of mediation in medical disputes over the services of health workers, especially the doctors and dentists, it becomes the basis for the authors to examine this matter more deeply. This paper aims to know and analyze how the implementation and efforts of mediation as medical dispute resolution in Indonesia.

**Observation and Results**

1. Health Services in the Implementation of Medical Practices

Health is a human right and one of the elements of welfare that must be realized following the ideals of the Indonesian people as referred to in Pancasila and the 1945 Constitution of the Republic of Indonesia.[7] Provisions of Law No. 36 of 2009 concerning Health, there are forms of health services, namely promotive health services, preventive health services, rehabilitative health services, curative health services, and traditional health services.[8] As one of the main components of providing health services to the community, medical services for doctors and dentists are vital because they are directly related to providing health services and the quality of services provided.
The principles and objectives of the implementation of medical practice are the foundation based on scientific values, benefits, justice, humanity, balance as well as protection, and patient safety.\[9\] In terms of health services, the profession of health workers, this case, doctors, has a lot to do with ethical problems that can potentially lead to medical disputes. Law No. 36 of 2009 concerning Health brings changes by providing more protection and legal certainty for both service providers as health workers (as stated in Article 21 to Article 29) and recipients of health services (as stated in Article 56 to Article 58), because in practice medical disputes often arise as a result of unsatisfactory results from the health service, due to lack of information from doctors or negligence arising from medical personnel themselves.\[10\] This is in line with the provisions of Article 66 of Law No. 29 of 2004 concerning Medical Practice, which affirms that “patients can complain to doctors or hospitals for alleged violations to the Indonesian Indonesian Medical Disciplinary Board (MKDKI)”.

2. Mediation of Out of Court Dispute Resolution

Disputes in health worker services provide space for parties who feel aggrieved (patients) to take the path of settlement in court both in civil, criminal charges, and administration.\[11\] This is reflected in Article 66 paragraph (3) of Law no. 29 of 2004 concerning Medical Practice, namely “Complaints do not diminish the right of everyone to report suspected criminal acts to the competent authority and/or sue for civil damages to the Court”.

In suspected disciplinary violations in health services, to uphold the principle of faster, cheaper, effective, and efficient dispute resolution by patients or patients’ families with solutions through means outside the court, it has not been used widely. Furthermore, with the increasing number of patients filing lawsuits in court, it will reduce public confidence in medical practice as the main component of health service providers to the community.

Etymologically, the term mediation comes from the Latin “mediare,” which means being in the middle.\[12\] This meaning refers to the role played by a third party as a mediator in carrying out its duties to mediate and resolve disputes between the parties. “Being in the middle” also means that the mediator must be in a neutral position and not take sides in resolving disputes. He must be able to protect the interests of the disputing parties fairly and equally, thus fostering the trust of the disputing parties.\[13\] Settlement of disputes over suspected violations of doctor’s discipline outside the court or known as non-litigation through mediation, as one of how the effectiveness of reaching an agreement on disputes arising is based on good intention.

Several provisions of laws and regulations that regulate and implement Mediation as a dispute settlement, namely:

a. Mediation Based on Law No. 30 of 1999 concerning Arbitration and Alternative Dispute Resolution

Based on Law No. 30 of 1999 concerning Arbitration and Alternative Dispute Resolution, there are several provisions, namely:

a) The provisions of Article 1 point 1 state that: “Arbitration is a way of resolving a civil dispute outside the general court based on an arbitration agreement made in writing by the disputing parties.”

b) Article 1 point 10 states that:

“Alternative Dispute Resolution is a dispute resolution institution or difference of opinion through a procedure agreed upon by the parties, namely settlement outside the court through consultation, negotiation, mediation, conciliation, or expert judgment.”

The provisions above, arbitration is a way of resolving disputes chosen by the parties to help resolve the dispute and provide an opinion on a specific legal relationship. The result of the agreement is a form of agreement agreed upon by the parties on the result of a binding win-win solution. Disputes that have been settled to be resolved through arbitration will not proceed to the judiciary.\[14\] The existence of Arbitration institutions in Indonesia,
known as BANI (BANI Arbitration Center), BAPMI (The Indonesian Capital Market Arbitration Board), and BASYARNAS (The Indonesian National Sharia Arbitration Board). In its application, the Arbitrator can be selected by each of the disputing parties. However, if the parties do not appoint the arbitrator themselves, they can request court assistance to appoint the arbitrator as examiner and decision-maker of the dispute case.

In line with Arbitration, Article 6 of Law No. 30 of 1999 concerning Arbitration and Alternative Dispute Resolution, several matters are regulated regarding alternative dispute resolution procedures, namely:

(1) The parties can resolve disputes or civil differences of opinion through alternative dispute resolution based on the good intention by overriding the settlement by litigation in the District Court;

(2) Settlement of disputes or differences of opinion through alternative dispute resolution as referred to in paragraph (1) shall be settled in a direct meeting by the parties within a maximum period of 14 (fourteen) days. The results are stated in a written agreement;

(3) If the dispute or difference of opinion as referred to in paragraph (2) cannot be resolved, then with the written agreement of the parties, the dispute or difference of opinion is resolved through the assistance of one or more expert advisors or through a mediator;

(4) If the parties within 14 (fourteen) days with the help of one or more expert advisors or through a mediator fail to reach an agreement, or the mediator fails to bring together the two parties, the parties can contact an arbitration institution or alternative dispute resolution institution to appoint a mediator;

(5) After the appointment of a mediator by an arbitration institution or an alternative dispute resolution institution, within 7 (seven) days, the mediation effort must be initiated;

(6) Efforts to resolve disputes or differences of opinion through a mediator as referred to in paragraph (5) by upholding confidentiality, within 30 (thirty) days an agreement must be reached in writing signed by all parties concerned;

(7) A written dispute settlement agreement or difference of opinion is final and binds the parties to be implemented in good intention and must be registered at the District Court within a maximum period of 30 (thirty) days from the signing;

(8) As referred to in paragraph (7), both parties must complete the dispute settlement agreement or difference of opinion within 30 (thirty) days from registration;

(9) Suppose both parties cannot achieve the peace effort, as mentioned in paragraph (1) to paragraph (6). In that case, the parties based on a written agreement can submit a settlement effort through an arbitration institution or ad-hoc arbitration.

b. Mediation Based on Supreme Court Regulation No. 1 of 2016 concerning Mediation Procedures in Courts

The preamble provisions of the Supreme Court Regulation No. 01 of 2016 concerning Mediation Procedures in Court, letter a states that: “Mediation is a process of dispute resolution that is faster and cheaper, and can provide greater access to the parties to find a satisfactory solution and fulfill a sense of justice”. And in letter b, it is stated: “that in the framework of reforming the bureaucracy of the Supreme Court of the Republic of Indonesia, which is oriented towards the vision of realizing a great Indonesian judicial body, one of the supporting elements is Mediation as an instrument to increase public access to justice as well as the implementation of the principles of simple, fast, and low-cost judicial administration”.

Furthermore, according to Article 1 point 1 in this Supreme Court Regulation, what is meant by mediation, namely: “Mediation is a way of resolving disputes through the negotiation process to obtain agreement from the Parties with the assistance of a Mediator”.

The process of the mediation procedure period in the Supreme Court Regulation No. 01 of 2016 with the
following conditions:

1) The mediation process lasts no later than 30 days from the stipulation of the order to mediate.

2) Based on the Parties’ agreement, the mediation period may be extended by a maximum of 30 days.

3) The mediator shall request an extension of the mediation period with reasons.

Supreme Court Regulation No. 1 of 2016 Article 7 regulates the obligation to carry out mediation with good intention. The parties involved in the mediation process must have good intentions to be carried out and run well with good intentions. Indicators stating that the parties did not have good intentions in carrying out the mediation, namely:

1) Not attending the mediation process even though he has been summoned twice in a row;

2) Present at the first mediation meeting, but subsequently not attending even though they have been summoned twice in a row;

3) Do not attend over and over again so that it interferes with the mediation schedule;

4) Not filing or not responding to the case resume;

5) Not signing a peace agreement.

The implementation of mediation when the parties do not have good intentions has a legal impact on the case examination process. In this case, it can be seen that the legal consequences of the parties who are not in good faith regarding the Plaintiff who does not have good intentions will declare that the lawsuit is not accepted (NO). Furthermore, the legal consequence of Defendant’s not having good intentions resulted in the payment of mediation fees.[15]

c. Mediation Based on the provisions of Law No. 36 of 2009 concerning Health

Mediation in the Health Law is stated in Article 29 of the Health Law, namely, “If a health worker is suspected of negligence in carrying out his profession, the negligence must first be resolved through mediation”. It is further stated in the elucidation of Article 29, “Mediation is carried out when a dispute arises between a health service provider and a patient as a health service recipient. Mediation is carried out to resolve disputes outside the court by a mediator agreed upon by the parties”.

Based on the provisions of the phrase “must” based on The Great Dictionary of the Indonesian Language of the Language Center, it means that 1/ha•rus/adv 1. appropriate; 2. mandatory; must (cannot or may not): Assign mediation steps first “must”, “mandatory”, taken as an effort to resolve disputes determined out of court by the mediator agreed upon by the parties.

d. Mediation Based on the Law of the Republic of Indonesia Law No. 36 of 2009 concerning Health in the Perspective of Legal Politics The New Normal

The order of community life in Indonesia is inseparable from legal provisions to create order, peace, tranquility, happiness, and prosperity, as created in the objectives of the Indonesian state are definitively stated in the fourth paragraph of the preamble to the 1945 Constitution.[17]

The role of law as a tool to achieve the goals of the state must also function and always be based on the four basic principles of legal ideals (rechtsidee), namely: [18]

1. Protecting all elements of the nation for the sake of integrity (integration)

2. Realizing social justice in the economic and
social fields

3. Acknowledging the sovereignty of the people (democracy) and state law (nomocracy)

4. Creating tolerance based on humanity and civility in religious life.

The four principles of legal ideals will guide the realization of the ideals and goals of the state because legal ideals are normative and constitutive belief frameworks. The ideal of law is normative. After all, it functions as an ideal base and prerequisite that underlies every positive law and is constitutive because it directs the law to the state’s goals. In achieving the country’s goals, as a whole, it cannot be separated from Indonesia’s national legal politics. The 1945 Constitution is the basis of Indonesia’s national politics. The existence of national law politics as a legal policy that has been or will be implemented nationally includes: first, legal development consisting of making and updating legal materials to suit current needs; second, implementation of existing legal provisions including affirming institutional functions and fostering law enforcers. [19]

Emphasizing the nature of legal politics, according to Satjipto Rahardjo, is the activity of choosing and the means to be used to achieve specific social and legal goals in society. The politics of law cannot be separated from the ideals of the Welfare State in the constitution. [20] Furthermore, according to Mochtar Kusumaatmadja, political law is a legal and statutory policy in legal reform with legal, political instruments carried out through law. The essence of legal, political thought put forward by Mochtar Kusumaatmadja is related to which laws need to be formed (renewed, changed, or replaced) and which laws need to be maintained so that gradually the goals of the state can be realized. [21]

Based on the political nature of law in the opinion of Satjipto and Rahardjo Mochtar Kusumaatmadja about Law no. 36 of 2009 concerning Health in Article 29 regarding mediation arrangements as mandatory, because it is not further followed by implementing regulations under it. Regulations of the Minister of Health, Regulations of the Medical Council make it challenging to apply them to the settlement of disputes that have the core of deliberation (Indonesian Medical Council Regulation No. 12 of 2015 concerning Procedures for Handling Cases of Suspected Discipline of Doctors and Dentists).

Furthermore, in the absence of any mediation procedural rules and procedures in line with Article 29 of Law No. 36 of 2009 concerning Health brings weaknesses that result in not running optimally as mandated by the Health Law, so the steps of the government to choose and the methods to be used to achieve specific social and legal goals in society through mediation efforts bring about weaknesses that arise, namely:

a) Mediation is mandatory but is not explicitly regulated in other regulations regarding the process and procedures for the mediation to be pursued;

b) The obligation to mediate for suspected health workers of negligence in carrying out their profession is not in line with Article 66 paragraph (3) of the Medical Practice Law. Will the process of mediation efforts delay the process of prosecution or claim for compensation? Considering that the processes and procedures are not regulated, are they based on Law No. 30 of 1999 concerning Arbitration and Alternative Dispute Resolution or based on the Regulation of the Supreme Court of the Republic of Indonesia No. 1 of 2016 concerning Mediation Procedures in Courts?

3. Inconsistency of Indonesian Medical Council Regulation No. 12 of 2015 for Not Regulating Mediation Efforts

Indonesian Medical Council Regulation No. 12 of 2015 concerning Procedures for Handling Cases of Alleged Disciplinary Violation of Doctors and Dentists was set on March 25, 2015. The stipulation of the council’s regulations was long after Law No. 36 of 2009 concerning Health. The basis for consideration of the Indonesian Medical Council Regulation No. 12 of 2015 as stated in the preamble:
a. That the enforcement of Doctor and Dentist discipline is part of the effort to provide protection for Doctors and Dentists and the public;

b. Whereas the procedures for enforcing the discipline of Doctors and Dentists are regulated in the Regulation of the Indonesian Medical Council No. 20 of 2014 concerning Procedures for Handling Cases of Alleged Discipline Violation of Doctors and Dentists need to be adjusted to the application of effectiveness;

c. That is based on the considerations as referred to in letters a and b and to implement Article 70 of Law No. 29 of 2004 concerning Medical Practice. It is necessary to stipulate a Regulation of the Indonesian Medical Council concerning Procedures for Handling Cases of Alleged Disciplinary Violation of Doctors and Dentists.

Referring to the above considerations about the procedure for complaints of cases of suspected disciplinary violations by doctors and dentists, several weaknesses were found, namely:

a. Indonesian Medical Council Regulation No. 12 of 2015 concerning Procedures for Handling Cases of Alleged Disciplinary Violation of Doctors and Dentists does not refer to Law No. 36 of 2009 concerning Health has regulated Mediation (Vide Article 29), only referring to Law No. 29 of 2004 concerning Medical Practice (Vide Article 70);

b. Mediation has no legal force, is challenging to achieve, is not an effective measure in resolving suspected violations by doctors and dentists because it is not strictly regulated how the process and procedures are. This can be compared to how the process and procedures for mediation settlement as described in Law No. 30 of 1999 concerning Arbitration and Alternative Dispute Resolution and Supreme Court Regulation No. 01 of 2016 concerning Mediation Procedures in Courts.

c. Law No. 36 of 2009 concerning Health has provided an opportunity for the necessity of resolving health service disputes through Mediation. Still, these opportunities have not been accommodated, and there is no precise regulation, specifically a reference for complainants as stated in the Regulation of the Indonesian Medical Council Number 32 of 2015.

Conclusion

Based on all the descriptions above, it can be concluded that health services run by doctors and dentists for patients are very susceptible to medical disputes. Health Law No. 36 of 2009 has covered the legal basis for health. Conceptually, it reflects the existence of a health law principle that rests on the right to health care as a social basis (the right to health care) which is supported by 2 (two) individual fundamental rights consisting of the right to information (the right to information) and the right to determine the right of self-determination to achieve safety for patients in an optimal health degree according to the principle “Agroti Salus Lex Suprema” - Patient safety is the highest law.

The legal incident that befell Dr. Setyaningrum, and Dr. Ayu et al. will not happen again to maintain the honor of the medical and dentistry professions. They have been allowed to handle suspected disciplinary violations by doctors and dentists using mediation as mandated in Article 29 of Law No. 36 of 2009 concerning Health. With no concrete implementing regulations regarding procedures and processes of mediation in the Regulation of the Indonesian Medical Council No. 12 of 2015 concerning Procedures for Handling Cases of Alleged Disciplinary Violation of Doctors and Dentists is set on March 25, 2015, so mediation in medical dispute resolution has not run optimally.

Suggestion

The Regulation of the Indonesian Medical Council in the procedure for handling cases of suspected disciplinary violations by doctors and dentists includes a clause in the Mediation process as an obligation that must be followed as in the application of civil procedural law in force in the Court.

To realize the objectives of the Government’s Political Law through legal reform, the provisions of
Article 29 of Law No. 36 of 2009 concerning Health do not only cover disciplinary violations of doctors and dentists but other health workers, hospital services. Mediation should be implemented by making Mediation guidelines to achieve effectiveness in resolving health service disputes and upholding dignity in human rights.

**Ethical Clearance**  : Not applicable

**Source of Funding**  : Self

**Conflict of Interest**  : Nill

**References**


Evaluation of the Social Wellbeing Status and Associated Factors in Students of Dezful University of Medical Sciences (Iran): A Cross-sectional Study

Fereshteh Sohrabivafa¹, Kamal Azam², Malihe Sohrabivafa³
¹Assistant Professor of Health Education and Promotion, Department of Community Medicine, School of Medicine, Dezful University of Medical Sciences, Dezful, Iran, ²Associate Professor of Biostatistics, Department of Epidemiology and Biostatistics, School of Public Health Tehran University of Medical Sciences, Tehran, Iran, ³PhD Student, Department of Health Education and Promotion, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Objectives: The aim of this study was to determine the social wellbeing status and related factors in students of Dezful University of Medical Sciences in 2016. Materials and Methods: The present study was a cross-sectional study that was conducted on 248 students of Dezful University of Medical Sciences in 2016. Students from different disciplines were selected by quota sampling method. The data collection tool was a questionnaire in two areas. The first part consisted of demographic and background characteristics and the second part was social well-being questionnaire of Keyes which was used for the assessment of social wellbeing and its various dimensions. Statistical analysis was conducted using SPSS 24 (multiple linear regression model, independent t-test, Analysis of Variance (ANOVA)) and P-value<0.05 was considered statistically significant. Results: The number of male and female were 114 (46%) and 134 (56%); respectively. The mean of age was 22.81 ± 3.09 years and the majority of students were in the age group of 21-25 years. The mean scores of well-being, social actualization, social integration, social coherence, social acceptance and social contribution were 67.24, 14.14, 9.91, 12.04, 13.42 and 17.70; respectively. The multiple linear regression model showed that age (β=0.59), sex (β=1.13), medical field (β=2.87) and student’s place of residence (β=1.07) were the most important predictors of social wellbeing (P-Value<0.05). Conclusions: Age, sex, medical field and student’s place of residence were the most important predictors of social wellbeing in the students, therefore, it is necessary to pay attention to these factors to promote social wellbeing of students.

Key words: Social Wellbeing, Students, A Cross-sectional Study, Dezful, Iran

Introduction

Health is the most fundamental issue in all cultures and an indicator of the well-being of today’s societies. Usually, more physical dimension is considered in health, while in addition to the dimension, health also depends on social factors (¹). Today, social wellbeing has gained a wide role and importance as one of the important dimensions of health along with physical, mental and spiritual health. In other words, health is not just being free from physical and mental illness and human performance in social relations and his thinking about society are also considered as criteria for assessing individual health at the macro level of society (²). Social
wellbeing is the ability to perform social roles effectively and efficiently, evaluation and reorganization the individual’s performance in society and the quality of his relationships with other people, relatives and social groups (3-6). According to Keyes theory, social wellbeing consists of five dimensions: 1- social actualization (believing in the gradual evolution of society and the existence of potential capabilities for positive change), 2- social coherence (belief in understanding, rationality and predictability of society), 3- Social acceptance (individual interpretation of society and characteristics of others), 4- social integration (assessment of the quality of mutual relations in society and social groups) and 5- social contribution (assessment of the individual of his social value and the importance of what he does in society (7-10).

Studies have shown that people with high social wellbeing can more successfully cope with the problems of playing key social roles (11). On the other hand, the active participation of young people in social and civic behaviors is considered as one of the indicators of youth development and promoting their health is one of the main goals in general health policies in different countries (12). Among the strata of society, the students of any society are the future makers of the country and paying attention to their physical, mental, social and cultural health will provide the necessary ground for a dynamic and healthy life in society for years to come (13). Due to the effective role of social wellbeing on other aspects of health and also on the educational status of students, not paying attention to the social dimension of health can increase or exacerbate mental problems such as depression, suicide, substance abuse, decreased quality of life, increased social harm, physical problems and decline in their academic performance (14-16).

Studying at university, which is associated with entering a larger society, different educational, social and cultural environment and economic problems can make many changes in a person’s life in various individual and social dimensions, such as changes in social and human relationships. These changes have a significant impact on students’ health, quality of life and educational status due to their level of skills and social performance (17). Medical students, while having the problems of other students, are also involved with certain problems such as psychological stress caused by the hospital environment and dealing with patients’ problems (18). Therefore, considering that medical students will play an important role in providing, maintaining and promoting community health in the future, and in order to play this important role properly, they themselves must have a desirable level of social health, the aim of this study was to determine the social wellbeing status and related factors in students of Dezful University of Medical Sciences in 2016.

Material and Methods

Study Design and Subjects

The present study was a cross-sectional study to evaluate the social wellbeing of students in Dezful University of Medical Sciences in 2016. Inclusion criteria consisted of student studying at Dezful University of Medical Sciences and informed consent to participate in the research. Exclusion criteria consisted of guest students from other universities and students with a history of less than one semester of study at the Dezful University of Medical Science.

Sample Size and Sampling Method

\[ N = 698 \] (Total number of students)
\[ \alpha = 0.05 \]
\[ Z^2 \times \frac{\alpha}{1-\alpha} = 1.96 \]
\[ d = 1.420 \]
\[ \sigma = 14.20 \]

The study of Farzi H. et al. (19) was used to estimate the sample size. Taking into account the 95% confidence interval and 80% power, the maximum sample size was estimated 248 students based on the following formula:

\[ n = \frac{N \times \sigma^2 \times Z^2}{(N-1) \times d^2 + \sigma^2 \times Z^2 \times 1-\alpha} \]

For sampling, we first took a list of students by gender and field of study, then using the quota sampling
method in proportion to the percentage of students in each field of study according to gender, the samples were selected (proportion to size).

**Data collection**

The data collection tool was a questionnaire in two areas. The first part was related to demographic and background characteristics including age, gender, marital status, field and level of education, household size, parents’ education level and student’s place of residence. The second part of the questionnaire was related to the assessment of social health and its various dimensions, which The Keyes Social Health Questionnaire was used for this purpose. This questionnaire has 20 questions in different dimensions, including social actualization dimension (4 question), social integration dimension (3 question), social coherence dimension (3 question), social acceptance dimension (5 question) and social contribution dimension (5 question). The questionnaire scoring method was the 5-point Likert scale (completely disagree = 1, disagree = 2, have no idea = 3, agree = 4 and completely agree = 5). Of course, this scoring method was inverse for questions 3, 5, 6, 7, 13, 14, 15, 16, 17, 18, 19, 20 (completely disagree = 5, disagree = 4, have no idea = 3, agree = 2 and completely agree = 1). In order to calculate the score related to each dimension and the total score of the questionnaire, the total scores of the questions related to that dimension and the total scores of all questions were used, respectively. The higher scores indicated higher social health. It should be noted that the validity and reliability of this questionnaire to assess the social wellbeing of students in a study in Iran has been confirmed (16, 20).

**Statistical Analysis**

For the descriptive analysis, mean (standard deviation) and frequency (%) were used. Then, to examine the relationship between demographic and background variables with social wellbeing and its various dimensions depending on the assumption of non-normality (according to Kolmogorov-Smirnov test), the Independent-Samples T-test or Mann–Whitney U test and Analysis of Variance (ANOVA) or Kruskal-Wallis test were used. Finally, multiple linear regression model was used to predict the effective factors on the students’ social wellbeing. It should be noted that the SPSS24 software was used to data analysis and P-Value <0.05 was considered as a significant level.

**Ethics Consideration**

First, the objectives of the research were fully explained to the students, and then informed consent was obtained from them. This study was done according to the principles expressed in the Declaration of Helsinki and was approved by the Deputy of Research and Ethics Committee of Dezful University of Medical Sciences.

**Results**

The aim of this study was to evaluate the social wellbeing of students in Dezful University of Medical Sciences in 2016. A total of 248 students were examined. The number of male and female were 114 (46%) and 134 (56%); respectively. The mean of age was 22.81 ± 3.09 years and the majority of students were in the age group of 21-25 years. The majority of participants were studying in the Bachelor of Science (B.Sc. course). Also, 86.60% of these students were single. In terms of field of study, the highest and lowest were related to nursing and operating room with 25.4 and 10.10%, respectively. In addition, the most of the students’ parents had non-academic education and the majority lived in student dormitories (Table 1).
<table>
<thead>
<tr>
<th>Variables</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (year)</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>35 (14.80)</td>
</tr>
<tr>
<td>21-25</td>
<td>181 (76.40)</td>
</tr>
<tr>
<td>≥ 26</td>
<td>21 (8.80)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>134 (54.00)</td>
</tr>
<tr>
<td>Female</td>
<td>114 (46.00)</td>
</tr>
<tr>
<td><strong>Degree</strong></td>
<td></td>
</tr>
<tr>
<td>Associate Degree</td>
<td>17 (7.50)</td>
</tr>
<tr>
<td>B.Sc.</td>
<td>158 (69.30)</td>
</tr>
<tr>
<td>Doctor of medicine</td>
<td>53 (23.20)</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>201 (86.60)</td>
</tr>
<tr>
<td>Married</td>
<td>21 (13.40)</td>
</tr>
<tr>
<td><strong>Field of Study</strong></td>
<td></td>
</tr>
<tr>
<td>Nursing</td>
<td>63 (25.40)</td>
</tr>
<tr>
<td>Medical</td>
<td>55 (22.20)</td>
</tr>
<tr>
<td>Laboratory Sciences</td>
<td>32 (12.90)</td>
</tr>
<tr>
<td>Operating room</td>
<td>25 (10.10)</td>
</tr>
<tr>
<td>Anesthesiology</td>
<td>41 (16.50)</td>
</tr>
<tr>
<td>Medical emergency</td>
<td>31 (12.90)</td>
</tr>
<tr>
<td><strong>Household size</strong></td>
<td></td>
</tr>
<tr>
<td>1-3</td>
<td>71 (29.71)</td>
</tr>
<tr>
<td>4-6</td>
<td>129 (53.97)</td>
</tr>
<tr>
<td>≥7</td>
<td>39 (16.32)</td>
</tr>
<tr>
<td><strong>Father’s education</strong></td>
<td></td>
</tr>
<tr>
<td>Non-academic</td>
<td>133 (62.10)</td>
</tr>
<tr>
<td>Academic</td>
<td>81 (37.90)</td>
</tr>
<tr>
<td><strong>Mother’s education</strong></td>
<td></td>
</tr>
<tr>
<td>Non-academic</td>
<td>155 (74.90)</td>
</tr>
<tr>
<td>Academic</td>
<td>52 (25.10)</td>
</tr>
<tr>
<td><strong>Student’s place of residence</strong></td>
<td></td>
</tr>
<tr>
<td>Dormitory</td>
<td>174 (71.90)</td>
</tr>
<tr>
<td>Rented house</td>
<td>12 (5.00)</td>
</tr>
<tr>
<td>Parents’ house</td>
<td>56 (23.10)</td>
</tr>
</tbody>
</table>
Table 2 shows the mean, standard deviation, minimum and maximum scores of social wellbeing and its various dimensions. The mean scores of social wellbeing, social actualization, social integration, social coherence, social acceptance and social contribution were 67.24, 14.14, 9.91, 12.04, 13.42 and 17.70; respectively.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social Actualization</td>
<td>14.14</td>
<td>2.52</td>
<td>6.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Social Coherence</td>
<td>9.91</td>
<td>2.15</td>
<td>5.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Social Integration</td>
<td>12.04</td>
<td>2.26</td>
<td>3.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Social Acceptance</td>
<td>13.42</td>
<td>2.97</td>
<td>5.00</td>
<td>21.00</td>
</tr>
<tr>
<td>Social Contribution</td>
<td>17.70</td>
<td>2.85</td>
<td>7.00</td>
<td>25.00</td>
</tr>
<tr>
<td>Social wellbeing</td>
<td>67.24</td>
<td>7.40</td>
<td>43.00</td>
<td>86.00</td>
</tr>
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</table>

The multiple linear regression model was used to determine the effective factors on mean of the students’ social wellbeing (Table 3). The results of this model showed that age, sex, field of study and student’s place of residence were the most important predictors of social wellbeing. As can be seen, the value of β for the sex was 1.13 which means that the social wellbeing score was 1.13 unit higher in male than female students. Also, the value of β for the age was 0.59, this means that for every 1 unit increase in the mean of age, the mean of social wellbeing is increased an average of 0.59 units. In addition, the social wellbeing score of students who lived with his parents was 1.07 unit higher compared to those who were in the university dormitory. Finally, the results of multiple linear regression model showed that the independent variables included in the model explained about 0.49 of the variance of social wellbeing score. Other details can be seen in Table 3.

<table>
<thead>
<tr>
<th>Variables</th>
<th>β</th>
<th>S.E</th>
<th>β</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>0.59</td>
<td>0.23</td>
<td>0.26</td>
<td>0.010</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Female</td>
<td>Reference</td>
<td>-</td>
<td>-</td>
<td>0.030</td>
</tr>
<tr>
<td>Male</td>
<td>1.13</td>
<td>1.25</td>
<td>0.07</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Associate Degree</td>
<td>Reference</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B.Sc.</td>
<td>-1.56</td>
<td>3.52</td>
<td>-0.05</td>
<td>0.650</td>
</tr>
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<td>Doctor of medicine</td>
<td>1.54</td>
<td>1.75</td>
<td>0.09</td>
<td>0.380</td>
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<td></td>
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<tr>
<td>Single</td>
<td>Reference</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Married</td>
<td>2.48</td>
<td>-0.11</td>
<td>2.03</td>
<td>0.220</td>
</tr>
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</table>
Cont... Table 3. Determination of the effective factors on the students’ social wellbeing by multiple linear regression model

<table>
<thead>
<tr>
<th>Field of Study</th>
<th>Nursing Reference</th>
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<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical</td>
<td>2.87</td>
<td>0.14</td>
<td>0.12</td>
<td>0.010</td>
</tr>
<tr>
<td>Laboratory Sciences</td>
<td>2.17</td>
<td>1.94</td>
<td>0.09</td>
<td>0.020</td>
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<tr>
<td>Operating room</td>
<td>1.47</td>
<td>2.12</td>
<td>0.05</td>
<td>0.480</td>
</tr>
<tr>
<td>Anesthesiology</td>
<td>1.45</td>
<td>1.79</td>
<td>0.07</td>
<td>0.410</td>
</tr>
<tr>
<td>Medical emergency</td>
<td>3.31</td>
<td>3.11</td>
<td>0.15</td>
<td>0.280</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Father’s education</th>
<th>Non-academic Reference</th>
<th>-</th>
<th>-</th>
<th>0.760</th>
</tr>
</thead>
<tbody>
<tr>
<td>Academic</td>
<td>-0.13</td>
<td>0.45</td>
<td>-0.02</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mother’s education</th>
<th>Non-academic Reference</th>
<th>-</th>
<th>-</th>
<th>0.460</th>
</tr>
</thead>
<tbody>
<tr>
<td>Academic</td>
<td>-1.00</td>
<td>1.37</td>
<td>-0.06</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Student’s place of residence</th>
<th>Dormitory Reference</th>
<th>-</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rented house</td>
<td>0.25</td>
<td>2.52</td>
<td>0.008</td>
<td>0.910</td>
</tr>
<tr>
<td>Parents’ house</td>
<td>1.07</td>
<td>0.41</td>
<td>0.06</td>
<td>0.040</td>
</tr>
</tbody>
</table>

Coefficient of determination (R2) for the model : 0.49

**Discussion**

The aim of this study was to evaluate the social wellbeing of students in Dezful University of Medical Sciences in 2016. A total of 248 students were examined. The number of male and female were 114 (46%) and 134 (56%); respectively. The mean of age was 22.81 ± 3.09 years and the majority of students were in the age group of 21-25 years. The mean scores of well-being, social actualization, social integration, social coherence, social acceptance and social contribution were 67.24, 14.14, 9.91, 12.04, 13.42 and 17.70; respectively. Also, the field of study showed a statistically significant association with all dimensions of social wellbeing except social contribution. In addition, age showed a statistically significant relationship with social coherence and social acceptance. Sex and marital status only with social acceptance showed a statistically significant association. As well as, there was statistically significant association between mother’s education and degree’s student with social coherence. Finally, the multiple linear regression model showed that age (β=0.59), sex (β=1.13), medical field (β=2.87) and student’s place of residence (β=1.07) were the most important predictors of social wellbeing (P-Value<0.05).

This study showed that with increasing age, social wellbeing of students increases (β=0.59), this finding was consistent with the results of studies by Javadi et al. (20) and Mazloomy et al. (21), which were conducted to assess the social health of students and related factors in students of Guilan and Hormozgan Universities of Medical Sciences. In addition, our results were in line with the findings of Fathi et al. (11), and Mozaffari et al. (22), who showed that with increasing age among Iranian teachers and nurses, their social wellbeing increases significantly. Generally, aging and its positive effect on students’ social wellbeing indicate that older students have more reasonable behaviors and more social adjustment in the face of problems.
In the present study, the social wellbeing of male students was higher than female students ($\beta=1.13$). This finding is also consistent with the studies of Abdelah Tabar et al. (23) and Fathi et al. (11), these studies attributed the difference to the higher prevalence of physical and mental disorders in girls than in boys. However, some other studies do not agree with our results (20, 21). These studies have mentioned that women are more likely than men to seek information about diseases prevention and also more than men, they feel responsible for their health and are concerned about their health. Also, the prevalence of smoking, alcohol and drugs and other high-risk behaviors that are dangerous to health in men is higher than women (24).

Regarding marital status, the results of the present study showed that the mean score of social wellbeing of married people, although higher than singles, however, the difference was statistically significant. This lack of significance was not consistent with most studies in this field. Studies have shown that being married has always been associated with health and promoting social health, and reduces risky behaviors, especially for men. In addition, marriage also provides emotional and moral support in stressful situations, which ultimately improves people’s social health (22, 23, 25). The inconsistency of this finding with the other studies may be due to the different effects of demographic and environmental factors on health and requires further investigation of these factors.

In the present study, also, social wellbeing in students living with family was more than students living in dormitories ($\beta=1.13$). This finding is in line with similar studies in this field. Students living in dormitories, due to being away from their families and lack of support, may be experience emotional and psychological crises and do not have adequate social skills and performance and reach lower levels of social wellbeing which this reflects the positive effect of family support on social health of students because family support for students at home helps them acquire good social skills and social functions (21, 26).

In addition, our study showed demonstrated that there is a statistically significant relationship between fields of study with field of study with social wellbeing in students under study so that the highest score was related to doctor of medicine and medical emergency with 68.38 and 70.67; respectively. It seems that the reason of different levels of social wellbeing the different disciplines under study is due to the difference in the type and nature of specialized units offered to students during the course of study because passing these units is a factor that can affect student behavior (20, 23). Perhaps one of the reasons for the high level of social wellbeing in medical students compared to other fields is due to the more opportunity faced with challenges and gain the necessary experience to solve various problems from the first year of entering the university. In contrast, a limited number of studies have found low social wellbeing score in emergency medical students. The reason is the low level of education (associate) in this field compared to other fields, because this problem may create a sense of low self-esteem and affect their social health (20).

This study, like other studies, has limitations. Perhaps the most important limitation is the nature of the study, in other words, due to the cross-sectional design of the present study and the measurement of all variables simultaneously, the possibility of examining the causal relationships between the studied variables and the level of social health is limited (27), therefore, the design of longitudinal and prospective researches with higher sample size is proposed.

**Conclusion**

The results of this study showed that age, sex, medical field and student’s place of residence were the most important predictors of social wellbeing in the students, therefore, it is necessary to pay attention to these factors to promote social wellbeing in medical students.

**Conflict of Interest: Nil**

**Ethical Clearance:** This study was performed according to the principles expressed in the Declaration...
of Helsinki and was approved by the Deputy of Research and Ethics Committee of Dezful University of Medical Sciences.

Acknowledgements: This article is extracted from Malihe Sohrabivafa’s MPH thesis in Tehran University of Medical Sciences, Iran. We would like to express our sincere gratitude to the students of Dezful University of Medical Sciences for their cooperation in this research.

Funding: The study was supported by Deputy of Research of Tehran University of Medical Sciences, Iran.

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Age Estimation from Radiological Study of Epiphyseal Fusion Around Wrist Joint in Male Population of North East Madhya Pradesh

Harshwardhan Khushalrao Khartade¹, Rahul Mishra², Priyanka Kumarsen Meshram³, Vidya Garg⁴, Prateesh Shukla⁵, Shashidhar Prasad Garg⁶

¹Associate Professor and Head, Dept. of Forensic Medicine, Shyam Shah Medical College, Rewa, Madhya Pradesh, ²Associate Professor and Head, Dept. of Radiodiagnosis, Shyam Shah Medical College and Associated Sanjay Gandhi Memorial Hospital, Rewa, Madhya Pradesh, ³Nodal Officer, COVID-19 Immunization, District Immunization Centre, Rewa, ⁴Associate Professor (Designated) Dept. of Physiology, ⁵Resident, Dept. of Forensic Medicine, ⁶Professor, Dept. of Forensic Medicine, Shyam Shah Medical College, Rewa, Madhya Pradesh

Abstract

Apart from identification, determination of age is required in various civil and criminal cases. Age estimation by observing appearance and fusion of ossification centres is most accurate and reliable method which is implemented universally. However, it is not possible to establish a uniform standard for age estimation from appearance and fusion of ossification centres for whole India as there is disparity in the timing of appearance and fusion of ossification centres in Indian population due to various factors. Hence, this descriptive observational study was conducted to estimate average age of fusion of ossification centres at lower end of radius and ulna in male population of North East Madhya Pradesh. Total 80 cases in the age group of 12 to 20 years attending the OPD of this tertiary care centre were included in the study. X-rays of both wrists were taken in anteroposterior view at Dept. of Radiology after taking written informed witnessed consent from parents and legal guardians of patients and examined in Dept. of Forensic Medicine.

Age of fusion of lower end of radius and ulna is found to be 17-18 years in males of North East Madhya Pradesh.

Keywords- Age estimation, X-rays, Ossification, Radius, Ulna

Introduction

Identification is the determination of individuality of person based on certain physical characteristics.¹ Age determination is one of the essential factor in establishing exact identity of a living individual. Apart from identification, determination of age is required for civil purposes like employment, consent for marriage, immigration, attainment of majority, competitive sports law suit and criminal purposes like rape, kidnapping, criminal responsibility, prostitution and judicial punishment.² Age estimation in the living becomes more important in developing countries where birth records are often not well maintained. Age estimation by observing appearance and fusion of ossification centres is most accurate and reliable method which is implemented universally.³ Ossification is a continuous process. Some of the bones are cartilaginous and some are membranous in origin. Ossification imparts terminal
shape and texture to them. Changes in ossification of bones occur in predictable order and hence, they are taken into account while estimating the age of a person.

There is disparity in the timing of appearance and fusion of ossification centres of the bones in Indian population. This disparity is mainly due to various factors like racial, hereditary, climatic, and nutritional. Due to these variations, it is not possible to establish a uniform standard for age estimation from appearance and fusion of ossification centres for whole India. Hence, this study was conducted to formulate references in future to estimate the age of males from radiological examination of wrist joint in North east Madhya Pradesh which will be helpful in civil and criminal cases.

Aims and Objectives

To estimate average age of fusion of ossification centres at lower end of radius and ulna in male population of North East Madhya Pradesh.

Material and Methods

This descriptive observational study was conducted in Dept. of Forensic Medicine of this tertiary care centre from October 2019 to October 2020 after obtaining approval from Institutional Ethics Committee. Total 80 males in the age group of 12 to 20 years attending the OPD of this tertiary care centre were included in the study. Cases were equally divided into 8 groups as 12-13, 13-14, 14-15,15-16,16-17,17-18,18-19 and 19-20 according to their age in years where upper limit indicates the completed age and lower limit indicates non completed age. Cases with nutritional, endocrinal and developmental disorders affecting skeletal maturity, cases showing physical deformities and fractures of radius and ulna and those in which birth date is not known or date of birth is not supported by valid proof like birth certificate etc. were excluded from the study. Written informed consent was taken from parents or legal guardians of participants. X-rays of both wrists were taken in anteroposterior view at Dept. of Radiology of this tertiary care centre and examined in Dept. of Forensic Medicine. Process of fusion of epiphysis with metaphysis was divided in five stages based on classification by Sangma W et al

Stage 1: Non union–When the epiphysial cartilage does not begin to decrease in thickness. (Figure No.1)

Stage 2: Commence of union – when the thickness of epiphysial cartilage is found to be reduced appreciably (1/4th united). (Figure No.2)

Stage 3: Incomplete union – when the epiphysis has begun to fuse with shaft and complete union is well underway (1/2 united). (Figure No.3)

Stage 4: Complete union – when the epiphysial cartilage is bony in architecture and its density indistinguishable from the epiphysis and diaphysis in its neighbourhood but an epiphysial line called epiphysial scar can still be distinguished. (3/4 united)(Figure No.4)

Stage 5: Complete union – with absence of epiphysial scar. (Figure No.5)

The youngest age group where there is complete fusion of epiphysis and diaphysis at distal end of radius in 100% cases is taken as criteria for generalization.

Data analysis was done in computer using SPSS software.
Observations and Results

Table No. 1 showing distribution of cases according to age groups

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-13</td>
<td>10</td>
</tr>
<tr>
<td>13-14</td>
<td>10</td>
</tr>
<tr>
<td>14-15</td>
<td>10</td>
</tr>
<tr>
<td>15-16</td>
<td>10</td>
</tr>
<tr>
<td>16-17</td>
<td>10</td>
</tr>
<tr>
<td>17-18</td>
<td>10</td>
</tr>
<tr>
<td>18-19</td>
<td>10</td>
</tr>
<tr>
<td>19-20</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
</tr>
</tbody>
</table>

Table No. 2 showing distribution of cases according to stages of fusion of lower end of radius

<table>
<thead>
<tr>
<th>Age</th>
<th>Stages of fusion of lower end of radius in males</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage 1</td>
<td>Stage 2</td>
</tr>
<tr>
<td>12-13</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>13-14</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>14-15</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>15-16</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>16-17</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>17-18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18-19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>19-20</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

It is evident from table no.2 that complete fusion at distal end of radius is seen in 30% of cases in age group 16-17 years and 100% of cases in age group 17-18 years, 18-19 and 19-20 years. The youngest age group where there is complete fusion of epiphysis and diaphysis at distal end of radius in 100% cases is taken as criteria for generalization. Hence, it can be interpreted as distal end of radius fuses completely at 17-18 years in males of North East Madhya Pradesh.
Table No. 3 showing distribution of cases according to stages of fusion of lower end of ulna

<table>
<thead>
<tr>
<th>Age</th>
<th>Stages of fusion of lower end of ulna in males</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage 1</td>
<td>Stage 2</td>
</tr>
<tr>
<td>12-13</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>13-14</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>14-15</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>15-16</td>
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<td>3</td>
</tr>
<tr>
<td>16-17</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>17-18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18-19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>19-20</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

It is evident from table no.3 that fusion of distal end of ulna is seen in 20% of cases in age group 16-17 years and 100% of cases in age group 17-18 years, 18-19 and 19-20 years. The youngest age group where there is complete fusion of epiphysis and diaphysis at distal end of ulna in 100% cases is taken as criteria for generalization. Hence, it can be interpreted as distal end of ulna fuses completely at 17-18 years in males of North East Madhya Pradesh.

Table No.4 showing comparison of the age of fusion of lower end of radius and ulna estimated by various studies in India with the present study.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population studied</th>
<th>Age of fusion of lower end of radius</th>
<th>Age of fusion of lower end of ulna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hapeworth12 (1929)</td>
<td>Punjabis</td>
<td>16-17</td>
<td>16-17</td>
</tr>
<tr>
<td>Lall R and Nat BS13 (1934)</td>
<td>Uttar Pradesh</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>M.J. Pilliai14 (1936)</td>
<td>South India</td>
<td>18 years</td>
<td>18 years</td>
</tr>
<tr>
<td>Galstaun15 (1937)</td>
<td>Bengal</td>
<td>18 years</td>
<td>18.5 years</td>
</tr>
<tr>
<td>Loomba16 (1958)</td>
<td>Uttar Pradesh</td>
<td>20-21</td>
<td>Beyond 18</td>
</tr>
<tr>
<td>Mehta17 (1963)</td>
<td>Mumbai</td>
<td>18-19</td>
<td>18-19</td>
</tr>
<tr>
<td>Das R et al18 (1965)</td>
<td>Punjab</td>
<td>Beyond 18</td>
<td>Beyond 18</td>
</tr>
</tbody>
</table>
Cont... Table No.4 showing comparison of the age of fusion of lower end of radius and ulna estimated by various studies in India with the present study.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population studied</th>
<th>Age of fusion of lower end of radius</th>
<th>Age of fusion of lower end of ulna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prasad RS et al22 (1976)</td>
<td>Bihar</td>
<td>17-18</td>
<td>17-18</td>
</tr>
<tr>
<td>Wankhade et al28 (2013)</td>
<td>Maharashtra</td>
<td>16-20</td>
<td>16-20</td>
</tr>
<tr>
<td>Shanmugasundaram et al30 (2015)</td>
<td>Tamilnadu</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Krishnamoorthy et al31 (2016)</td>
<td>Khammam</td>
<td>18-19</td>
<td>17-18</td>
</tr>
<tr>
<td>Present study(2020)</td>
<td>Madhya Pradesh</td>
<td>17-18</td>
<td>17-18</td>
</tr>
</tbody>
</table>

Table No.5 showing comparison of the age of fusion of lower end of radius and ulna given by various studies in foreign countries with the present study.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population studied</th>
<th>Age of fusion of lower end of radius</th>
<th>Age of fusion of lower end of ulna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pryor34 (1923)</td>
<td>American</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Paterson35 (1929)</td>
<td>English</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Sidhom and Derry36 (1931)</td>
<td>Egyptian</td>
<td>19-20</td>
<td>19-20</td>
</tr>
<tr>
<td>Ledger and Wassom37 (1941)</td>
<td>Pakistan</td>
<td>Beyond 20 years</td>
<td>18-19 years</td>
</tr>
<tr>
<td>Flecker38 (1942)</td>
<td>Australian</td>
<td>19 years</td>
<td>19 years</td>
</tr>
<tr>
<td>Brash39 (1953)</td>
<td>European</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Breathnach40 (1958)</td>
<td>European</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Greulich and Pyle41 (1959)</td>
<td>American</td>
<td>Beyond 18</td>
<td>18</td>
</tr>
<tr>
<td>Gray42 (1995)</td>
<td>European</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Davies and Parsons44</td>
<td>-</td>
<td>19-20</td>
<td>20</td>
</tr>
<tr>
<td>Present study(2020)</td>
<td>Madhya Pradesh</td>
<td>17-18</td>
<td>17-18</td>
</tr>
</tbody>
</table>
Figure 1- X-ray AP view of wrist of 12 years male showing stage 1 of ossification of radius (Non-union)

Figure 2- X-ray AP view of wrist of 13 years male showing stag 2 of ossification of radius (Commence of union)
Figure 3- X-ray AP view of wrist of 15 years male showing stage 3 of ossification of radius (Incomplete union)

Figure 4- X-ray AP view of wrist of 17 years male showing stage 4 of ossification of radius (Complete union with epiphyseal scar)
Discussion

The cardinal principle of age estimation techniques in juveniles is that the skeleton is continuously growing slowly until the adult age is reached. Fusion of centres of ossification occur during age periods which are remarkably persistent for a particular bone. As per recommendations of the study group on Forensic Age Diagnostics, procedure for estimation of age should consist of a physical examination including measurements of height and weight, determining degree of sexual maturation by Tanner staging, dental examination and radiological examination. Ideally, a longitudinal study assessing the fusion of epiphysis with diaphysis will predict more accurately the age of epiphyseal union. However, this is not ethically possible owing to the health risks to participants caused by repeated radiological examinations. A cross-sectional study involving large number of individuals in each age group will resolve this problem. Typical long bones develop by primary and secondary centres. Initially, the centre is small, round and pinhead sized. It grows peripherally and gradually takes up the osteological details of bony part. This is possible due to complex but dependable system by which the osseous framework of the body develops, grows and matures. Radiologically, primary and secondary centres become apparent as white spots. As bone grows, this white area increase in size and only black plate remains between primary and secondary centres termed as growth plate. It is a layer of hyaline cartilage that remains between epiphysis and diaphysis. Complete disappearance of the epiphyseal plate is termed as fusion. Fusion is not an event but it...
is a process. Epiphyseal scar is a radiopaque line visible at the junction of epiphysis and metaphysis which represents union. It was interpreted as complete fusion in present study. Union of epiphysis is seen about 6 months earlier radiologically than anatomically. In present study, we observed various stages of fusion of epiphysis with diaphysis giving particular importance to last two stages that is stage 4 and 5 as they are having higher practical utility forensically.

We observed fusion of lower end of radius in 17-18 years. Similar to our findings, Pillai MJ14, Gaulstaun15, Prasad RS et al22, Bhise et al25, Vaishnawa et al29, Shanmugasundaram et al30, and Wankhade et al28 also observed fusion of lower end of radius in the same age group. Hapeworth12 observed fusion of lower end of radius in earlier age group i.e.16-17 years in a study conducted on Punjabi population. Lall R and Nat BS13, Loomba16, Mehta17, Das et al18, Saksena and Vyas19, Gupta SMD et al20, Kothari21, Banerjee and Agrawal22, Nemade et al24, Patel et al26, Kadam and Vishwanathan27, Hassan et al2, Krishnamoorthy et al31, Leena et al32, and Dere et al33 noted fusion of lower end of radius in higher age groups. Also, all studies conducted on American population by Pryor34 and Greulich and Pyle41, European population by Brash39, Breathnach40 and Gray42, Australian population by Flecker38, English population by Paterson35, Jordanian population by Al-Qtaita43, Egyptian population by Sidhom and Derry36 and Pakistani population by Ledger and Wassom37 showed that fusion of lower end of ulna occurred in higher age groups than present study.

Differences in fusion of lower end of radius and ulna may be due to climatic, hereditary, racial, and nutritional factors.

**Conclusion**

Age of fusion of lower end of radius and ulna is found to be 17-18 years in males of North East Madhya Pradesh. It can also be concluded that age of fusion of lower end of radius and ulna is highly variable in populations of different countries and population of different regions of same country. Hence, standard data for one population may not be applicable to other population and every region must have their own standard set of data for comparison for accurate age estimation. Small sample size in each age group is a limitation of present study and authors recommend that further studies should be conducted by taking larger sample size for each group for more reliable results and higher applicability of results to population of North East Madhya Pradesh.

**Ethical Clearance** - Necessary ethical clearance has been taken from Institutional Ethics Committee.

**Source of Funding** - Self

**Conflict of Interest** - None

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Complex Training and its Effect on the Speed Force and Quantities of Strength for Specific Distances and the Achievement of 400 Meters Freestyle Under the Age of 20 Years

Ali Hussein Sabri¹, Rafid Saad Hadi², Nadia Shaker Jawad³
¹M.Sc. Student, ²Asst. Prof. Dr., ³Prof. Dr., Faculty of Physical Education and Sports Sciences / University of Kerbala, Iraq

Abstract
Athletics in general, and in particular the 400-meter freestyle event, requires special physical preparation for the parts of the body that greatly participate in the skillful performance so that the running process can be performed in the correct manner and with minimal effort by matching the muscles of the legs, distributing the exerted force during the run and increasing its quantities, from this basis, we must focus on the strength component, because strength has a good effect in the final achievement of the 400-meter freestyle, as modern training methods must be used here to improve the strength component, and among these methods is the complex training whose basis is the mixing of weight exercises with plyometric exercises where weight exercises are stimulated muscles well and with plyometric exercises, allowing contractions moving in a circle (lengthening - shortening), therefore, the importance of the research lies in providing data and information within the complex training to know its effect on the force distinguished by speed and the quantities of strength for specific distances and the achievement of 400 free meters, by applying these double exercises together and because they have an effect on the development of the most important types of muscle capacity, either the problem of research and through the follow-up of the researcher for many training units for 400-meter freestyle runners in Kerbala, the researchers noted the lack of trainers’ use of modern training methods that would raise the level of the muscular capabilities of runners and their reliance on traditional training methods, and the importance of research is to perform exercises in a complex training method and to identify the effect of temporary training on strength distinguished by speed and strength quantities for specific distances and to achieve 400 free meters.

Keywords: complex training, strength distinguished by speed, strength quantities.

Introduction
Athletics is one of the sports spread throughout the world, as it consists of several activities and this is what makes it distinct from the rest of the games, this sport consists of short sprint races, hurdle races, throwing events, medium and long distance races. Each activity has its own rules and laws, as well as records, and these numbers came through the contribution of modern science and studies to the development of achievement and thus benefit from these sciences and studies in the development of various athletics activities.

The 400-meter freestyle race is one of the short distance races, and it is the third of the short sprint competitions after 100 meters and 200 meters, the 400-meter freestyle race takes place on the track, that is, in the open air. This race is characterized by the strength of numbers, and this is due to the strength of competition and the strength of achievement that
runners aspire to and the best evidence; the world record is 43.03 seconds.

New methods have appeared that are used in the development of physical abilities, especially components of muscular ability, and one of these methods is complex training, as this training method depends on the interaction between weight training and playmaking exercises, and this method supports another activation of the nervous-muscular system by increasing muscle contraction associated with the previous discipline and deals with muscular capabilities within the training unit and thus the development of physical capabilities (strength characterized by speed).

The quantities of strength (the force exerted on the ground by the runner) is an important indicator in the 400-meter race, as these quantities differ from one runner to another and according to the distances divided for the race, and this requires good preparation and structured training of the muscular strength represented by the distinctive forces with speed, which is determined by the force of striking the ground and the organized step also, the force of characteristic speed is a positive role in the stages of the 400-meter race, as the force characterized by speed is an important role at the beginning and middle of the stages of the race through the performance of muscle contractions mixed with speed during the sprint and all of this will be developed that will greatly affect the achievement of a 400-meter freestyle sprint.

Therefore, when preparing for training programs for runners, the training goal must be based on the development of the components of muscle strength and focus on the development of the strength component, as this leads to an increase in strength quantities at specific distances from the 400-meter freestyle race and thus to the development of the achievement time by taking advantage of the strength component and must it takes into account the recovery process, where the influential training process is effective in achieving the goal that was previously set, and the most important one of these exercises is the complex training.

The research problem crystallized, through the researcher’s follow-up of many training units for the 400-meter freestyle runners in Kerbala, the researcher noticed the lack of trainers’ use of modern training methods that would raise the level of physical capabilities of runners and their reliance on traditional training methods as these traditional training methods raise the level of capabilities the physical fitness of the runners, but not of the required level, which in the end simulates the good achievement of the 400-meter freestyle.

This is why the importance of the research lies in providing data, statistics and information within the complex training to know its impact on the development of strength distinguished by speed and quantities of force for specific distances and the achievement of 400-meter freestyle runners under the age of 20 years.

Research objective:

- Preparing complex training exercises in developing the force characterized by speed and quantities of strength for specific distances and the achievement of 400 free meters under the age of 20 years.
- Identify to effect of exercises with complex training in developing the force characterized by speed and quantities of force for specific distances and the achievement of 400 freestyle meters under the age of 20 years.
- Identify the effect preference between the experimental and control groups.

Research methodology and field procedures:

Research Methodology

The researcher used the (experimental method) in the manner of the two equivalent groups (control and experimental) with two tests (pre and post), in order
to fit this approach into the nature and problem of the research.

**Community and sample research:**

The researcher identified a society, the research with hostile clubs, Kerbala governorate under 20 years of age for the 400-meter freestyle race, of which (8 clubs) (in front of Al-Mutqeen, Al-Hindiya, Al-Hussainia, Iraq, Al-Ghadriyah, Al-Hur, Al-Mass, Al-Khairat), and who participated officially in the tournaments held by the Central Federation for Athletics and adults. their number (18 runners), and chose the researcher his research sample by random way. The simple (lottery) and by (12 runners) and divided into two groups (control and experimental) and also by the simple random method. (Draw), and the researcher conducted parity between the two research groups in the pre-test for the research variables that were adopted, as shown in table (1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Unit</th>
<th>Group</th>
<th>Mean</th>
<th>Std. Deviations</th>
<th>T value</th>
<th>Sig level</th>
<th>Sig type</th>
</tr>
</thead>
<tbody>
<tr>
<td>legs strength speed</td>
<td>Second</td>
<td>Experimental</td>
<td>10.9300</td>
<td>0.45537</td>
<td>0.663</td>
<td>0.522</td>
<td>Non sig</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>11.1800</td>
<td>0.80337</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strength at the end of the first 50 meters (50 meters)</td>
<td>Newton</td>
<td>Experimental</td>
<td>2603.0000</td>
<td>208.55599</td>
<td>0.208</td>
<td>0.839</td>
<td>Non sig</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>2625.1667</td>
<td>157.17432</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strength to the end of the fourth 50 meters (200 meters)</td>
<td>Newton</td>
<td>Experimental</td>
<td>2541.1667</td>
<td>131.94759</td>
<td>0.304</td>
<td>0.767</td>
<td>Non sig</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>2520.1667</td>
<td>106.08754</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strength of the end of the sixth is 50 meters (300 meters)</td>
<td>Newton</td>
<td>Experimental</td>
<td>2339.8333</td>
<td>93.73669</td>
<td>0.161</td>
<td>0.875</td>
<td>Non sig</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>2350.6667</td>
<td>135.08763</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strength at the end of the seventh of 50 meters (350 meters)</td>
<td>Newton</td>
<td>Experimental</td>
<td>2471.5000</td>
<td>176.68588</td>
<td>0.186</td>
<td>0.856</td>
<td>Non sig</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>2453.5000</td>
<td>157.71335</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Completed 400-meter freestyle</td>
<td>Second</td>
<td>Experimental</td>
<td>53.8350</td>
<td>0.80786</td>
<td>0.832</td>
<td>0.425</td>
<td>Non sig</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>54.1967</td>
<td>0.69460</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
From table (1) it becomes clear to us that the level of significance of the test is greater than the value of the level of significance (0.05), and for all the variables under study, this indicates the lack of significance of the test between the two research groups and that the two groups are equal.

Means, devices and tools used by the researcher:

Methods of data collection:
- Observation.
- Test and measurement.
- The questionnaire.
- Sources and references.

Tools and devices used in the research:
- Force sensor (Dyna Foot).
- Dell laptop (1) of Korean origin.
- Two cameras video with speed (500 p / s), a Korean-made (Casio), and accessories.
- Manual stopwatches, number (3) type Kislo 610 of Chinese origin.
- Sharp hand-held electronic calculator, number (1) of Chinese origin.
- Different weights.
- Boxes of different heights.
- (15) barriers.
- tape measure.
- Two whistles.

Field research procedures:

Determine the search tests:

- The strength speed of the legs: To determine the tests, the researchers looked at a number of scientific sources, and through them, they nominated a group of tests and put them in a questionnaire that was presented to a group of experts and specialists in the science of sports training and strength games of (12) experts. After collecting the forms and unpacking the data, they were treated statistically with the (Ki ²) law, and table (2) shows that.

- Distances to measure the strength applied on the ground: For the purpose of determining the distances for measuring the amounts of force imposed on the ground while running 400 free meters for the research sample, the researcher prepared a questionnaire and presented it to a group of experts and specialists in the science of sports training and strength games and the number of experts and specialists was (12), and after collecting these forms and downloading the data, they were processed. Statistically, by the (Ki ²) and table (2) shows that.
Table (2) shows the statistical treatment ($Ki^2$) of approval and non-acceptance of the opinions of experts and specialists in determining the strength speed test characteristic.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Test</th>
<th>Expert approval</th>
<th>Ki2</th>
<th>Sig type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Agree %</td>
<td>Disagree %</td>
<td>Calculated Tabular</td>
</tr>
<tr>
<td>strength speed of legs</td>
<td>Partridge on one leg for 10 sec</td>
<td>9</td>
<td>75</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Successive jumps in place in 15 seconds</td>
<td>8</td>
<td>66.667</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Partridge for a distance of 36 meters (18 meters for the right-hand man and 18 meters for the left leg)</td>
<td>10</td>
<td>83.333</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Distances</th>
<th>Expert approval</th>
<th>Ki2</th>
<th>Sig type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agree</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of the first 50 meters . (50 meters)</td>
<td>11</td>
<td>91.6</td>
<td>1</td>
</tr>
<tr>
<td>End of the second 50 meters. (100 meters)</td>
<td>8</td>
<td>66.6</td>
<td>4</td>
</tr>
<tr>
<td>The end of the third 50 meters.(150 meters)</td>
<td>7</td>
<td>58.3</td>
<td>5</td>
</tr>
<tr>
<td>End of the fourth 50 meters. (200 meters)</td>
<td>10</td>
<td>83.3</td>
<td>2</td>
</tr>
<tr>
<td>End Fifth of 50 meters. (250 meters)</td>
<td>8</td>
<td>66.6</td>
<td>4</td>
</tr>
<tr>
<td>End of the sixth 50 meters. (300 meters)</td>
<td>11</td>
<td>91.6</td>
<td>1</td>
</tr>
<tr>
<td>The end of the seventh is 50 meters.(350 meters)</td>
<td>10</td>
<td>83.3</td>
<td>2</td>
</tr>
</tbody>
</table>
Determine the tests:

The partridge test for a distance of 36 meters (18 m for the right leg and 18 m for the left leg); (1)

**Purpose of the test:** To measure the strength speed characteristic of the legs.

**Test requirements:** Timer, recorder, stopwatch, tape measure, whistle.

**Test description:** The tester stands behind the starting line, and upon hearing the whistle, it sets off to roll on the right leg to the finish line, then turn to partridge on the left leg and return to the starting line as shown in figure (1).

**Register distance:** The distance is measured to the nearest tenth per second.

Figure (1) The partridge shows a distance of 18 m on the right leg and 18 m on the left leg.

Test run of 400 meters from low start: (2)

**Purpose of the test:** Measuring the time achieved to travel a distance of 400 meters and measuring the force exerted during specific stages by means of a force sensor (Dina foot).

**Test requirements:** Legal stadium, stopwatches

**Test description:** A runner stops behind the starting line with a low starting position after installing a force sensor device on it, and after hearing the signal, start as quickly as possible to the finish line

**Register:** Distance travel time is measured to the nearest tenth of a second. With the preparation of a means of transmission that ensures that the sensor signal remains within the sensing area, to ensure that the changes in force values are read during the run for the distances that have been determined.

**Exploratory experience:**

The researchers conducted an exploratory experiment on Tuesday (1/12/2020) on six runners (400 free meters) from the research community and from outside the research sample and applied the tests listed on them. The purpose of the exploratory experiment was as follows:

- Determine the difficulties and obstacles that will arise during the implementation of the tests and measurements.
- Identify on when to take tests and measurements and how long to take.
- Identify the possibility of measuring the force using a DYNA FOOT sensor.
- Identify the necessary equipment and tools to be available and test their validity.
Pre-test:

The researchers conducted pre-tests and measurements of the research variables (strength distinguished by speed, quantities of strength and achievement) on Sunday (13/12/2020) in order to confirm the degree of measurement and identify the level and work in light of these levels when preparing exercises.

Main experience:

The researcher prepared complex training exercises and implemented them on the experimental group to develop the variables of the research under study, relying on the analysis and review of a large number of scientific sources and references, and the researcher took into account the training level, the age stage and the physical ability of the research sample. Likewise, he took care of training the sample members for the rest of the week, so that the goal of training was one. The exercises were distinguished by the following:

- The exercises were carried out in the special numbers stage.
- The implementation of the training units began on Saturday (19/12/2020).
- The training modules included in the training program continued for a period of (8 weeks).
- The number of training units (Appendix No. 6) during one week was (3 training units) and thus the total number of units prepared was (24 training units).
- The days of the training units were: Saturday, Monday, Wednesday.
- The time for performing the exercises differed in the main section of the training unit, and the time ranged between (48-76) minutes.
- The intensity used in the implementation of the exercises ranged between (80% - 89%) from the maximum repetition of one runner (1RM) and in light of the pre-tests that were applied to the research sample.
- The rest was between repetitions (1 minute) and between groups (3 minutes), and the researcher used the high intensity interval training method.

Post-test:

The post-tests were conducted under the same conditions as those conducted in the pre-tests, on Saturday (2/13/2021).

Statistical means:

The researcher used the SPSS to compare the results in the tests for the two research groups.

Presentation and discussion of results:

Presentation and discussion of the results of the pre and post-tests of the control and experimental groups of force characterized by velocity and quantities of force for specific distances and achievement:

Presenting the results of the pre and post-tests of the control group for the force distinguished by the speed and the amounts of force for the specified distances and achievement:
Table (3) shows the mean, standard deviations, the calculated T value, and the level of significance for the pre and post-tests of the control group of the search variables.

### Difference of standard deviations

<table>
<thead>
<tr>
<th>Distances</th>
<th>Pre-test</th>
<th>Post-test</th>
<th>Means difference</th>
<th>Std. deviations difference</th>
<th>T value</th>
<th>Sig level</th>
<th>Sig type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviations</td>
<td>Mean</td>
<td>Std. Deviations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>legs strength speed</td>
<td>11.180</td>
<td>0.803</td>
<td>10.503</td>
<td>0.624</td>
<td>0.676</td>
<td>0.086</td>
<td>7.864</td>
</tr>
<tr>
<td>End of the first 50 m (50 m)</td>
<td>2625.16</td>
<td>157.17</td>
<td>3030.83</td>
<td>219.33</td>
<td>-405.6</td>
<td>106.56</td>
<td>3.80</td>
</tr>
<tr>
<td>End of the fourth 50 m (200 m)</td>
<td>2520.16</td>
<td>106.08</td>
<td>2787.16</td>
<td>230.32</td>
<td>26.00</td>
<td>77.46</td>
<td>-3.44</td>
</tr>
<tr>
<td>End of the sixth 50 m (300 m)</td>
<td>2350.6</td>
<td>135.08</td>
<td>2677.16</td>
<td>570.53</td>
<td>-326.50</td>
<td>271.57</td>
<td>1.20</td>
</tr>
<tr>
<td>End of the seventh 50 m (350 m)</td>
<td>2453.5</td>
<td>157.71</td>
<td>2479.0</td>
<td>138.58</td>
<td>25.00</td>
<td>24.472</td>
<td>1.04</td>
</tr>
<tr>
<td>Completion of 400 freestyle meters</td>
<td>54.19</td>
<td>0.694</td>
<td>53.23</td>
<td>0.662</td>
<td>0.961</td>
<td>0.072</td>
<td>13.19</td>
</tr>
</tbody>
</table>

Below the level of significance (0.05) and the degree of freedom (n = 5)

Displaying the results of the pre and post-tests of the experimental group for the force distinguished by the speed and the quantities of force for the specified distances and achievement

Table (4) shows the mean, standard deviations, the calculated T value, and the level of significance for the pre and post-tests of the experimental group of research variables.

<table>
<thead>
<tr>
<th>Distances</th>
<th>Pre-test</th>
<th>Post-test</th>
<th>Means difference</th>
<th>Std. deviations difference</th>
<th>T value</th>
<th>Sig level</th>
<th>Sig type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviations</td>
<td>Mean</td>
<td>Std. Deviations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>legs strength speed</td>
<td>10.930</td>
<td>0.455</td>
<td>9.726</td>
<td>0.401</td>
<td>1.203</td>
<td>0.051</td>
<td>23.45</td>
</tr>
<tr>
<td>End of the first 50 m (50 m)</td>
<td>2603.00</td>
<td>208.555</td>
<td>3516.66</td>
<td>181.951</td>
<td>-913.66</td>
<td>146.080</td>
<td>6.255</td>
</tr>
<tr>
<td>End of the fourth 50 m (200 m)</td>
<td>2541.16</td>
<td>131.94</td>
<td>4048.66</td>
<td>338.65</td>
<td>-1507.00</td>
<td>189.92</td>
<td>-7.937</td>
</tr>
</tbody>
</table>
Discussing the results of the pre and post tests for the control and experimental groups for force characterized by speed and strength quantities for specific distances and achievement:

By observing table (3) which shows the results of the pre and post tests for the control group for the research variables, as it was found from the table that there are significant differences between the pre and post tests and in favor of the post-test with respect to the variables (characteristic strength in speed, the end of the first 50 meters, the end of the fourth 50 meters and the achievement of 400 Meter), and it appears from the table as well that there are no significant differences between the pre-test and the post test for variables (end of the sixth of 50 meters and the end of the seventh 50 meters).

The researchers attribute the moral development that took place to the variables (strength, characteristic speed, end of the first 50 meters, end of the seventh 50 meters, and achievement) to the exercises that were given by the trainer and their commitment to it as it was purposeful and effective in relation to the above variables, and it was ineffective for the variables (the end of the sixth is 50 meters and the end of the seventh 50 meters) and this is what appeared in the table in the absence of significance between the pre and post- test for this group.

As for Table (4), which shows the results of the pre and post tests for the experimental group of the research variables, if it appears from the table that there are significant differences between the pre and post tests and in favor of the post tests and for all research variables.

The researchers attribute this moral development to the group’s use of complex training exercises, which depended on a combination of weight exercises with plyometric exercises, which rely on these exercises on the rapid character of muscle fibers through resistance training with weights, jumping and jumping exercises, and the variety of exercises and targeted movements and the ability to rapid responses through these exercises. Which confirms (Ahmed Amin Fawaz, 2008) (3), “The diversity of tools and exercises, all of this will stimulate the players and increase the motivation towards progress and upgrading the athletic level”.

As for the variable strength characteristic of speed, the researcher attributes its development through the use of complex exercises with weight and play, which was of a fast nature and high movement through its exercises, which was represented in frequent and fast movements, and the researcher relied on his viewpoint (Karl Heinz, Kibo Schdotter, 1987) (4), “The force of characteristic velocity has a great correlation in skill performance”. 

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Std Dev</th>
<th>T Value</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>End of the sixth 50 m (300 m)</td>
<td>2339.83</td>
<td>93.736</td>
<td>3605.33</td>
<td>193.720</td>
</tr>
<tr>
<td>End of the seventh 50 m (350 m)</td>
<td>3605.3</td>
<td>193.72</td>
<td>3297.6</td>
<td>307.02</td>
</tr>
<tr>
<td>Completion of 400 freestyle meters</td>
<td>53.83</td>
<td>0.807</td>
<td>51.81</td>
<td>0.737</td>
</tr>
</tbody>
</table>

Below the level of significance (0.05) and the degree of freedom (n = 5)
On the other hand, the exercises used by the researcher, the plyometric exercises that depend on the circuit (development and shortening), which in turn worked to improve the compatibility between the nerves feeding the muscle groups that lead the movement in terms of the increase in the frequency and speed of nerve signals, and this was confirmed (Abu Al-Ola Ahmad Abdel-Fattah, 1997) (5) “The neuromuscular compatibility is one of the most important factors that lead to the development of rapid force”.

The researcher focused on weight training to be strong, quickly and with high intensity, ranging between (80% -9%), and by the method of high-intensity interval training, and this was given a positive impact to achieve the best level in the rapid strength of the material muscles for the two men and this is what we noticed in increasing the quantities of strength for the specified distances for the 400-meter freestyle race, he asserts (Stein H, 1999) (6), “There is a need to increase the intensity to develop muscle capacity, with an emphasis on the importance of the size of the exercises and the amount of muscle contraction”, the development of the characteristic strength with speed and the increase in the strength of the runners helped the development of the achievement time in the 400-meter freestyle race through neuromuscular compatibility and rapid muscle contractions.

**Presentation of the results of the post-tests of the control and experimental groups of force characterized by speed and quantities of force for specific distances and achievement**

<table>
<thead>
<tr>
<th>Distances</th>
<th>Group</th>
<th>Mean</th>
<th>Std. Deviations</th>
<th>T value</th>
<th>Sig level</th>
<th>Sig type</th>
</tr>
</thead>
<tbody>
<tr>
<td>legs strength speed</td>
<td>Experimental</td>
<td>9.7267</td>
<td>0.40118</td>
<td>2.563</td>
<td>0.028</td>
<td>Sig</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10.5033</td>
<td>0.62455</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of the first 50 m</td>
<td>Experimental</td>
<td>3516.6667</td>
<td>181.95128</td>
<td>4.176</td>
<td>0.002</td>
<td>Sig</td>
</tr>
<tr>
<td>(50 m)</td>
<td>Control</td>
<td>3030.8333</td>
<td>219.33848</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of the fourth 50 m</td>
<td>Experimental</td>
<td>4048.6667</td>
<td>338.65538</td>
<td>7.545</td>
<td>0.000</td>
<td>Sig</td>
</tr>
<tr>
<td>(200 m)</td>
<td>Control</td>
<td>2787.1667</td>
<td>230.32101</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of the sixth 50 m</td>
<td>Experimental</td>
<td>3605.3333</td>
<td>193.72007</td>
<td>3.773</td>
<td>0.004</td>
<td>Sig</td>
</tr>
<tr>
<td>(300 m)</td>
<td>Control</td>
<td>2677.1667</td>
<td>570.53358</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of the seventh 50 m</td>
<td>Experimental</td>
<td>3297.6667</td>
<td>307.02030</td>
<td>5.953</td>
<td>0.000</td>
<td>Sig</td>
</tr>
<tr>
<td>(350 m)</td>
<td>Control</td>
<td>2479.0000</td>
<td>138.58427</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Completion of 400</td>
<td>Experimental</td>
<td>51.8183</td>
<td>0.73774</td>
<td>3.499</td>
<td>0.006</td>
<td>Sig</td>
</tr>
<tr>
<td>freestyle meters</td>
<td>Control</td>
<td>53.2350</td>
<td>0.66286</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance level (0.05) and degree of freedom (n = 10)
Discussing the results of the post-tests of the experimental and control groups for force characterized by speed and quantities of force for specific distances and achievement:

Through Table (5), which shows the statistical indicators of the results of the post-tests for the experimental and control groups, the table shows that there are significant differences between the experimental and control groups and in favor of the experimental group in the post-tests.

The researchers attribute this development to the exercises used according to the complex training method, through which the muscular capacity developed (strength characterized by speed and strength quantities to aggregate specific distances), as weight training and plyometric exercises contributed significantly and clearly to the above variables.

The development of the characteristic strength with speed came due to the adaptation of the working muscles due to the added weights in addition to the plyometric exercises, which in turn led to an increase in the capacity of the neuromuscular systems, and this is consistent with what was stated by (Sarih Abdul Karim, 2003) (7), “Muscle fibers have the ability to produce strength Large through the change of resistance, and thus the working motor units will increase and increase accordingly their ability to produce energy”.

In its training units, the researchers took into account the gradient in loads and privacy with the change in the intensity used as well as the repetitions, (Jamal Sabry Faraj, 2012) (8) asserts, “The nervous-muscular system responds better when it is excited in a variable manner all the time, and this system needs to be surprised in order to be forced to adapt to weight training and playmaking exercises, and this is what was taken into account in the training units”.

The exercises used were in different proportions in the complex training, which led to the occurrence of adaptation in the fast fibers and this gave a major role in the production of strength during jogging and this was reflected in the increase in strength amounts and for the specified distances, as weight training gives strength to runners by defining a special muscle They are greatly stimulated, and in return comes the work of the (lengthening and shortening) circuit in the plyometric exercises, and the plyometric exercises are complementary to the work of the strength of runners, and this is in agreement with (Burger, 2000) (9) “Weight training exercises with plyometric left in training based on scientific foundations has a positive effect, on other physical abilities”.

The researchers also find weight training exercises not sufficient for the performance of the runner, as they must be completed with the playmate exercises, and that the fact that the plyometric exercises are a reinforcing factor for strength training and able to improve the level of neuromuscular compatibility, and this is what was confirmed (Jamal Sabri Faraj, 2010) (10) “The weight training does not it alone suffices to give elasticity to the muscles, but hinders muscular capacity and joint mobility, on the contrary, we find that plyometric exercises give multiple movements to the joints and occur at the same time”.

The development of muscle capacity (strength distinguished by speed) and the development of strength quantities have helped the development of the time of achievement, as he (Amer Fakher Shagati et al., 2006) (11) points to the development of ability and the enemy: “The use of vehicle exercises for the 400-meter race is free to use the means (weights and exercises) And with fixed devices, climbing stadiums, jumping exercises, partridges to the front or the top, plyometric exercises, push-ups using crates, use of stadium seats and barriers”.

Conclusions and recommendations:

Conclusions:

- The complex training had a positive effect
on the force distinguished by strength speed and for specific distances of the 400-meter freestyle runners.

- That the development of force characterized by speed and magnitudes of strength for specific distances was positively reflected in the development of the time of achievement of the members of the experimental group.

**Recommendations:**

- The use of complex training because of its positive effect on the development of muscular capacity (strength characterized by speed) and strength quantities of runners.

- Conducting similar research and studies, for other events, and for different training categories.

**Financial disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** “All experimental protocols were approved under the Faculty of Physical Education and Sports Sciences were carried out in accordance with approved guidelines”.

**References**

8. Issa FAW. The effect of physical exercises according to the force sensor device in the development of some physical abilities, the time ratios of partial distances, the length and frequency of the step, and the completion of 400 meters, youth, Master Thesis, College of Physical Education, University of Babylon, 2014.
Febrile Seizures in Thalassemic Patients in Babylon Teaching Hospital for Maternity and Children

Yahya A. Altufaily1, Hakim Yosif Radhi2, Laith Jasim khejani2

1Prof. Dr., University of Babylon, Faculty of Medicine, Iraq. 2Ph.D., Dr., Babylon Teaching Hospital for Maternity and Children, Iraq.

Abstract

Febrile seizures are the most common seizures in children. Their incidence is (2-5 %). To date, the pathophysiology of febrile seizures is unknown. But several hypotheses have been supposed that it may relate with plasma iron level. Such low risk in thalassemic patients whose plasma iron level is high could give some clues to this hypothesis.

Aim of the Study: Determine the risk of febrile seizure in thalassemic patients in comparison with the corresponding control group.

Patients and Methods: This case–control study was conducted on one hundred fifty patients with thalassemia major between six month and six years of age who were visit Babylon center for inherited blood diseases in the Teaching hospital for maternity and children from April 2019 to January 2020. All medical records of thalassemic patients were thoroughly reviewed and the occurrence of febrile seizures are interviewed and compared with the control group. The children in the control group are neither thalassemic nor has iron deficiency anemia.

Results: Febrile seizure was detected in one case of the thalassemic group (0.66%) versus six cases (4%) of the control group. The risk of febrile seizure in the control group was 6 times more than that in the thalassemia group, which was statistically significant (P =0.036).

Conclusion: The risk of febrile seizures in thalassemic patients was (0.16) that of the general children population. Thus, iron overload may be a major factor involving the brain metabolism that prevents febrile seizures.

Keywords: Patients, Children, Maternity, Thalassemic, Febrile Seizures

Introduction

Febrile seizures are seizures that occur between the age of 6 and 60 mo with a temperature of (38oC) or higher, that are not the result of central nervous system infection or any metabolic imbalance, and that occur in the absence of a history of prior afebrile seizures (1). Febrile seizures are the most common cause of convulsions in children and a frequent cause of emergency hospital admissions (2). Between 2% and 5% of neurologically healthy infants and children experience at least one, usually simple febrile convolution (1). Febrile seizures are typically divided into two types: ‘simple’ and ‘complex.’ A simple FS is a generalized seizure (without focal features) which lasts less than (15) minutes and occurs only once during a 24-hour period of fever in a neurologically normal child (3). The pathophysiology of febrile seizures remains unclear. It is generally believed that FS is an age-dependent response of the immature brain to fever (4). This postulation is supported by the fact that most (80-85%) of febrile seizures occur between (6) months and (3) years of age, with the peak incidence at
(18) months (7,8). A positive family history for febrile seizures can be elicited in (25-40)% of children with febrile seizures. Familial clustering studies indicate a doubling of risk in children when both parents, rather than one parent, had febrile seizures (5). Studies show a higher concordance rate in monozygotic as compared to dizygotic twins. Although there is clear evidence for a genetic basis, the precise mode of inheritance is unclear. Most studies suggest that the mode of inheritance of susceptibility to febrile seizures is mostly polygenic and rarely autosomal dominant (6). Certain ion channels in the brain are temperature sensitive and may generate fever-associated synchronized neuronal activity (7). There is also evidence to suggest that hyperthermia-induced hyperventilation and alkalois may play a role (8). Children with febrile seizures have been reported to have significantly low levels of plasma ferritin, suggesting a possible role of iron insufficiency (9). Also low ferritin level or low serum iron, was associated with increased risk of febrile seizures (10). Iron also plays specific roles in the central nervous system (CNS). It is involved in myelin formation, as well as in the production of several neurotransmitters such as dopamine, norepinephrine and serotonin, and generation of GABA activity. In addition, iron overload is implicated as a cause of neuronal death (11). The incidence of febrile seizures in thalassemic children is significantly lower, and iron overload may be a major factor that prevents their occurrence (12,13).

**Aim of the Study**

To determine the risk factors of febrile seizure in thalassemic patients in comparison with the corresponding control group, to prove that high iron storage is a protective factor against febrile seizure.

**Patients and Method**

This case-control study was conducted on one hundred fifty patients with thalassemia major between six months to six years of age, who were attending the center for inherited blood diseases in Babylon Teaching hospital for maternity and children from the first of April 2019 to the end of January 2020. To avoid bias, just one child from each family was selected & interviewed about. All medical records of thalassemic patients were thoroughly reviewed. The children who were diagnosed as thalassemic patients on the basis of clinical manifestations, complete blood count and hemoglobin electrophoresis were studied. We summarized data regarding percentage of hemoglobin, MCV, MCH, MCHC, RDW, type of thalassemia, and serum ferritin level. The control group consisted of one hundred fifty non-thalassemic patients selected at the same hospital with similar sex and age distribution as the cases. Exclusion criteria for both groups were history of any brain lesion, mental retardation, cerebral palsy, family history of epilepsy and history of afebrile seizure. Another exclusion for control group if there CBCs and blood films show a hypochromic microcytic anemia with a high RDW, and reduced RBC it means they have a presumptive diagnosis of iron deficiency anemia.

This exclusion made because of many researches that found iron deficiency anemia is a risk factor for febrile seizure to guarantee better comparism. Questionnaires containing demographic data and past history of febrile seizure, onset of febrile seizure, hospitalization, number of episodes, family history of febrile seizure, history of afebrile seizure and its etiology were included. More information to differentiate febrile seizure from shivering, temper tantrums, breath holding spells, and other types of seizures has been taken if parents reports history of seizure like activity. We exclude (38) controls from the study because they have a presumptive diagnosis of iron deficiency anemia, two others have family history of epilepsy and one case has history of afebrile seizure.

**Data Analysis**

Statistical analysis was carried out using SPSS version 18. Categorical variable were presented as frequency and percentages. The Pearson's, Chi-square test (X2) test was used to determine the
associations between two groups. A P-value of <0.05 was considered as statistically significant. Logistic regression analysis was also done for the analysis by using Fishers exact test.

**Results**

One Hundred fifty thalassemic patients range from six months to six years old, and one Hundred fifty non thalassemic children as a control group were evaluated in this study. Table (1) shows seventy eight thalassemic patients (52%) were male, and seventy two (48%) were female, the sex distribution was comparable with control group in which eighty one (54%) were males and sixty nine (46%) were females and the small differences are statistically not significant.

![Table (1): distribution of thalassemic and control groups by sex.](image)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>78</td>
<td>52</td>
</tr>
<tr>
<td>Female</td>
<td>72</td>
<td>48</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100</td>
</tr>
</tbody>
</table>

P-value = 0.729

Table (2) shows The age distributions are nearly similar between the two groups and the little differences are statistically insignificant.

![Table (2) distribution of thalassemic and control groups by age](image)

<table>
<thead>
<tr>
<th>Age</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>6 months-2 years</td>
<td>38</td>
<td>25.33</td>
</tr>
<tr>
<td>2 years -4 years &gt;</td>
<td>52</td>
<td>34.66</td>
</tr>
<tr>
<td>4 years - 6 years &gt;</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100</td>
</tr>
</tbody>
</table>

P-value = 0.928

Eighty one cases were from rural areas (54%) versus seventy two controls (48%). The residence distribution among two groups shown in table (3)
Table (3): Distribution of thalassemic and control groups by residence.

<table>
<thead>
<tr>
<th>Residence</th>
<th>Cases</th>
<th></th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Rural</td>
<td>81</td>
<td>54</td>
<td>72</td>
<td>48</td>
</tr>
<tr>
<td>Urban</td>
<td>69</td>
<td>46</td>
<td>78</td>
<td>52</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100</td>
<td>150</td>
<td>100</td>
</tr>
</tbody>
</table>

P-value = 0.729

Febrile seizure was detected in (1) case (0.66%) and (6) cases (4%) in the thalassemic and control groups respectively. FS in the control group was (6) times more than that in the thalassemic group, which was statistically significant (P = 0.036) as in table (4).

Table (4): Comparison between FS in the thalassemic and control groups.

<table>
<thead>
<tr>
<th>Risk</th>
<th>Cases</th>
<th></th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td></td>
<td>Number</td>
<td></td>
</tr>
<tr>
<td>Febrile seizure</td>
<td>1</td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>No febrile seizure</td>
<td>149</td>
<td></td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100</td>
<td>150</td>
<td>100</td>
</tr>
</tbody>
</table>

P-value = 0.036

The data of patient with thalassemia and FS with history of admission to our hospital have been reviewed. The information are shown in table (5).

Table (5): Details of the case with thalassemia and FS.

<table>
<thead>
<tr>
<th>Data</th>
<th>Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, sex</td>
<td>(3) years, male</td>
</tr>
<tr>
<td>Onset of seizure</td>
<td>(1) year old</td>
</tr>
<tr>
<td>Hospitalization, period of it</td>
<td>Yes, (2)days</td>
</tr>
<tr>
<td>CSF exam</td>
<td>Negative</td>
</tr>
<tr>
<td>Biochemistry (serum calcium and blood sugar)</td>
<td>Normal</td>
</tr>
<tr>
<td>Temperature</td>
<td>39°C</td>
</tr>
<tr>
<td>Recurrence, number of episodes</td>
<td>Yes, once</td>
</tr>
<tr>
<td>Age of recurrence</td>
<td>(2) years and (4) months</td>
</tr>
<tr>
<td>Prophylaxis</td>
<td>No</td>
</tr>
</tbody>
</table>
Three controls with FS were males and three were females. Four controls with FS have family history of FS while two have no family history of FS.

More informations about the controls with FS are shown in table (6).

**Table (6): Details of the children with FS in the control group.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case I</th>
<th>Case II</th>
<th>Case III</th>
<th>Case IV</th>
<th>Case V</th>
<th>Case VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Age (months)</td>
<td>24</td>
<td>30</td>
<td>48</td>
<td>14</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Number of attack(s)</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Time of seizure(s)</td>
<td>24</td>
<td>18, 24&amp;27</td>
<td>48</td>
<td>10&amp;13</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Family history of FS</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>Yes</td>
<td>Yes in the 1st (2) attacks</td>
<td>Yes</td>
<td>Yes in both attacks</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Prophylaxis</td>
<td>___</td>
<td>no</td>
<td>___</td>
<td>no</td>
<td>___</td>
<td>___</td>
</tr>
</tbody>
</table>

**Discussion**

In accordance with the present study, the risk of febrile seizures in thalassemic patients is lower than that of control group.

The results of this study are consistent with the results of a previous study performed by Auvicha in Thailand\(^{(13)}\). They reviewed (430) patients with β-thalassemia. Three of them had febrile seizure and the rate of febrile seizure was 4.4 times less than the normal population.

In another study by Dauod, (Department of Pediatrics, Jordan University of Science and Technology) it was shown that serum ferritin was significantly lower in cases with febrile seizure compared to individuals in the control group\(^{(14)}\), but in another study, Hartfiel (Department of Pediatrics, University of Alberta, Canada) showed that the rate of Iron deficiency anemia in patients with febrile seizure was two times less than its rate in febrile patients without seizure\(^{(15)}\).

The explanations to support the conclusion that said “high iron storage might probably act as a protective factor against febrile seizure” illustrated below.

It has been known that the complex balance between glutamate-GABA systems plays a crucial role in controlling convulsions. Hyperthermia was reported to reduce the activity of the GABA system while increasing the activity of glutamate via the decrease in glutamate reuptake and therefore inducing the up-regulation of glutamate receptor and resulting in a convulsion.

Iron has been reported to correlate with various functions of the nervous system especially neurons for a long time\(^{(16,17)}\). Iron deficiency was also reported to reduce the GABA metabolism via the alteration of glutamate decarboxylase and GABA transaminase enzymes\(^{(18,19)}\).
Therefore, the effect of iron on febrile seizures could possibly be due to iron overload increasing the activity of GABA system which is the main inhibitory neurotransmitters in the brain. This system is particularly important in suppressing seizures. In the case of febrile seizure in the thalassemic group, there were no data on the serum ferritin level at the time of febrile seizure but the nearer levels of serum ferritin before and after the event are (1420) nanogram/ml and (1575) nanogram/ml respectively. Taking into account the prevalence of iron deficiency anemia in children and the relationship between febrile seizure and the serum ferritin level, as shown in the present and similar previously study, it could be suggested that iron supplement and treatment of iron deficiency anemia could reduce the corresponding risk of febrile seizure\(^1\)\(^{,}10\).

### Conclusions

1. The risk of febrile seizures in thalassemic patients was less than that of the controls.

2. Iron overload may be a factor involving the brain metabolism that prevents febrile seizures.

### Recommendation:

1. Further similar studies are recommended, better to be a multicenter studies which will include larger sample size about the risk of febrile seizure in thalassemic patients and the effect of iron overload in prevention of febrile seizure.

2. Therapy for those with iron deficiency anemia could decrease the risk of seizure recurrence and will prevent other sequelae of iron deficiency in the young children.

3. Special attention for thalassemic patients presented with fever and convulsion to exclude CNS infection and other potential causes as the frequency of FS are very low in those patients.

### Financial Disclosure:

There is no financial disclosure.

### Conflict of Interest:

None to declare.

### Ethical Clearance:

“All experimental protocols were approved under the faculty of medicine and carried out in accordance with approved guidelines”.

### References


12. A momen, RA malamiri, B Dehdezi. Thalassemia major may decrease the frequency of febrile
convulsion in children. 2014; (89): 17-20


Scout Culture and its Relationship with Scout Curricula According to the Perspective of the Sustainable Development Goals among Scout Leaders in Iraq

Magda Abbas Muhammad Ali¹, Mahmood Dawood Salman², Amna Fadel Mahmoud³

¹Lecturer, University of Babylon - Faculty of Physical Education and Sports Sciences / Iraq, ²Prof. Dr., Al-Mustaqbal University - Physical Education and Sport Sciences Department, College, 51001 Hillah, Babil, Iraq, ³Prof. Dr., University of Babylon - Faculty of Physical Education and Sports Sciences / Iraq

Abstract

The Scout movement is considered a tool for upbringing and building for generations in a way that reflects positively on the civilized, scientific and social development of society, for it is no longer just a means to occupy the leisure time of youth, but rather has become a contribution to developing their abilities and behaviour by developing their social skills, instilling sound values and ethics for them, and enriching their knowledge with movements and information that it can contribute to providing them with experiences that qualify them physically, mentally and socially as individuals who are good citizens in their societies to build a generation capable of serving their homeland. Values have a great and direct influence on the work that the mobile performs in terms of controlling his behavior and defining his goals and taking decisions related to its work, and the extent of the relationship of scouting curricula according to the perspective of the sustainable development goals with the application of scout culture in the behavior of scouts, the research objectives. The research community was identified with leaders and scout leaders in the education directorates in the governorates of Iraq except for the Kurdistan region for the academic year 2019-2020, and the researchers identified the research sample in a random way, Whereas, (10) leaders and female leaders were selected for the reconnaissance experiment, (40) leaders and female leaders for the construction sample.

Keywords: Scouting culture, Scouting curricula according to the sustainable development goals perspective.

Introduction

The Scout movement plays multiple roles and functions within the social system, as it is considered a form of general culture and is a practical application of moral ideals, and its fields are full of attitudes through which its practitioners acquire the good qualities that motivate our youth to assume full responsibility for increasing production and investing their energies to double national income in addition to preparing them to assume positions Leadership in different areas of life and their pride in belonging to the group and the homeland (¹)

The Scout movement is considered a tool for upbringing and building for generations in a way that reflects positively on the civilized, scientific and social development of society, for it is no longer just a means to occupy the leisure time of youth, but rather has become a contribution to developing their abilities and behaviour by developing their social skills, instilling sound values and ethics for them, and enriching their knowledge with movements and information that it can contribute to providing them with experiences that qualify them physically, mentally and socially as individuals who are good citizens in their societies to
build a generation capable of serving their homeland. The problem of research The Scout movement did not get much attention in our beloved country, Iraq, and the diversity in its curricula, knowing that the Scout movement in Iraq had started in The year (1918 AD) and its curricula are schools for group work and self-reliance in the time of technology and difficult living conditions, knowing that it was previously receiving attention for all its scouting stages, especially the (wandering) stage because of this stage of great importance in the life of society because it includes the group of university youth who The culture is acquired through education and experience from together. Values have a great and direct influence on the work that the mobile performs in terms of controlling his behaviour and defining his goals And taking decisions related to its work, and the extent of the relationship of scouting curricula according to the perspective of the sustainable development goals and the application of scout culture in the behaviour of scouts, the research objectives The research aims to identify the reality of scouting culture and scouting curricula by the goals of sustainable development and to identify the relationship between scouting curricula by the goals of sustainable development And between the scouting culture of scout leaders in Iraq.

The practical side:

The researchers used the descriptive approach in the survey method, due to its blame and the nature of the research, and the research community was identified with leaders and scout leaders in the education directorates in the governorates of Iraq except for the Kurdistan region for the academic year 2019-2020, and the researchers randomly identified the research sample, as (10) leaders and leaders were selected. For the pilot experiment, (40) leaders and leaders of the construction sample.

Field research procedures:

First: Measuring Scout Culture.

The researchers adopted the Scout Culture Scale for the researcher (Wathiq Hadoud Shuailah), where the researcher relied on applying the Scout Culture Scale to theoretical definitions, which focus that scout culture is the outcome of information that scouts have, through which he can be an idea about the scouting movement to help him develop his personality, and improve His social relations and the development of his cognitive, educational, health and moral aspects, the scale consists of (54) paragraphs and each paragraph has five responses and each response represents a specific situation, and there are five alternatives to the answer in front of each paragraph, which are (always, often, sometimes, rarely, never), positive weights of them (5-4-3-2-1) respectively, and the negative weights are (1-2-3-4-5).

Second: Measuring the Scout curricula according to the sustainable development goals perspective.

The researchers adopted the Scout Curriculum Scale according to the sustainable development goals perspective of the researcher (Magda Abbas Muhammad), where the researchers adopted the application of the scale on the sustainable development goals used in the Scouting curricula, the scale consists of (71) paragraphs and each paragraph has five responses, and each response represents a specific situation. In front of each paragraph there are five alternatives to the answer, which are (always, often, sometimes, rarely, never), the positive weights of which are (4-3-2-1-0) respectively, and the negative weights are (0-1-2-3-4) The highest score obtained by the subject is (284), and the lowest score is (0). The hypothetical mean of the scale (142), and whenever the score obtained by the subject is equal to or higher than the hypothetical mean, indicates the prevalence of the scout curricula according to the perspective of the sustainable development goals among scout leaders.

Psychometric properties of the Scout Culture Scale:

Honesty:
The validity of the Scout Culture Scale and Scouting Curricula was verified according to the sustainable development goals perspective, by presenting their paragraphs to a group of experts and specialists to ensure the validity and suitability of their paragraphs for measurement.

**Stability:**

To verify the stability of the Scout Culture Scale and the Scout Curriculum Scale by the sustainable development goals perspective,

**Halftone segmentation:**

The half-segmentation was calculated by isolating the individual paragraphs from the marital paragraphs and to find out the homogeneity between the individual and marital paragraphs, and then calculate the correlation coefficient between them, so the value of the correlation coefficient between the two halves was (0.767) for the Scout Culture scale and (0.744) for the Scout Curriculum Scale according to the development goals perspective. Since this indicator is concerned with half of the vocabulary, it is necessary to use the (Spearman-Brown) equation to identify the amount of the stability coefficient for all vocabulary. After the statistical procedure for this equation, the stability value of the entire scale was (0.868) for the Scout Culture scale and (0.849) for the Scout Curriculum Scale the scale. According to the perspective of the sustainable development goals, which is a high value, which indicates that the scale is constant.

**Statistical methods:** The Statistical Package for Social Sciences (SPSS) was used.

**Results**

Presenting, analyzing and discussing the results of Scouting culture and Scouting curricula according to the perspective of the Sustainable Development Goals of the Scout leaders, to verify this goal, the researchers used the T-test for one sample t-test to compare the sample mean of (100.144) with a standard deviation of (33.84) with the average hypothesis for the scale was (150), so the calculated T value was (-23.62), which is a function of the level of significance (0.05) and the degree of freedom (250) of (± 1.96). They have psychological security and statistically significant. Table (1) illustrates this.

**Table (1):** It shows the results of the T-test for one sample to indicate the difference in the scouting culture of students of the College of Physical Education and Sports Sciences.

<table>
<thead>
<tr>
<th>variable</th>
<th>Number of paragraphs</th>
<th>default means</th>
<th>mean</th>
<th>standard deviation</th>
<th>t value</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scout Culture</td>
<td>54</td>
<td>162</td>
<td>98.36</td>
<td>23.39</td>
<td>9.37</td>
<td>significance</td>
</tr>
<tr>
<td>Scouting curricula according to sustainable development</td>
<td>71</td>
<td>177.5</td>
<td>125.34</td>
<td>39.18</td>
<td>8.247</td>
<td>significance</td>
</tr>
</tbody>
</table>
This result can be interpreted as naturally expected, as the Scouting curricula, which depend on the perspective of sustainable development, are those that meet the needs of the present without compromising the ability of future generations and helping them meet their own needs.

The relationship of the Scout curricula according to the perspective of the sustainable development goals in Scout culture:

Table (2): It shows the relationship of the Scout curricula according to the perspective of the sustainable development goals in Scout culture:

<table>
<thead>
<tr>
<th>variable</th>
<th>unit of measure</th>
<th>mean</th>
<th>standard deviation</th>
<th>R value</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scout Culture</td>
<td>degree</td>
<td>98.36</td>
<td>162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scouting curricula according to sustainable development</td>
<td>degree</td>
<td>125.34</td>
<td>177.5</td>
<td>0.73</td>
<td>Significant</td>
</tr>
</tbody>
</table>

We conclude from Table (2) that there is a relationship between the Scouting curricula, which is based on a sustainable development perspective, and the Scouting culture adopted by the Scout Leader, scouting curricula being a group of diverse programs that meet the needs of Scouts according to their age, to achieve the principles of the Scout Movement that seeks to educate the individual from all ways. It is also the group of experiences and activities that the Scout Movement provides under its supervision to Scouts with the intention of their contact with these experiences and their interaction with them, and the results of these frictions are the interaction that takes place learning or modification in their behaviour that leads to achieving comprehensive integrated growth. (5) It is a comprehensive general plan for the subjects that the student should study. In school in order to obtain an academic degree or a certificate qualifying to work in a specific profession, “Scout programs” is all the activities that boys and youth do in terms of activities that suit their needs by using the Scout method to achieve the educational goal of the movement, and it includes the various activities on which the activities of the stages depend “is the development that works to achieve A better life for the individual than he is by working to improve the social, economic, cultural and political aspect, especially the health and environmental aspect, as it has a very great impact on the life and health of the individual, sustainable development has become a major issue for many international organizations included in the United Nations, such as the United Nations Development Program (UNDP) and the Organization for Economic Cooperation (OECD), the Sustainable Development Committee emanating from the Earth Summit issued a book that includes four main dimensions of development. Sustainability represented in the economic, social, environmental and technological dimension, and these dimensions give his view on the most important indicators related
to each dimension. (6)

First - the economic dimension:

Several indicators are reflected in the environment in which a person lives, namely:

1- The rate of per capita consumption of natural resources: the rate of per capita consumption must be commensurate with the available resources and in a way that ensures their survival for future generations. It is known that developed countries are more consumers of natural resources than developing countries.

2- Eradication of poverty: Developing countries seek to fight poverty through their indication that poverty does not mean prosperity and the exploitation of all environmental potentials, but rather taking people to their needs and directing resources properly so that an individual can fulfil the requirements of his life and that development is inclusive of the sufficiency of individuals.

3- Equitable Income Distribution: Sustainable development aims at distributing income among all individuals in an equitable manner, and not building social disparities between the rich and the poor, and this requires developing countries to distribute equitably in the resources available to them. (7)

Second - the social dimension:

The social dimension is of prominent importance in achieving sustainable development and there are a set of indicators that define the role of this dimension, namely:

1- Good governance: It is represented in the use of democracy and participation in decision-making, which will be positively reflected in society.

2- Providing health and education: Providing health and education at all school levels is important, and sustainable development has had a prominent role through concern for human development and human building, which will ensure the continuity of development and lasting awareness.

3- Population growth and population distribution:

Sustainable development means determining the population and not overly increasing the capacity of societies, which will negatively affect the environmental depletion as well as the population distribution between rural and urban areas in a thoughtful manner, to reduce the prejudice to green areas and reduce the emitted environmental pollution.

Third - the environmental dimension:

“The environmental dimension represents natural resources (water, energy, agriculture and biodiversity), which is the backbone of sustainable development. It is defined as the interest in managing natural resources by focusing on the quantity and quality of resources on the globe. The concept of environmental development is related to environmental pollution or the overuse of resources that leads to depletion. Natural resources and their collapse, on the other hand, it is related to the concepts of waste recycling and safe disposal of waste.

Fourth - The technological dimension

“The technological dimension and its uses have positive results in raising the income of countries and individuals and improving living conditions. Among its most important indicators, which should be taken into account:

1- The use of clean technology in industry: as technology may result in a pollution tax, therefore it is necessary to choose technology with a clean use that does not cause environmental pollution, and many industrialized countries have passed laws for the use of technology, and financial penalties for those who commit such violations.

2- Adopting advanced technology: it is the process of selecting technology with high quality and appropriate efficiency, as well as using tools and machines with a clean use. (8)
Conclusions

In light of the results reached by the researchers, a set of conclusions were reached:

The members of the research sample, the Scout leaders, enjoy a good level of Scouting culture, as well as there is a moral correlation between the Scout curricula according to the sustainable development goals perspective and the scouting leader’s scout culture.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the University of Babylon and carried out in accordance with approved guidelines”.

References

The Effect of Feedback at Different Times by Using Multimedia in Learning the Performance of Some Basic Skills in Handball For Students

Hamida Obaid Abdulamir¹, Nisreen Hassan Naji², Naseer Ahmad Kamil³
¹Assistant Prof., University of Kufa/Faculty of Education for Girls / Department of Physical Education and Sports Sciences / IRAQ, ²Assistant Prof., University of Kufa/Faculty of Education for Girls / Department of Physical Education and Sports Sciences / Iraq, ³Lecturer, Al-Furat Al-Awsatt Echnical University/ Iraq

Abstract

The handball game has taken to keep pace with the development and progress in the world, where many changes have occurred in the methods of learning and training in various aspects, including in particular changes in the methods of playing teams in terms of applying defensive and offensive plans in order to surprise the competitor, and the use of feedback in the process of learning and developing basic skills By varying the time it is given in order to make the learner have the ability to absorb the skill and the process of choosing the appropriate motor program to solve the motor duty in the best way and according to the requirements of the situation facing him. Conclusions: The use of times (10, 20, 30 seconds) to provide feedback of all kinds has an impact. Positively in learning basic handball skills and for the three groups, the use of multimedia (visual display device “Data show”, video cameras, color papers for the movement sequence of skills, display of the live model) has a great role in developing basic skills of handball through increasing motivation In competition between players, a time of (10, 30) seconds in providing feedback is more influential in learning basic handball skills.

Keywords: Feedback at different times, basic handball skills

Introduction

The handball game has taken to keep pace with the development and progress in the world, where many changes have occurred in the methods of learning and training in various aspects, especially changes in the methods of playing teams in terms of applying defensive and offensive plans to surprise the competitor, and the use of feedback in the learning process and the development of basic skills By diversifying the time it is given to make the learner have the ability to absorb the skill and the process of choosing the appropriate motor program to solve the motor duty in the best way and according to the requirements of the situation he faces. (¹)

The importance of the research lies in providing feedback at different times using multimedia in developing the basic skills of female students with handball, to be an expressive method used by coaches to achieve the best results. (²)

Practical Part:

Research Methodology:

The researchers used the experimental approach to suit the nature of the research problem.

Research community and sample:

The research community was identified by the students of the Department of Physical Education and Sports Sciences in the College of Education for Girls at the University of Kufa, whose number is (40) students. The sample is (75%) of the size of the community, with (10) female students for each group.
Determine search variables:

Identify some basic skills and shape them with a hand reel:

The researchers prepared a questionnaire to present the basic skills and form them to the experts and specialists in handball to determine the most important of these skills and their shapes, as in Table (1).

Table (1). Questionnaire to present the basic skills and form them to the experts and specialists in handball to determine the most important of these skills and their shapes.

<table>
<thead>
<tr>
<th>Basic Skills</th>
<th>Forms of Skills</th>
<th>Agree</th>
<th>Not Agree</th>
<th>Chi-square</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shooting</td>
<td>Shooting from a high jump</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>insignificant</td>
</tr>
<tr>
<td></td>
<td>Shooting from a fall</td>
<td>7</td>
<td>4</td>
<td>0.818</td>
<td>Not insignificant</td>
</tr>
<tr>
<td>Handling</td>
<td>Whip handling from shoulder level</td>
<td>5</td>
<td>6</td>
<td>0.090</td>
<td>Not insignificant</td>
</tr>
<tr>
<td></td>
<td>Whip handling from head level</td>
<td>9</td>
<td>2</td>
<td>4.45</td>
<td>insignificant</td>
</tr>
<tr>
<td>Receiving and Delivery</td>
<td>Receiving the ball with two hands</td>
<td>6</td>
<td>5</td>
<td>0.090</td>
<td>Not insignificant</td>
</tr>
<tr>
<td></td>
<td>Receiving the ball from a jump</td>
<td>4</td>
<td>7</td>
<td>0.818</td>
<td>Not insignificant</td>
</tr>
<tr>
<td>The plump</td>
<td>straight line</td>
<td>2</td>
<td>9</td>
<td>4.45</td>
<td>insignificant</td>
</tr>
<tr>
<td></td>
<td>zigzag</td>
<td>0</td>
<td>11</td>
<td>11</td>
<td>Not insignificant</td>
</tr>
<tr>
<td>Deception</td>
<td>Simple deception without a ball</td>
<td>5</td>
<td>6</td>
<td>0.095</td>
<td>Not insignificant</td>
</tr>
<tr>
<td></td>
<td>Simple deception with the ball</td>
<td>1</td>
<td>10</td>
<td>7.36</td>
<td>Not insignificant</td>
</tr>
<tr>
<td>Defensive skills</td>
<td>blocking wall one way</td>
<td>4</td>
<td>7</td>
<td>0.818</td>
<td>Not insignificant</td>
</tr>
<tr>
<td></td>
<td>blocking wall two way</td>
<td>10</td>
<td>1</td>
<td>7.36</td>
<td>insignificant</td>
</tr>
<tr>
<td></td>
<td>Defensive moves For both sides</td>
<td>2</td>
<td>9</td>
<td>4.45</td>
<td>Not insignificant</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td></td>
<td>insignificant</td>
</tr>
<tr>
<td>Interview</td>
<td>4</td>
<td>7</td>
<td>0.818</td>
<td></td>
<td>Not insignificant</td>
</tr>
<tr>
<td>Coverage</td>
<td>6</td>
<td>5</td>
<td>0.095</td>
<td></td>
<td>Not insignificant</td>
</tr>
<tr>
<td>Getting rid of seizure</td>
<td>1</td>
<td>10</td>
<td>7.36</td>
<td></td>
<td>Not insignificant</td>
</tr>
</tbody>
</table>
Tests identify the most important forms of basic hand reel skills:

The researchers prepared a questionnaire form for several tests of the basic forms of handball skills to choose the best of these tests from the point of view of experts and specialists, as shown in Table (2).

Table (2). questionnaire form for several tests of the basic forms of handball skills to choose the best of these tests from the point of view of experts and specialists.

<table>
<thead>
<tr>
<th>Skills</th>
<th>Tests</th>
<th>Agree</th>
<th>Not Agree</th>
<th>Chi-square</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shooting</td>
<td>Shooting from jumping high on overlapping rectangles</td>
<td>7</td>
<td>4</td>
<td>0.818</td>
<td>Not insignificant</td>
</tr>
<tr>
<td></td>
<td>Shooting from jumping high on the squares with the accuracy of the correction</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>insignificant</td>
</tr>
<tr>
<td>Handling</td>
<td>Handling whip from the level of the head in the form of an oval for 30 seconds and a distance of 3 m</td>
<td>10</td>
<td>1</td>
<td>7.36</td>
<td>insignificant</td>
</tr>
<tr>
<td></td>
<td>Whip handling from head level towards a target 20 m</td>
<td>5</td>
<td>6</td>
<td>0.095</td>
<td>Not insignificant</td>
</tr>
<tr>
<td>Blocking Wall</td>
<td>Two-way blocking wall test</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>insignificant</td>
</tr>
<tr>
<td></td>
<td>One-way blocking wall test</td>
<td>8</td>
<td>3</td>
<td>2.27</td>
<td>Not insignificant</td>
</tr>
<tr>
<td>Defensive moves</td>
<td>Test defensive moves for both sides</td>
<td>5</td>
<td>6</td>
<td>0.095</td>
<td>Not insignificant</td>
</tr>
<tr>
<td></td>
<td>Various defensive moves test</td>
<td>9</td>
<td>2</td>
<td>4.45</td>
<td>insignificant</td>
</tr>
</tbody>
</table>

The tabular value of $\text{Ka}_2$ is below the level of $(0.05)$ and the degree of freedom $(1)$ equals $(3.84)$.

Determine feedback times:

To determine the times of the feedback, the two researchers prepared a questionnaire form that includes several suggested times and is presented to a group of experts and specialists in the fields of motor learning, teaching methods and handball to choose three appropriate times for the feedback. (3)
Table (3). Shows the percentage of opinions of experts and specialists about feedback times

<table>
<thead>
<tr>
<th>Suggested times</th>
<th>chosen time</th>
<th>number of experts and specialists</th>
<th>percentage of expert opinions</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 seconds</td>
<td></td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>10 seconds</td>
<td>*</td>
<td>7</td>
<td>100%</td>
</tr>
<tr>
<td>20 seconds</td>
<td>*</td>
<td>7</td>
<td>100%</td>
</tr>
<tr>
<td>30 seconds</td>
<td>*</td>
<td>7</td>
<td>100%</td>
</tr>
<tr>
<td>40 seconds</td>
<td></td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>50 seconds</td>
<td></td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>60 seconds</td>
<td></td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Exploratory experience:**

The exploratory experiment is one of the most important necessary procedures that the researcher undertakes before performing his final experiment to test research methods and tools and indicate the requirements of accurate and correct work free of difficulties. Conducting exploratory experiments on a sample of (10) female students for basic handball skills tests, the aim of which was:

1. Ensure the efficiency of devices and tools
2. Knowing the time spent for each test as well as the time for the total exams
3. Efficiency of auxiliary work team
4. The level of difficulty of the tests for the research sample
5. Knowing the difficulties facing the researcher to avoid them in the future
6. Finding scientific transactions for tests (stability and objectivity).

**Scientific foundations of basic skills tests:**

**Test validation:**

Honesty is one of the important qualities that a good test should possess. A test that does not have a good percentage of honesty cannot perform its function. To extract the validity of the candidate tests for basic skills, the researcher will present the contents of the tests to a group of experts and specialists. Often it is “by logical judgment about the existence of the characteristic, characteristic, or capacity in question, to investigate whether the proposed method of measurement measures it or not.”

**Stability test:**

To extract the stability factor for basic skills tests, the principle of the static test must be applied, which gives similar results or the same results if applied more than once in similar conditions. The test reliability is also the value expressed on the extent of the test’s accuracy in extracting fixed results if the test is repeated More than once on the same sample to give close results.

**Objectivity:**

Objectivity is defined as “the extent to which the judge or examiner is free from subjective factors. That is, the test is not subject to self-assessments. To extract objective values, the objectivity of the test must be
used, which indicates that the assessors do not differ in judging something or on a particular topic.

**Pre-tests:**

The researcher conducted the pre-tests for the research sample (the first experimental, the second experimental and the third experimental) before starting the main experiment with controlling all the variables.

**Equivalence of the sample:**

To equalize the research groups among themselves, the researcher will work by relying on the results of the pre-test for basic skills and by applying a (t-test) for the results of the pre-tests. Table (4)

**Table (4). The equivalence of the three research groups in the tests under investigation.**

<table>
<thead>
<tr>
<th>Statistical parameters</th>
<th>sources of variance</th>
<th>deviations</th>
<th>degree of freedom</th>
<th>variance</th>
<th>F value</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A test of shooting skill from jumping high</td>
<td>between groups</td>
<td>2.94</td>
<td>2</td>
<td>1.47</td>
<td>2.13</td>
<td>Not insignificant</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>59.97</td>
<td>27</td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head level handling skill test</td>
<td>between groups</td>
<td>0.42</td>
<td>2</td>
<td>0.21</td>
<td>0.22</td>
<td>Not insignificant</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>91.54</td>
<td>27</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A test of defensive moves</td>
<td>between groups</td>
<td>2.84</td>
<td>2</td>
<td>1.47</td>
<td>2.19</td>
<td>Not insignificant</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>59.93</td>
<td>27</td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A test of barrier skill</td>
<td>between groups</td>
<td>0.44</td>
<td>2</td>
<td>0.21</td>
<td>0.25</td>
<td>Not insignificant</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>91.66</td>
<td>27</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tabular value of F = 3.15 at the level of significance (0.05)

**Competition style tutorial:**

The researchers reviewed the educational curriculum followed, after which they provided feedback at various times using multimedia on the vocabulary of this program after reviewing the relevant sources and references. The researchers also conducted personal interviews with many experts in the field of motor learning and training to develop appropriate exercises and special duties And how to give feedback and how to apply multimedia during the main experience in line with the capabilities of the handball players.

* The researchers also adopted multimedia during the educational units, which are as follows.

* Displaying a live model via the laptop for the basic skill being applied.
* Illustrations of the skills in question.

The two researchers prepared coloured papers showing the kinematic sequence of the studied skills to take advantage of them in the player’s knowledge of her mistakes during the motor performance of the skills under study. This helps the player to overcome these errors at the same time.

* Live model display, the researchers performed studied skills in front of the players to identify the correct typical performance.

* Provide the feedback in the form of written text.

Post test:

The researchers conducted the post-tests after completing the educational program, taking into account the same conditions in the pre-tests.

Statistical means:

The researcher used the statistical bag (SPSS) to process the data.

Results

View and analyze the results of the first experimental group tests.

Table (5). The arithmetic mean, standard deviations, and (t) value calculated between the pre and post-tests of the first experimental group tests.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-test</th>
<th>Post test</th>
<th>(t) calculate</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>STD.EV</td>
<td>Mean</td>
<td>STD.EV</td>
</tr>
<tr>
<td>Test of high jump-shooting skill</td>
<td>2</td>
<td>1.5</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>Handling test from head level</td>
<td>9</td>
<td>1.75</td>
<td>13</td>
<td>1.5</td>
</tr>
<tr>
<td>defensive moves test</td>
<td>15</td>
<td>3.5</td>
<td>19</td>
<td>1.75</td>
</tr>
<tr>
<td>Blocking wall test</td>
<td>6</td>
<td>2.5</td>
<td>8</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The tabular value (t) = (2.04) at the level of significance (0.05) and below the degree of freedom (29)
Presentation and analysis of the results of the tests of the second experimental group.

Table (5). The arithmetic mean, standard deviations, and (t) value calculated between the pre and post-tests of the second experimental group tests.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-test</th>
<th>Post test</th>
<th>(t) calculate</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>STD.EV</td>
<td>Mean</td>
<td>STD.EV</td>
</tr>
<tr>
<td>Test of high jump-shooting skill</td>
<td>2</td>
<td>1.5</td>
<td>5</td>
<td>0.75</td>
</tr>
<tr>
<td>Handling test from head level</td>
<td>9</td>
<td>3.5</td>
<td>12</td>
<td>1.5</td>
</tr>
<tr>
<td>defensive moves test</td>
<td>14.5</td>
<td>1.75</td>
<td>17.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Blocking wall test</td>
<td>6</td>
<td>0.75</td>
<td>9</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The tabular value (t) = (2.04) at the level of significance (0.05) and below the degree of freedom (29)

View and analyze the results of the third experimental group tests.

Table (6). the arithmetic mean, standard deviations, and (t) value calculated between the pre and post-tests for the third experimental group tests.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-test</th>
<th>Post test</th>
<th>(t) calculate</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>STD.EV</td>
<td>Mean</td>
<td>STD.EV</td>
</tr>
<tr>
<td>Test of high jump-shooting skill</td>
<td>3</td>
<td>0.75</td>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>Handling test from head level</td>
<td>9</td>
<td>3.5</td>
<td>13</td>
<td>1.5</td>
</tr>
<tr>
<td>defensive moves test</td>
<td>14</td>
<td>1.77</td>
<td>18.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Blocking wall test</td>
<td>7</td>
<td>0.74</td>
<td>10</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The tabular value (t) = (2.04) at the level of significance (0.05) and below the degree of freedom (29)

Discussion

It was evident from the previous tables that the three experimental research groups had developed in a different and different way in the basic skills tests under study, but it is noticed that the group that has the most influence in learning these skills is the first and third group, as the results showed significant differences in favor of these two groups. The researchers attribute the reason for these differences...
to the use of the appropriate time for the feedback that these two groups worked with, which is a time (10, 30) seconds, (8) as the first group that used the time (10) seconds has an immediate feedback. This helped the learner to know his mistakes quickly and try to correct his path through Looking at the colored papers hanging on the stadium wall and the coach’s directions. The second group that used time (30) had the opportunity to look at the clips that the coach had previously filmed and watched to find out the correct performance and try to repeat as seen in the video and through it the learner has knowledge of his wrong performance He corrects the error if it occurs, as this is the learner’s self-evaluation by being an “appropriate” time that allows the learner to recognize weaknesses in performance and correct his mistakes, if any, thus generating a complete perception of his performance in an attempt to improve and develop it, which made this group outperform groups The remaining search. (9)

**Conclusions**

1- The use of times (10, 20, 30 seconds) to provide feedback of all kinds has a positive effect on learning basic handball skills and for the three groups.

2- The use of multimedia (visual display “data show”, video cameras, colour sheets for the movement sequence of skills, display of the live model) has a great role in developing basic handball skills by increasing motivation in competition between players.

3- The time (10, 30) seconds in providing feedback is more influential in learning basic hand reel skills

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** “All experimental protocols were approved under the University of Kufa and carried out in accordance with approved guidelines”.

**References**

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The Attitudes of Iraqi Lecturers towards E-Learning

Alaa Khalaf Awad¹, Fadhel Farhan Kadhum Al-Bahadli²

¹Ph.D., Dr., Community Health Nursing / Higher Health institutes, Alanbar. ²Ph.D., Dr., Community Health Nursing / Ministry of Health

Abstract


Aim: to note the views of Iraqi lecturers towards e-Learning. Did they approve of E-learning? What are the principal conflicts of E-examinations?

Methods: This descriptive, observational, prospective study. It involved 78 haphazardly collected Iraqi lecturers from various colleges and institutes of Al- Anbar. The data gathered by a combination of learners from the higher health institute of Al-Anbar. The research began from 1st February 2021 to 1st March 2021. The survey form about the lecturers’ views towards E-learning by implementing three points agreement Likert scale. Microsoft Excel software used for data analysis. The institute gave the Ethical permission. The dismissed lecturers were those who did not reply.

Results: There were seventy-eight lecturers, aged (25-55) years old. Thirty-three females (43 %) and 45 (57 %) males lecturers. Forty-eight (61.5 %) lecturers had two modules. Forty-nine (62.6 %) lecturers had more than 100 students per a lecturer. Sixty-four (82.2 %) lecturers dislike E-learning. Fifty-five (70.7 %) lecturers did not support E-learning nor E-written examinations.

Keywords: Attitudes, Iraqi, lecturers, Electronic, learning.

Introduction

During COVID-19 lockdown, there was an emerging type of Learning started in the world. This type of learning is called online learning or E-learning.¹ E-learning or online learning is a type of learning gained by electronic tools and media. E-learning passes from the teacher by the net, to the students. Students get the learning syllabus online at far distant places. E-learning is a developed learning method in Europe, the USA, and Japan. There are good Infrastructures to send these learning types like online degrees, courses. (Singh V, 2019).

E-learning has many kinds:

1. Synchronous type: in this kind, the lecturer must be present online with the students at the same time.

2. Asynchronous learning: it allows the lecturer to participates with extra students like webinars.

3. Blended learning: it is a mixed kind of traditional and eLearning-like workshop. (Khalid A, 2012)

E-Learning strategies embrace many plans for data transfer. So it aids the students to get the learning aims.

1. E-lectures:

2. E-discussion

3. E-problem-solving

4. E-simulation

Each of these strategies had its own characteristics and indications.⁴
There were 169 papers on E-learning topics in the Iraqi scientific journals. The oldest paper was published on this topic was in 1986. This indicates that Iraq is the first country in the middle east that E-learning.

Iraqi researchers developed well-designed strategies and programs of E-learning. Iraqi scientists understand who needs E-learning and when to apply. Although, there were simple computer technologies and primitive use of E-learning measures. In 2005 there was a paper about E-learning use in Iraq. Since 2005, many Iraqi studies have deliberated the E-learning views in different aspects. Iraq developed adjusted methods of E-learning. But, E-learning continued in primitive ways. It was not applied until the COVID-19 lockdown. Newton’s platform started in February 2020. The higher institute of Nursing in Al-Anbar started E-learning at the same time. During the COVID-19 pandemic, the academic teachers commenced Google classroom as a platform. Electronic lessons, and examinations completed in the expected time.

E-Learning platforms:

There are many popular e-learning platforms:

The electronic platform gives comfortable entrance to online learning. So, these platforms enable students to interact and motivate their Lecturers. These platforms permit classes and examinations by linking the educational data. The various kinds of electronic platforms are important learning tools. (Ajaj IE, 2020)

1. Google classroom:

Google Classroom is a popular learning platform. It is free and extended in a brief time to many countries, incorporating Iraq. Google Classroom is a popular learning platform. It is free and extended in a brief time to many countries, incorporating Iraq. 10

2. Moodle:

Moodle is another well-known e-learning platform. Many colleges allow Moodle usage. It is as effective as google classroom, because of its comfortable uses. Some universities choose Moodle platform to enable more learning effects. 11

Importance of study:

1. E-learning is a modern type of learning in Iraq.

2. E-learning had many challenges to the Iraqi lecturers.

3. There are few articles in the Al-Anbar government about this topic.

4. This study allows lecturers to improve this method.

5. This study had good addition to the Iraqi and Al-Anbar libraries.

Statement of the problem:

In 2020, there was a COVID-19 pandemic that causes a universal lockdown. This lockdown obligates Iraqi students to be at home. The Iraqi lecturers started E-learning learning to overcome the COVID-19 lockdown. This important topic made this group of students explore the lecturers’ attitudes toward e-learning. Also, this article shows how the Iraqi lecturers change their strategies in learning.

The purposes of the Cross Sectional study are:

1. to show the attitudes of the Iraqi lecturers towards e-Learning.

2. How did the lecturers believed about e-learning?

3. What are the lecturers struggles of E-learning?

4. How can the Iraqi lecturers develop e-learning in the institute?

Methods

This descriptive, observational, prospective study. It involved 78 haphazardly collected Iraqi lecturers from various colleges and institutes of Al-Anbar. The
data gathered by a combination of learners from the higher Health institute of Al-Anbar. The research began from 1st February 2021 to 1st March 2021. The survey form about the lecturers’ views towards E-learning by implementing three points agreement Likert scale.

Microsoft Excel software used for data analysis. The institute gave the Ethical .

**Limitation of the study:**

It is a small sample size & not represents the whole community.

Conflict of interest: none

Ethical committee: vague names, oral consent
Plagiarism: none
Bias risk is low

**Results of the Study**

There were seventy-eight lecturers, aged (25-55) years old. Thirty-three females (43 %) and 45 (57 %) males lecturers. Forty-eight (61.5 %) lecturers had two modules.

Forty-nine (62.6 %) lecturers had more than 100 students per a lecturer. Sixty-four (82.2 %) lecturers dislike E-learning. Fifty-five (70.7 %) lecturers did not support E-learning nor E-written examinations.

---

**Table 1. shows the numbers and percentages of modules per lecturer. N= 78.**

<table>
<thead>
<tr>
<th>Males</th>
<th>Two</th>
<th>Three</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 (19.7 %)</td>
<td>27 (34.8 %)</td>
<td>3 (0.3 %)</td>
<td>45 (57.6 %)</td>
</tr>
<tr>
<td>12 (15.8 %)</td>
<td>21 (26.7 %)</td>
<td>0 (0 %)</td>
<td>33 (42.4 %)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>27 (34.8 %)</strong></td>
<td><strong>48 (61.5 %)</strong></td>
<td><strong>3 (3 %)</strong></td>
</tr>
</tbody>
</table>

**Table 2. shows the numbers of students per lecturer. N= 78.**

<table>
<thead>
<tr>
<th>Males</th>
<th>50-99</th>
<th>100 and more</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (2.9 %)</td>
<td>17 (21.5 %)</td>
<td>26 (33.7 %)</td>
<td>45 (57.6 %)</td>
</tr>
<tr>
<td>3 (3.9 %)</td>
<td>7 (8.5 %)</td>
<td>23 (29.9 %)</td>
<td>33 (42.4 %)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>5 (6.8 %)</strong></td>
<td><strong>24 (31 %)</strong></td>
<td><strong>49 (62.6 %)</strong></td>
</tr>
</tbody>
</table>
Table 3. shows the opinions of the lecturers. N= 78

<table>
<thead>
<tr>
<th>Question</th>
<th>Agree</th>
<th>Neutral</th>
<th>Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1 Did you prefer E-learning to Traditional learning?</td>
<td>7 (8.9 %)</td>
<td>7 (8.9 %)</td>
<td>64 (82.2 %)</td>
</tr>
<tr>
<td>Q2 Is E-learning harder than traditional learning?</td>
<td>26 (33.4 %)</td>
<td>15 (19.1 %)</td>
<td>37 (47.5 %)</td>
</tr>
<tr>
<td>Q3 Does it better to register the students’ attendance in e-learning than traditional learning?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 (20 %)</td>
<td>18 (22 %)</td>
<td>44 (56 %)</td>
</tr>
<tr>
<td>Q4 Is the modules demonstration better in E-learning than traditional learning?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 (16.5 %)</td>
<td>17 (21.5 %)</td>
<td>48 (62 %)</td>
</tr>
<tr>
<td>Q5 Does the modules preparation is better in E-learning?</td>
<td>12 (16 %)</td>
<td>13 (16.5 %)</td>
<td>53 (67.5 %)</td>
</tr>
<tr>
<td>Q6 Does Electronic examinations more difficult in E-learning?</td>
<td>46 (59 %)</td>
<td>10 (12.8 %)</td>
<td>22 (28.2 %)</td>
</tr>
<tr>
<td>Q7 Do score evaluation harder in the electronic exam?</td>
<td>42 (53.8 %)</td>
<td>12 (16 %)</td>
<td>24 (30.2 %)</td>
</tr>
</tbody>
</table>

Table 4. shows the attitudes of the Lecturer towards E-learning. N= 78

<table>
<thead>
<tr>
<th>Question</th>
<th>Agree</th>
<th>Neutral</th>
<th>Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q8 Did you support E-learning?</td>
<td>15 (19.1 %)</td>
<td>8 (10.2 %)</td>
<td>55 (70.7 %)</td>
</tr>
<tr>
<td>Q9 Did you encourage E-oral examinations?</td>
<td>20 (25.6 %)</td>
<td>11 (14.1 %)</td>
<td>47 (60.3 %)</td>
</tr>
<tr>
<td>Q10 Did you boost E-written examinations?</td>
<td>18 (23 %)</td>
<td>6 (7.6 %)</td>
<td>54 (69.4 %)</td>
</tr>
</tbody>
</table>

Discussion

In COVID-19 lockdown, there was an emerging type of Learning started in the world. This new type is called an E-learning type. \(^1\)

It is gained by electronic tools and media, passed from the lecturer by the net, to the students. All the Iraqi lecturers used E-learning. In this study, a group of Iraqi lecturers expressed their attitudes towards E-learning.

In this study, there were seventy-eight Iraqi lecturers. This study covered a group of a haphazard collection of diverse lecturers from different colleges and various specialties.

This diversity of elections gives the study its originality and unique character.
Thirty-three female’s lecturers (43 %) participated in this study. Female selection is not biased. But, the selection of a group depends on who wants to take part. Some of the females felt shy or did not want to take part.

Table 1 and fig. 1 show the numbers and percentages of modules per a lecturer.

There were 45 (57 %) males lecturers. Male dominance is not intended. The researchers called for lecturers’ participation in different ways and optional ways.

Forty-eight (61.5 %) lecturers held two modules. These lecturers express the majority of the lecturers. Twenty-seven (34.8 %) of them were males, while females were 21 (26.7 %). Twenty-seven lecturers (34.8 %) possessed one module. This group received the practice module or had more than 100 students. Fifteen (19.7 %) of them were males, and twelve (15.8 %) were females. However, the male lecturers were more than females. Three (0.3 %) of lecturers who received three modules, were males only. This means that males were more tolerant of the extra work than females.

This result is similar to many previous researches like 12 Table 2 and fig. 3 show the numbers of students per a lecturer.

Forty-nine lecturers (62.6 %) had more than 100 students per a lecturer. This means more stress and extra effort of learning. This task in E-learning needs satisfactory net and stable electricity for the lecturer and the student. Google classroom; is the main platform could not tolerate more than fifty students. Some lecturers used Moodle platform or divide the students into two groups. In both solutions, there was extra work in lesson explanation and attendance registration.

Some lecturers dislike E-learning for this matter. The other lecturers find E-learning is the easiest type in electronic written examinations for a class of more than 100 students. Student crowding is one of the reason to state E-Learning during Covid-19 crisis. This result is parallel to the universal studies like 13

Twenty-four lecturers (31 %) held module/s of (50-99) students. Those lecturers also had many difficulties in lesson explanation and attendance registration. However, there were fewer struggles in electronic written examinations.

Five lecturers (6.8 %) held a class of fewer than fifty students. Those lecturers felt more comfortable with the lesson explanation and attendance registration. But, they felt that electronic oral and written examinations are troublesome. These finding went with many articles like 14

Table 3 and fig. 3 show the opinions of the lecturers.

Sixty-four (82.2 %) lecturers dislike E-learning, while seven lecturers (8.9 %) prefer it. This result may be due to the earlier trials of E-Learning in Iraq. There were many difficulties due to the weak infrastructure of E-Learning in Iraq. This finding went with the established Iraqi papers like 15

Forty-four lecturers (56 %) believed that E-Learning is harder than the traditional model. This result may be due to the structure of the E-Learning, the weak net, or the fluctuating Electricity. It could be the fewer skills in E-Learning or the usual practice of the traditional Type. This result is parallel to the reported studies, like (Jabir AC, 2020)

Forty-eight lecturers (62 %) rejected the modules demonstration is better in E-learning. This event could be interpreted as the little experience of lecturers in their computer skills. While the thirteen lecturers (16.5 %) agreed to the module’s demonstration is better in E-learning. This debate may be due to their sufficient computer skills. 17

Fifty-three lecturers (67.5 %) disapprove of module preparation in E-learning.
This result might be due to lecturers’ proficiency in electronic skills. Yet, twelve lecturers (16%) prefer modules preparation in E-learning. This finding went with previous studies, like (Timothy M, 2018)

Forty-six lecturers (59%) accepted that Electronic examinations are more difficult in E-learning due to their primitive expertise. This challenge arose from the type of questions, and to prevent fraud. The use of some kinds of questions leads to exam cheats. The nature of questions that reduces cheats was the essay questions and multiple-choice tests.

This result is the same as the mentioned articles, like 19

Forty-two lecturers (53.8%) think that the score evaluation is harder in the electronic exam due to their trials to stop cheats among the students. Cheats could be the principal barrier against the reliability of the electronic exam. Nevertheless, there were 24 lecturers (30.2%) who did not agree by using tricky multiple-choice questions or adjustments of essay questions. This result went well with the mentioned reports, like 20

Table 4 and fig. 4 show the attitudes of the Lecturer towards E-learning.

Fifty-five (70.7%) did not support E-learning. This is because of the primitive experiences of this type. The lecturers may change their opinions in the future because of the increased experiences and skills practices. This upshot is parallel to the reported work of literature like (Qianzhou AH, 2021)

Forty-seven lecturers (60.3%) discourage Electronic oral examinations. This is the result of the lecturers’ struggles in E-learning. The lecturers felt that their sufferings affect the student’s level of training and downgrades learning as a whole. However, there were twenty lecturers (25.6%) who encourage E-oral examinations. Those lecturers asked the student to open the camera and answer the oral exam. Some lecturers use educational pictures, tools, or models and asked their students to answer. These exams need a stable net and stable electricity to perform. The lecturers who dislike electronic exams felt unhappy with the security of the electronic exams. Cheats and fraud are the main struggles.

This result is parallel to the documented studies like (Konishi K, 1998)

Fifty-four lecturers (69.4%) did not boost E-written examinations. The lecturers believed that the electronic tools had insufficient security to prevent cheats and fraud. The simple expertise and the lack of time control in the Google Classroom were the principal barriers. This outcome went in parallel with the mentioned reports like (Villarroel V, 2020)

Conclusions

Electronic Learning is a challenging style of learning. It demands progressive development and configuration to prevent cheats and fraud. The infrastructure of electronic learning is the chief obstacle. The Webinars, conferences, and workshops improve the control of E-examinations and enhance E-learning in Iraq.

Recommendations:

1. E-learning necessitates reliable infrastructure to achieve.

2. Webinars, conferences, and workshops improve the control of E-examinations and learning in Iraq.

3. The lecturers’ good experiences drive good E-learning.

4. The lecturers’ skills in electronic tools create excellent E-learning outcomes.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.
Ethical Clearance: “All experimental protocols were approved under the Community Health Nursing and carried out in accordance with approved guidelines”.

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21. Qianzhou AH. ATTITUDES TOWARDS E-LEARNING SYSTEMS IN A CONVENTIONAL TEACHING-LEARNING ENVIRONMENT IN TUNISIA COLLEGES AND

Alliance of Matrix Metalloproteinase-9 Promoter-1562C/T Polymorphism and Metalloproteinase-9 Saliva Concentration of Iraqi Patients with Chronic Periodontitis

Atyaf Lateef Alturfa¹, Laith Sami Mehdi², Majida Sulaiman Aadai³

¹M.Sc., Department of Clinical Biochemistry, Al-Nahrain University, Baghdad, Iraq. Clinical Biochemistry, ²Consultant, Department of Hematology, Al-Karkh General Hospital Laboratories, Baghdad, Iraq. ³M.Sc., Department of Immunology, AL Karama Teaching Hospital, Kut, Iraq. Master Degree in Medical Laboratory Technology

Abstract

Matrix metalloproteinases (MMPs) are key enzymes responsible for matrix degradation, derived from polymorphonuclear leukocytes, during the early stages of periodontitis. There is plenty of evidence for the role of matrix metalloproteinases in the destructive processes of periodontal disease as a viable target in early diagnosis and chemotherapeutic approach. The aim of this study was to determine the levels of salivary matrix metalloproteinase-9 (MMP-9) in patients with periodontitis and healthy controls and the role of gene polymorphisms in the aetiology of the disease. Levels of MMP-9 in saliva was determined by enzyme linked immune sorbent assay (ELISA) technique in healthy subjects without any periodontal disease (n = 45) and in the patients with diagnosed periodontitis (n = 45). Gene Amplification, gel electrophoresis and genotyping were also carried out on MMP-9 gene. The MMP-9-1562C/T appeared in three genotypes: CC, CT and TT, allele specific gene polymorphism was amplified by PCR technique. The distribution of different genotypes in patients and controls for each of MMP-9-1562C/T polymorphism was in a good accordance with Hardy-Weinberg equilibrium (HWE). Genotypes in both dominant and recessive models were non-significantly associated with periodontitis. Significantly higher salivary MMP-9 was observed in cases of periodontitis compared to healthy adults (p = 0.043). Salivary MMP-9 may serve as a biomarker of periodontal disease monitoring and aid in the early detection of periodontitis.

Key words: Polymorphism, matrix metalloprotinase-9, chronic periodontitis, ELISA, saliva, promoter −1562 C/T.

Introduction

Chronic periodontitis (CP), the most commonly occurring and slowly progressive form of periodontal disease, can lead to continual inflammatory host response, which may finally result in periodontal attachment loss and bone resorption (¹,²). The CP is a highly prevalent disease and has shown to affect 90% of the worldwide individuals (³). Even though the key etiological factor that results in progression of CP is the formation of complex biofilm on the surfaces of teeth adjacent to their periodontal tissues, determinants like demographic, social, environmental, behavioural, systemic and genetic factors have also been coupled with the epidemiology of this disease (⁴).

Matrix metalloproteinase-9 (MMP-9) is a member of a family of proteolytic enzymes that
regulate cell matrix composition by requiring zinc for their proteolytic activities. The MMP-9 cleaves denatured collagen (gelatin), in particular, type IV collagen, which constitutes the major component of the basement membranes \(^5,^6\). This cleavage helps lymphocytes and other leukocytes like dendritic cells (DCs) to enter and leave the blood and lymph circulations. The MMP-9 also cleaves myelin compounds such as myelin basic protein (MBP) and type 2 gelatin, which leads to remnant epitopes that can generate autoimmunity \(^5,^6,^7\). Expression and secretion of MMP-9 by activated lymphocytes and monocytes is tightly regulated by cytokines, chemokines, eicosanoids and peptidoglycans \(^7\). In most cell types, gene transcription of MMP-9 is inducible by cytokines and cellular interactions. The MMP-9 is secreted as a zymogen (proenzyme), which remains inactive unless it is activated by the removal of the propeptide domain by proteolytic enzymes like stromelysin-1, MMP-2 and other MMPs \(^6\).

The aim of this study was to determine the role of gene polymorphisms in matrix metalloproteinase-9 gene in chronic periodontitis and investigate the association of gene polymorphism in this gene using the saliva MMP-9 concentration.

**Methods**

This study included 45 patients with chronic periodontitis and 45 healthy controls. Blood and saliva samples were taken from all participants, DNA was extracted from whole blood for all participants, the Matrix metalloproteinase-9 (MMP-9) gene fragment corresponding the MMP-9-1562C/T SNP was amplified with specific primers using conventional PCR technique. Genotyping was achieved through restriction fragment length polymorphism (RFLP)-PCR. Enzyme linked immune sorbent assay (ELISA) technique was used to measure the saliva concentration of MMP-9.

**Results**

A specific pair of primary was used to amplify Matrix metalloproteinase-9 (MMP-9) gene fragment corresponding the MMP-9-1562C/T polymorphism using PCR, as shown in figure (1).

![Figure (1): Gel electrophoresis of MMP-9-1562C/T gene polymorphism amplified with specific pair of primers using conventional PCR. The PCR product was stained with ethidium bromide. The fragment length was 435 base pair (bp).](image-url)
Genotyping was achieved through RFLP-PCR. According to the cutting pattern, the MMP-9-1562C/T appeared in three genotypes: CC, CT and TT (Figure 2).

Figure (2): Sph I restriction endonuclease restriction fragment length polymorphism analysis of the MMP-9-1562C/T biallelic polymorphism, M, DNA ladder; lanes 1, 3, 5, 8 and 9: GG genotype; lanes 2 and 10: TT genotype; lanes 4, 6, 7, and 11: GT genotype.

bp: base pair.

Table (1) shows that the frequency of CC, CT and TT genotype in patient with periodontitis was 53.33 %, 40 % and 6.67 % respectively which did not differ significantly from that of the healthy controls (68.69 %, 28.89 % and 2.22 %, respectively). Moreover, genotypes in both dominant and recessive models were non-significantly associated with periodontitis. Although the frequency of T allele was higher in the patients than that of the controls (26.67 % versus 16.67 %), the difference did not reach the significant level (OR= 1.82, 95 % CI= 0.88-3.75, p= 0.106).

Table (1): The frequency of different genotypes and alleles of MMP-9-1562C/T polymorphism in both patients and controls.

<table>
<thead>
<tr>
<th>MMP-9-1562C/T Polymorphism</th>
<th>Controls (45)</th>
<th>Patients (45)</th>
<th>P-value (*)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>31 (68.69 %)</td>
<td>24 (53.33 %)</td>
<td>0.274 (NS)</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td>CT</td>
<td>13 (28.89 %)</td>
<td>18 (40 %)</td>
<td>0.201 (NS)</td>
<td>1.8 (0.73-4.36)</td>
</tr>
<tr>
<td>TT</td>
<td>1 (2.22 %)</td>
<td>3 (6.67 %)</td>
<td>0.254 (NS)</td>
<td>3.87 (0.38-39.63)</td>
</tr>
<tr>
<td>HWE</td>
<td>0.788</td>
<td>0.879</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant model</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC+CT</td>
<td>44 (97.78 %)</td>
<td>42 (93.33 %)</td>
<td>0.33 (NS)</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td>TT</td>
<td>1 (2.22 %)</td>
<td>3 (6.67 %)</td>
<td>3.14 (0.31-31.42)</td>
<td></td>
</tr>
<tr>
<td>Recessive model</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>31 (68.69 %)</td>
<td>24 (53.33 %)</td>
<td>0.132 (NS)</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td>CT+TT</td>
<td>14 (31.11 %)</td>
<td>21 (46.67 %)</td>
<td>1.94 (0.82-4.58)</td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>75 (83.33 %)</td>
<td>66 (73.33 %)</td>
<td>0.106 (NS)</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td>T</td>
<td>15 (16.67 %)</td>
<td>24 (26.67 %)</td>
<td>1.82 (0.88-3.75)</td>
<td></td>
</tr>
</tbody>
</table>
The concentration of MMP-9 in saliva in the patient group (the mean ± Standard deviation) was (10.27 ± 4.07) ng/mL while in the control group, it was (7.67 ± 3.35) ng/ml. There is a significant increase in patient in comparison to the control group (p= 0.043).

![Figure (3): Mean concentration of Matrix metalloproteinase-9 (MMP-9) in saliva of both patients and controls. We note significant increase at P value <0.05.](image)

**Discussion**

Our results show that there was no statistically significant difference in the frequency of the MMP-9-1562C/T genotypes between the periodontitis patient and the control persons, with no association between MMP-9-1562C/T polymorphism and the occurrence of periodontitis. Furthermore, genotypes in both dominant and recessive models were not significantly associated with periodontitis. The meta-analysis study of (Song et al., 2013) (8) demonstrates that a lack of association between the MMP-9-1562C/T polymorphisms and periodontitis. However, (Pan et al., 2013) study (9) found that the MMP-9-1562C/T polymorphism was associated with modified risk of periodontitis among Caucasian population, which population that differ from middle east population such as Iraq. While (Emingil et al., 2014) study (10) suggested that the other MMPs polymorphism such as MMP-8 might be associated with the susceptibility to periodontitis in Turkish population that also differ from Iraq population.

In this present study, the salivary MMP-9 levels (mean ± Standard deviation) in the periodontitis patient group was (10.27 ± 4.07) ng/mL while in the control group, it was (7.67 ± 3.35) ng/ml with a significant increase in patients comparison to the control group (p = 0.043). Our results were in agreement with many studies such as (Rai et al., 2008) (11), (Wan et al., 2014) (12) and (Baeza et al., 2016) (13) studies. Other studies, such as (Wang et al., 2013) (14) and (Cheng et al., 2014) (15) studies, proved that the MMP-9 levels could also predict the prognosis and progression of many disorders in nasopharyngeal region including chronic inflammation and carcinoma, e.g. squamous cell carcinoma.

However, (Franco et al., 2017) meta-analysis study (16) established that the other genotype of MMP-9 such as MMP-9-753 C/T polymorphism reduced the risk of chronic periodontitis but not mentioned the relationship between the MMP-9-1562C/T
polymorphism and chronic periodontitis, also the same study mentioned that the MMP-9 is the most abundant MMPs in periodontal tissues reflecting periodontal disease severity, progression, and treatment response.

**Conclusion**

Our study concludes that the salivary Matrix metalloproteinases (MMPs) determination can definitely be used as a biomarker for the diagnosis and progression of periodontal diseases. It may also facilitate the better treatment of periodontal disease and the assessment of periodontal conditions in medical and research settings.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** “All experimental protocols were approved under the Al-Nahrain University and carried out in accordance with approved guidelines”.

**References**

14. Wang WL, Chang WL, Yeh YC, Lee CT, Chang CY, Lin JT, Sheu BS. Concomitantly elevated serum matrix metalloproteinases 3 and 9 can


The Anthropometric and Biochemical Parameters in Normal Weight-Central Obesity Females

Sanaa Jameel Thamer  
Department of Biology, College of Science, University of Basrah, Iraq

Abstract

Central obesity is abdominal fat that accumulates and affects the health status. The present study aims to estimate some blood biomarkers among normal-weight females with central obesity and determine the prevalence of central obesity. The method: healthy females with normal body weight and age group of (33-44 y) have participated and their body anthropometric parameters were determined. The Navy formula detected the body composition. Serum glucose, lipid profile, insulin, and cortisol hormone were tested. The results: a high prevalence has been found of the central obesity among females with normal weight (71.098%). Central obesity is characterized by increasing WC, WHR, and WHtR with high-fat percentage and fat mass (40.55%, 25.6 kg) than females without central obesity (31.5%, 19.4 kg, respectively). The central obesity females showed significant elevation in serum glucose 8.937 mmol/L, insulin 177.884 pmol/L, and HOMI 10.216 with dyslipidemia than the ones with the non-central obesity. A high significant cortisol level (29.126 µg/dl) was observed in the normal weight central obesity group. Conclusions: the incidence of central obesity among normal-weight females has been associated with changes in some blood profiles, which may contribute to increasing adiposity-related risk factors and health outcomes and the effect on body shape.

Keywords: Biochemical parameters , anthropometric, females , obesity

Introduction

The local body fat distribution revealed an important role in initiating health problems [1]. The distribution of body fat is identified as the android shape (central) and ganoid shape (peripheral) [2]. Central obesity is one of the regional fat accumulations that contribute mainly to metabolic abnormalities like hypertension, diabetes type2, and cardiovascular diseases [3] by promoting mediating factors including inflammations, insulin resistance, and dyslipidemia [4].

Central (abdominal) obesity was observed in subjects with normal body weight (BMI < 25 Kg/m²) [5], which resulted from many factors, mainly diet type and lifestyle modifications [6]. The central fat is characterized by excessive abdominal fat deposit even among the subjects with normal weight [7]. The abdominal fat has been considered as the optimal criterion for the prediction of the fitness and for the metabolic syndrome incidence, especially visceral fat type [8, 9].

Abdominal obesity is gender-specific obesity [10] that is affected by the age [11]; in the females, abdominal obesity showed a specific android pattern (apple shape) with increasing WC [11, 12].

The modern direct measurement for abdominal obesity is magnetic resonance imaging or computer tomography scan [13]. Since they are expensive, not available, and limited in use, the simple alternative is using the anthropometric indicators including WC, WHR, and WHtR [7, 14]. These indicators were
suggested by many epidemiological studies and health organizations [15, 16].

The most widely used obesity index measurement is the BMI method. Still, with some limitations, represented in the fact that it does not provide the pattern of body fat distribution, therefore the combination between abdominal measurements with BMI was recommended [17]. In general, the regional fat depots were assessed among overweight and obese people and not considered in normal-weight subjects, which is an important indicator for the health condition. This study aims to determine the prevalence of central obesity among females with normal body weight, assess the risk factors associated with abdominal fat deposits through blood biomarkers, and detect the correlation between WC and some blood parameters.

**Materials and Methods**

The population sample of the study consists of healthy females (n=173) with normal body weight (BMI≥25 kg/m²) and age (33-44) years. The subjects were divided according to age into two age groups, the first age group: (33- 38 years) and the second age group (39- 44 years). The data has been collected from the participants according to the protocol of the ethical committee of Biology Department-Science College-Basrah University. The data has been collected firstly by questioner forms and then the anthropometric parameters and blood samples have been taken. Questioner forms asked all participants about social status, diet type, lifestyle, physical activity, smoking, drugs, hormonal therapy, allergy condition, and polycystic ovaries syndrome. The exclusion criteria were pregnancy, athletic females, hormonal therapy, females with diseases, and medications. The study was carried out from July 2019 to February 2021.

**The anthropometric measurements:**

The body mass index (BMI) is measured by weight kg/height m² [18]. Waist circumference (WC) was measured at the umbilicus region (cm), hip circumference (HC) was measured at iliac ridge over the buttocks (cm), the neck circumference at the neck place. All measurements were done by plastic strip [19]. The WHR ratio by WC/HC, the WHtR by WC/height. The central obesity was determined according to WC, WHR and WHtR: WC ≥ 80 cm, WHR ≥ 0.85, and WHtR > 0.50 [20]. Basing on questioners and medical history, all participants have been considered in good health. The participants were classified to normal body weight with central obesity and normal body weight without central obesity. The percentage of body fat (%BF) has been measured according to the formula of American Navy: females= 163.205 × log10 (waist + hip - neck) - 97.684 ×log10 (height) - 78.387 [21]. Using a digital scale to measure the weight. The blood pressure was taken in a sitting position.

**The Blood Sample**

The venous blood sample (5ml) from all participants was drawn after overnight fasting (at 8-10 Am). Serum specimens were collected in a gel tube after blood clotting for 15 minutes at room temperature and then centrifuged at 3000 rpm for 10 minutes. The serum was stored at deep freeze -70 °C [22].

**The serum assays:**

Serum glucose and lipid profiles (triglycerides TG, total cholesterol T-ch, and high-density lipoprotein HDL) were measured by using the commercial kits (Biolab, France). According to the formula, low-density lipoprotein (LDL) is Tch- HDL- TG/5 [23]. The insulin has been measured by Elisa kit (Abcam, USA) and cortisol by Elisa Kit (Crystal Chem, USA). The homeostatic index of insulin resistance (HOMI) by the formula: [glucose (mmol/L) × insulin (pmol/L)] ÷ 155 [24].

**The Statistical Analysis**

The data were analyzed by ANOVA test by SPSS version 22. T-Test was used for the two groups, Chi-square was used for obtaining values of percentage, and a general linear model was used for groups with many factors. The correlation by bivariate analysis.
The data has been expressed by mean ± standard deviation SD.

Results

The female participants of this study had a normal body mass index (23-24 kg/m²) (Table 1). A group of females revealed a significant (p ≤ 0.05) increase in WC (94.189 cm), WHR (0.908), and WHtR (0.579) than other female participants, therefore, they have been divided to normal BMI with central obesity and normal BMI without central obesity. The female group without central obesity showed normal ranges in WC (78.419 cm), WHR (0.778), and WHtR (0.479) values.

According to anthropometric parameters, the healthy females with normal body weight showed a high prevalence of central obesity 123 (71.098 %), while the females without central obesity reveal less occurrence 50 (28.901%), table (2).

The Navy formula (table 1) revealed that the females with normal weight and central obesity are considered obese as they showed higher body fat percentage (% BF) and higher fat mass 40.55%, 25.6 kg, respectively with significantly (p ≤ 0.05) compared to the non-central obesity females group (31.5%, 19.4 kg respectively). The lean body mass that has been displayed by this formula showed that the non-central obesity females had more lean mass, 42.1 kg, than the females with central obesity 37.4 kg.

The systolic and diastolic pressure were significantly (p ≤ 0.05) elevated in normal weight-central obesity group 123.927 mmHg and 80.893 mmHg respectively compared to normal weight without central obesity 119.371 mmHg and 77.440 mmHg.

According to age groups (table 3), in the central obesity group, the second age group, 39-44y of central obesity displayed a higher WC (95.588), WHR (0.917), WHtR (0.585) with significantly (p ≤ 0.05) than the first age group (33-38 y). The Navy formula also displays insignificant differences in the percentage of body fat, fat and lean mass between the two age groups. There were significant differences (p ≤ 0.05) between the systolic and diastolic pressure between the two age groups.

In normal weight without central obesity, the second age group showed higher significance (p ≤ 0.05) values in the ratio of WC, WHR, and WHtR than the first age group. According to the Navy formula, the second age group considers obese by high significant body fat percentage and fat mass values than the first age group, which have been considered with average body fat. No significance was observed in lean mass between the two age groups. The systolic and diastolic pressure showed insignificant differences.

The biochemical parameter between the two main groups (table 4) showed significant (P≤0.005) elevation in serum lipid profiles of central obese females TG, T-ch, LDL: 163.420 mg/dl, 177.656 mg/dl, 112.7 mg/dl, and a decrease in the HDL 47.233 mg/dl compared to non-central obesity.

There was a significant (p ≤ 0.05) increase in serum glucose and insulin levels in females with normal weight and central obesity group 8.937 mmol/L, 177.884 pmol/L respectively compared to normal weight without central obesity 8.079, 171.464. Therefore, the homeostatic index of insulin resistance was significantly higher in the central obesity group 10.216 than the non-central obesity group 8.937.

The results observed an increased level of serum cortisol in normal weight-central obesity females 29.126 µg/dl with significant difference (p ≤ 0.05) than the non-central obesity females 21.621 µg/dl.

From table (5), according to age groups, in normal weight-central obesity females, it was observed that the second age group (39-44 y) showed significant (p ≤ 0.05) elevation in glucose, TG, T-ch, and LDL levels compared with the second age group. Also, there was an increase in the insulin level 179.629 pmol/L and HOMI (10.316) with significant differences compared to the first age group 176.140 pmol/L and 10.116,
respectively. The serum cortisol level displayed significant elevation in the second age group 30.531 µg/dl compared to the first age group 27.721 µg/dl.

There were significant differences in normal weight without central obesity (p ≤ 0.05) in the levels of glucose, TG, insulin, and HOMIR between the two age groups. A significant elevation in these parameters characterizes the second age group. No significant differences were observed in the levels of T-ch, HDL, LDL, and cortisol between the two age groups.

The results of the bivariate correlation analysis (table 6) showed that BMI had a positive correlation with fat mass percentage. The WC displayed a positive correlation with WHR and serum cortisol level. There was a positive correlation between WHR and HOMIR and between serum fasting glucose and TG level.

Table 1: The Anthropometric parameters in normal weight females with and without central obesity.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal BMI with central obesity (n=123)</th>
<th>Normal BMI without central obesity (50)</th>
<th>P value (Significant at p ≤ 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>63.367±1.501</td>
<td>61.838±1.643</td>
<td>0.532</td>
</tr>
<tr>
<td>Height</td>
<td>162.478±1.731</td>
<td>163.325±1.309</td>
<td>0.231</td>
</tr>
<tr>
<td>BMI</td>
<td>24.004±1.567</td>
<td>23.183±1.537</td>
<td>0.819</td>
</tr>
<tr>
<td>WC</td>
<td>94.189±2.773</td>
<td>78.419±1.842</td>
<td>0.003</td>
</tr>
<tr>
<td>HC</td>
<td>103.680±5.171</td>
<td>100.737±3.192</td>
<td>0.420</td>
</tr>
<tr>
<td>WHR</td>
<td>0.908±0.058</td>
<td>0.778±0.087</td>
<td>0.001</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.579±0.093</td>
<td>0.479±0.097</td>
<td>0.001</td>
</tr>
<tr>
<td>NC</td>
<td>33.810±1.218</td>
<td>33.362±1.063</td>
<td>0.530</td>
</tr>
<tr>
<td>% BF</td>
<td>40.55±1.040</td>
<td>31.5±1.624</td>
<td>0.000</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>25.6±1.537</td>
<td>19.4±1.857</td>
<td>0.000</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>37.4±1.978</td>
<td>42.1±1.607</td>
<td>0.001</td>
</tr>
<tr>
<td>Body category</td>
<td>obese</td>
<td>average</td>
<td>-</td>
</tr>
<tr>
<td>SBP mmHg.</td>
<td>123.927±3.863</td>
<td>119.371±1.118</td>
<td>0.032</td>
</tr>
<tr>
<td>DBP mmHg.</td>
<td>80.893±2.674</td>
<td>77.440±1.311</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Table 2: the prevalence of central obesity among normal weight females in the study population.

<table>
<thead>
<tr>
<th>The prevalence</th>
<th>Normal BMI with central obesity</th>
<th>Normal BMI without central obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td>The prevalence</td>
<td>123 (71.098 %)</td>
<td>50 (28.901 %)</td>
</tr>
</tbody>
</table>

Chi-Square=17.640, Sig= 0.000
Table 3: the anthropometric parameters in normal weight females with and without central obesity according to each groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal BMI with central obesity (n=123)</th>
<th>Normal BMI without central obesity (50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age group1 33-38 y, n=53</td>
<td>Age group 2 39-44 y, n=70</td>
</tr>
<tr>
<td>Weight</td>
<td>62.554±1.499</td>
<td>64.181±1.545</td>
</tr>
<tr>
<td>Height</td>
<td>161.751±1.520</td>
<td>163.205±1.698</td>
</tr>
<tr>
<td>BMI</td>
<td>23.912±1.614</td>
<td>24.096±1.102</td>
</tr>
<tr>
<td>WC</td>
<td>92.791±2.527</td>
<td>95.588±3.502</td>
</tr>
<tr>
<td>HC</td>
<td>103.202±1.528</td>
<td>104.159±2.485</td>
</tr>
<tr>
<td>WHR</td>
<td>0.899±0.010</td>
<td>0.917±0.010</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.573±0.068</td>
<td>0.585±0.076</td>
</tr>
<tr>
<td>NC</td>
<td>32.901±0.551</td>
<td>34.720±0.863</td>
</tr>
<tr>
<td>% BF</td>
<td>40.5±0.400</td>
<td>40.6±0.503</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>25.1±0.100</td>
<td>26.1±0.129</td>
</tr>
<tr>
<td>LM (kg)</td>
<td>36.9±0.476</td>
<td>37.9±0.208</td>
</tr>
<tr>
<td>Body category</td>
<td>obese</td>
<td>obese</td>
</tr>
<tr>
<td>SBP mmHg</td>
<td>121.424±5.532</td>
<td>126.430±5.389</td>
</tr>
<tr>
<td>DBP mmHg</td>
<td>79.113±3.706</td>
<td>82.673±3.487</td>
</tr>
</tbody>
</table>
Table 4: the serum assays in normal weight females with and without central obesity.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal BMI with central obesity (n=123)</th>
<th>Normal BMI without central obesity (50)</th>
<th>P value (Significant at p ≤ 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose mmol/L</td>
<td>8.937±1.837</td>
<td>8.079±1.508</td>
<td>0.000</td>
</tr>
<tr>
<td>TG mg/dl</td>
<td>163.420±2.780</td>
<td>145.933±1.678</td>
<td>0.001</td>
</tr>
<tr>
<td>T-ch mg/dl</td>
<td>177.656±1.247</td>
<td>173.830±1.841</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL mg/dl</td>
<td>47.233±1.231</td>
<td>51.44±1.088</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL mg/dl</td>
<td>112.7±1.830</td>
<td>106.25±1.390</td>
<td>0.000</td>
</tr>
<tr>
<td>Insulin pmol/L</td>
<td>177.884±1.982</td>
<td>171.464±1.369</td>
<td>0.040</td>
</tr>
<tr>
<td>HOMI</td>
<td>10.216±0.215</td>
<td>8.937±0.151</td>
<td>0.001</td>
</tr>
<tr>
<td>Cortisol µg/dl</td>
<td>29.126±0.818</td>
<td>21.621±0.872</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 5: the serum assays in normal weight females with and without central obesity according to age groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal BMI with central obesity (n=123)</th>
<th>Normal BMI without central obesity (50)</th>
<th>P value (Significant at p ≤ 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose mmol/L</td>
<td>8.902±1.115</td>
<td>8.972±1.321</td>
<td>0.011</td>
</tr>
<tr>
<td>TG mg/dl</td>
<td>160.310±3.615</td>
<td>166.530±4.586</td>
<td>0.012</td>
</tr>
<tr>
<td>T-ch mg/dl</td>
<td>176.802±3.527</td>
<td>178.510±3.577</td>
<td>0.051</td>
</tr>
<tr>
<td>HDL mg/dl</td>
<td>48.768±2.358</td>
<td>45.699±1.732</td>
<td>0.030</td>
</tr>
<tr>
<td>LDL mg/dl</td>
<td>110.4±2.577</td>
<td>115±2.309</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin pmol/L</td>
<td>176.140±2.732</td>
<td>179.629±5.196</td>
<td>0.032</td>
</tr>
<tr>
<td>HOMI</td>
<td>10.116±0.166</td>
<td>10.316±0.329</td>
<td>0.045</td>
</tr>
<tr>
<td>Cortisol µg/dl</td>
<td>27.721±1.577</td>
<td>30.531±1.100</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Table 6: the observed significant correlations among the variables in the study.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pearson Correlation</th>
<th>Sig. (p ≤ 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI-Fat mass</td>
<td>.917(*)</td>
<td>.028</td>
</tr>
<tr>
<td>WC-WHR</td>
<td>.958(*)</td>
<td>.010</td>
</tr>
<tr>
<td>WC-Cortisol</td>
<td>.906(*)</td>
<td>.034</td>
</tr>
<tr>
<td>WHR-HOMIR</td>
<td>.907(*)</td>
<td>.034</td>
</tr>
<tr>
<td>Glucose -TG</td>
<td>.942(*)</td>
<td>.017</td>
</tr>
</tbody>
</table>
Discussion

The general anthropometric parameter to diagnose obesity in the population uses the body mass index BMI [18]. In this study, we found that the healthy females with normal body weight showed central obesity by increasing WC, WHR, and WHtR despite lowering their BMI (18.5-24.9 kg/m\(^2\)). The high prevalence of central obesity among the study participants suggests the importance of detecting the WC, WHR, and WHtR in normal-weight subjects to determine regional obesity as critical measurements for body fat distributions [25].

In this study, we compared the results of the Navy formula with the WC, WHR, and WHtR and found that the normal weight females with central obesity showed more fat percentage in both age groups. Simultaneously, the normal weight females without central obesity also showed a fat ratio and were considered obese in older age (39-43y). These results indicate that the Navy formula was adequate to determine body fitness by including all body anthropometric parameters in addition to sex and age. Also, this formula was effective in expressing fat accumulation in the normal body weight. The Navy formula is an adequate and certain formula for the detection of the body composition with similar results as using the bioelectrical analysis [26, 27], and suitable for use any time even after the physical activity [28], it has also been considered as a screening tool for body fitness and obesity complications [29].

The normal-weight females without central obesity showed high-fat percentage, especially in the second age, this may related that the Navy formula reflect the fitness degree of these participants. In our study, all participants showed a sedentary lifestyle. In the central obesity females, the formula showed a higher fat percentage as expected with increasing the abdominal fat deposit in this group.

The total body mass, including the fat and non-fat mass, was expressed by BMI. In contrast, the WC expressed only the fat accumulation in the abdominal region and HC revealed the fat tissue in peripheral body parts [20]. In normal BMI with central obesity, the increase in WC and decrease in the HC may decrease fat deposits in hips and legs [6].

In this study, the females with central obesity showed elevation in lipid profile, glucose, insulin, and HOMI, indicating that the central fat accumulation causes a disturbance in lipid and glucose metabolism and developing the risk factors leading to glucose intolerance, dyslipidemia, hypertriglyceridemia, insulin resistance, and then systematic inflammation [7].

The lipolytic activity resulting from lipids disturbance, causing high TG production and transferring to the liver, which in turn increases VLDL synthesis and TG elevation in the blood circulation, enhances the exchange of the cholesterol esters and TG hydrolyzing, leading to lower HDL levels [30]. High TG production in liver during hyperglycemia decreases the insulin clearance and causes hyperinsulinemia [31].

Some previous studies revealed the importance of determining the central obesity among people with normal weight [7, 32] as it is pointed to developed morbidities like that in obese people with central obesity [7, 33]. These because of a strong association between the central obesity and CVD, regardless of BMI [34]; also, the WC and WHR are more correlated with the risk factors of metabolic problems beyond the BMI [34, 35].

The excessive visceral fat depots in the abdominal region may cause metabolic alternations, including dyslipidemia and hyperinsulimia that elevated risk factors of cardiovascular diseases CVD [36, 37, 38]. The presence of hypertension and diabetes type 2 in normal-weight central obese individuals leading to develop cardiovascular diseases (CVD) was more than the individuals without the central obesity [39, 40].

In this study, the elevated systolic and diastolic blood pressure in normal–weight–central obesity females may be explained by the fact that the ratio of body adiposity was an independent risk factor for hypertension [41]. The risk factor of hypertension was 63% in normal-weight Malaysian women [42]. The normal weight subjects with high-fat percentages showed elevated blood pressure and were more expected to develop CVD [43].

The study results showed that the normal BMI with central obesity has high serum cortisol compared to female without central obesity, this may be related to increased visceral fat accumulation [44], or the elevated cortisol in blood circulation that caused abdominal obesity [45]. The visceral adipose tissues are supplied with more blood vessels than the peripheral adipose tissues and with more glucocorticoid receptors; therefore, the visceral fat was more sensitive to TG and glucocorticoids. The glucocorticoids, like cortisol stimulate proliferation and differentiation of human adipose tissue that causes a redistribution of fat cells from peripheral to central accumulation [46].

Conclusions

Inclusion of central adiposity measurements and body fat percentage with BMI are important parameters for
detecting body fat deposition and body fitness even in the absence of whole-body obesity. It is important to consider the metabolic alternations associated with central obesity in normal BMI to avoid the risk factors of metabolic complications. Considering normal weight subjects as a control in clinical studies based only on BMI and regardless the abdominal obesity will lead to misclassification and underestimation of blood metabolites like lipid profile and hormones that will affect the study results.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the College of Science and carried out in accordance with approved guidelines”.

References


Morphological Study of the Esophagus in the Domestic Fowl

*Gallus Gallus Domesticus* (Linnaeus 1758)

Wijdan Basheer Abed¹, Hanan Raad Dheyaa Hussain², Dalia H. Al-Hamawandy³

¹Assistant Prof. Dr., Department of Biology, College of Education for pure Science (Ibn Al-Haitham), University of Baghdad, ²Assistant Lecturer, Directorate General of Education Al-Karkh First, Ministry of Education, ³Lecturer, Directorate General of Education Al-Karkh First, Ministry of Education

Abstract

The esophageal morphology of domestic fowl (*Gallus gallus domesticus*) was investigated to fill the dearth of information on the morphology of esophagus from available literature and help in understanding its digestive tract biology. The esophagus is under study as musculomembranous tubular shape, it began from oropharyngeal cavity and terminated on the proventriculus. The esophagus was divided into three regions are represented by cervical part, crop, and Thoracic part. Internal lining of the esophagus consisted of un-branched straight longitudinal folds The length of the cervical part, crop and thoracic part were 11.5 cm, 3.5 cm, 6 cm respectively.

**Key words:** Avian, chicken, esophagus, *Gallus gallus domesticus*.

Introduction

The avian digestive tract is a continuous tube that opens at either end (beak and vent) to the outside world and consists of a mouth, esophagus, crop, proventriculus, ventriculus or gizzard, intestine, ceca, rectum, and cloaca. As food progresses The esophagus is a narrow, muscular, highly-distensible tube extends from the pharynx and joins with the stomach¹. Sisson et al (1986)² observed that the avian esophagus is on the right side of neck (mammal present it was on left side) and it was placed between the pharynx and stomach glandular portion, it was thin and dilatable walls with a diameter relatively larger than of mammals. According to³ the avian esophagus consist of two parts, cervical and thoracic, while in mammals through these organs, a specific sequence of digestive events occurs, including grinding, acidifying, hydrolyzing, emulsifying and transporting of the end products⁴.

There was three parts, cervical, thoracic and abdominal. At the thoratical part of the avian alimentary tract, oesophagus enlarge to form the crop, so that, the cervical part functionally acts to lubricate the food and contact it into the thoratical part in which the food is stored and released into the other parts of the alimentary tract¹. The distensibility of the avian oesophagus, however, comes from the longitudinal folds in the inner layer of it. Unlike mammals, the avian oesophagus don’t have any sphincter in both upper and lower regions of it⁵. Crop structure and function vary according to the species and the diet, the crop is absent in the gulls and penguin so food passe directly or it is stored in the tubular esophagus⁶. The crop in duck and goose as in most birds is merely a fusiform enlargement of the esophagus⁷, crop takes a simple spindle shape while in pigeon as an herbivorous, it took a more complicated structure, whereas some granivorous species have no true crop and can store their food in a very distensible oesophagus⁸. Crop may provide a type of protection for birds by its storage function that allows the ingestion of the stored food in the evening providing the overnight energy needs, in the other hand, it also allows the birds to ingest their...
food rapidly and softly by swallow the water giving
the important security for them\textsuperscript{9}. The digestive system
of birds has adaptations to facilitate flight. Many birds
operate on a thin margin of metabolic safety and may
require a constant source of feed to sustain activity.
Birds cannot afford to store heavy food materials
for long periods and digest their food rather quickly
relative to mammals\textsuperscript{10}.

Materials and Method

Twenty adult specimens of species domestic
fowl (\textit{Gallus gallus domesticus}) were obtained from
different localities at Baghdad city, Iraq. They were
collected during December 2020 until February
2021, the animals were brought to the laboratory
alive the animals were anesthetized and decapitated
according to the international protocol for biomedical
investigation with human being and animals, then
dissected carefully by making at longitudinal incision
at the middle line of skin from ventral surface starts
from first cervical vertebrae access to pelvic region,
then the skin separated from muscles, which under it.
The thoracic region mad transverse incision, then the
skin was drawn from muscles. The peripheral muscles
were removed within ventral wall, then to cut right
part of ventral wall, followed by cut left part of ventral
wall and to complete incision across to thoracic
girdle. The gastrointestinal tract was extirpated and
esophagus was remove and measurement related
to formation study were taken which are length of
cervical crop thoracic parts.

Results

The esophagus of domestic fowl (\textit{Gallus gallus
domesticus}) (Fig. 1) was muscular membranous,
tubular shape, which began from oropharyngeal cavity
and terminated on the proventerculus (Fig. 2). The
esophagus divided into the three regions represented
by cervical part, crop and thoracic part (Fig. 3). The
cervical part of esophagus was located in the right
side of neck and began with the end of the oropharynx
and intertwined on the trachea, the length of cervical
part of the esophagus was 11.5 cm. The crop was sac-
like structure protruded in the upper part of the body
of birds began before the entering of the thoratical
cavity, it had a thin wall and very low inner folds
(Fig.4), crop length was 3 cm and thoracic part where
the crop returned to narrow was length 6 cm . The
lining of esophagus in chicken seems white color in
three part (cervical, crop, thoracic), and cervical part
of esophagus was composed of about 5 un- branched
high straight longitudinal fold, but the thoracic part
have contains about 7 un-branched straight lower
longitudinal folds (Fig.4).

![Figure 1: Class: Aves Order: Galliformes Family:
Phasianidae Genus: Gallus Species: gallus.](image)

![Figure 2: Ventral view of thoracoabdominal cavity.
L, liver; H, heart; G, gizzard; Du, duodenum; TR, trachea.](image)
Results of the present study showed that the oesophagus in the domestic fowl under investigation was an organ located on the right side of the neck and situated between the pharynx and stomach glandular. This result agrees with 2, 11.

Peristaltic contraction of inner circular and outer longitudinal muscles in the tunica muscularis propels food posteriorly through the esophagus. Esophagus was divided into three anatomical regions: cervical oesophagus, crop, and thoracic oesophagus as it was found by 12. Cervical part longer than thoraces. This result in agreement with Samuelson (2007) who study the esophagus in party Rhynchotus rufescens, also in similar with Das et al. (1967) during his study on goose esophagus. To aid in swallowing large food items, the esophagus is expandable as a result of a series of longitudinal folds. This accordion-like arrangement is enriched with mucous glands to provide lubrication that results agree with Rasha et al. (2015) in wood pigeon (Columba palumbus) and The barn owl Tyto alba, also agree with Al Kinany (2017) in Breasted Kingfisher (Halcyon symamensis).

The esophagus widens into a crop just before entering the thoracic cavity, It appears primarily in the esophagus Grain- eating birds (Granivorous) Where is a place To store and hydrate food (Parachami and Dehkordi, 2011) while being lost in Others, such as gulls (Larus melanoccephalus) (Ali, 2014) and Bustards( Bailey et al., 1997).

The crop is a sac-like enlargement on the esophagus. This description similar with Rasha et al. (2015) who studied esophagous of wood pigeon and Shehan (2012) but different with Al Kinany (2017) and Al-Jumaily and Al-Borznge (2016) who studied Esophagus in White Breasted Kingfisher (Halcyon symamensis) (Piscivorous) who founded that the bird don’t had crop, Similar with Rodrigues et al. (2012) and Bailey et al. (1997) on his study on in rheas and captive bustards respectively. Some granivorous species (eg. Cardueline Finches) do not have a true crop, but they usually have a very expandable esophageal pouch that can store food items 4. The presence of large folds of mucosa in crop allows considerable expansion and shrinkage, depending on the amount of contents. Immediately cranial to the thoracic inlet, the crop narrows to reform an esophageal tube and passes between the coracoid bones to the right of the syrinx and dorsal to the heart (Evans, 1996). Within the crop, food softens as a result of hydration by saliva added to food during

Discussion

Results of the present study showed that the oesophagus in the domestic fowl under investigation was an organ located in right side of neck and situated between the pharynx and stomach glandular this result agree with 2,11.

Figure 3: Forms, location of esophagus in Gallus gallus domesticus. CER, cervical part; C, crop; THO, thoracic part; L, liver; GB, gall bladder; G, gizzard; DU, duodenum; CO, Colon; CL, cloaca; P, pancreas ; SI , small intestine.

Figure 4: Inner view of esophagus showing LF, Longitudinal folds; CER, cervical part; THO, thoracic part; C, crop.
swallowing, mucus secreted into the crop, and water consumed after a meal. More significant digestion may occur because of the presence of enzymes in the food itself or from microbial action in the warm moist environment of the crop, the crop also provides a temporary storage area that permits a bird to rapidly forage for large amounts of food and then fly off to digest the meal in safe cover. A crop also permits “tanking up” in the evening, so that food can be slowly released to supply nutrients during the nighttime (Soedarmo et al., 1961 and Hainsworth et al., 1972). The crop and the esophagus play an important role in nourishing the young by permitting the storage and softening of food, which is later regurgitated into the beak of the nestlings. The nutritional and immunologic contribution of the hen to the regurgitated food is not known for most species, but is important in pigeons. The crop represents the first major defence against poultry pathogens and zoonotic organisms with well-established adaptive and innate immune function, and a lactobacilli dominated microbiota capable of reducing the passage of these organisms further along the digestive tract (Classen et al., 2016).

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the Department of Biology and carried out in accordance with approved guidelines”.

References
Mothers’ Knowledge toward Home Care for Children with Wilms Tumors at Pediatric Hospitals in Bagdad City

Rehab A. Ali¹, Zaid W. Ajil²
¹M.Sc., Student, Department of Nursing, Ministry of Health, Baghdad, Iraq, ²Lecturer, Dr., Department of Nursing, College of Nursing, University of Baghdad - Iraq

Abstract

Objectives: To find out relationships between mothers’ knowledge and their demographic characteristics that includes (age, marital status, level of education, family income, home location, number of family members.

Methods: A descriptive research was performed from October 1, 2020, to June 1, 2021, Non - probability (purposive) sample. In which the sample was objectively chosen from (100) mothers who participated in the study and were obtained from three Baghdad hospitals. It included 47 mothers at the Child welfare Teaching Hospital and 28 at the Central Child Teaching Hospital and 25 in the city of Miamian al-Kadhimin (peace be upon them both) in Baghdad.

Results: Mothers’ knowledge towards homecare for “Children with Wilms Tumours” at pediatric hospital’s items was not assigned at the established level in which that achieving the goal of this study.

Conclusion: Mothers’ knowledge towards homecare for “Children with Wilms Tumours” at pediatric hospital’s items was not assigned at the established level in which that achieving the goal of this study.

Keywords: Mothers’, knowledge, homecare, Wilms Tumours.

Introduction

Wilms tumour or nephroblastoma is a type of cancer that occurs in the kidneys, and this type is considered one of the most common forms in children. This type of cancer was named after the germen doctor Max Wilms, who was the first to write about the disease in 1899 [2]. Around the world about 90% children’s’ cancers suffer from this type of tumours. However, nephroblastoma often affects one side (unilateral) or two sides (both), every 100 children 7% affects both kidneys [11]. The cause of childhood cancer is unknown, although there are causative factors, including chemotherapy, radiation, smoking and congenital anomalies that have a major role in the occurrence of childhood cancer and are associated with cancer [9]. Among the causative factors of this disease are genetic or environmental factors, as well as congenital anomalies that have an effective role in this disease, as it has been proven that about 20% of children who suffer from solid tumours are infected with congenital abnormalities in the kidney where they have a defect of chromosome 11. In some cases, the tumour can progress to other tissue, such as the liver, bones, and brain, in the solution of not being diagnosed and treated early [6].

Importance of Study

The World Health Organization states that health is “a complete physical condition, social and mental well-being and not merely the absence of disease and disability [12]. This means that a disease is “a disease or a period of illness affecting the body and mind ⁵. The disease can be classified into many categories and these groups are associated with the disease: acute (it starts in the beginning and lasts a month) long-term (this type lasts about more than 6 months (chronic)
lasts for a long time and dangerous for the patient’s life \cite{7}. Wilms’ tumour is one of paediatric cancers that affect the kidneys and its present in children. Around the world about 90\% children’s cancers suffer from this type of tumour. It often affects one side (unilateral) or two sides (both), every 100 children 7\% affects both kidneys \cite{11}.

**Method and Material**

Descriptive Design that applies a methodology to achieve the study objectives to assess mothers ‘knowledge in home care of children with Wilms’ tumour in children’s hospitals in Baghdad during the period from October 28, 2020 to June 1, 2021. The research was carried out in the city of Baghdad, at three hospitals. The total sample number of children with Wilms’ tumour was (100) mothers. In the city of Baghdad, including the regions of Baghdad on both sides of al-Karkh and al-Rusafa. The sample was purposive selected which included three hospitals in Baghdad and a sample size of 100 mothers. It was divided into three categories and distributed to hospitals, including (47) samples in the Child welfare Teaching Hospital in the Medical city (28) samples in the Central Teaching Hospital, and (25) samples in the city of Al-Kadhimin, peace be upon them, in the city of Al-Kadhimiya in Baghdad.

Data were collected during the period /1December/2020 to 1/March/2021. For this study to collect data, the researcher collected data from mothers, what is their knowledge towards home care for their children with Wilms ’tumour. Data were collected randomy from three hospitals in Baghdad.

Ethical considerations were concerned throughout study process, and the Research Ethical Committee at College of Nursing was approved the questionnaire format.

**Result**

**Table (1). Mothers’ Sociodemographic Characteristics**

<table>
<thead>
<tr>
<th>Age Groups Yrs.</th>
<th>Groups</th>
<th>No.</th>
<th>Cum.%</th>
<th>C.S. P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 _ 24</td>
<td>9</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 _ 29</td>
<td>19</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 _ 34</td>
<td>43</td>
<td>71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 35</td>
<td>29</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>No.</th>
<th>Cum.%</th>
<th>C.S. P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>97</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>2</td>
<td>99</td>
<td>(\chi^2=182.42) P=0.000 (HS)</td>
</tr>
<tr>
<td>Widowed</td>
<td>1</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
Results shows that a highly significant differences are accounted at $P<0.01$ between observed distribution with their expected outcomes in each variable. Regarding of “Age Groups” most of studied respondents were focused at the third and fourth age groups (i.e. $\geq 30$) yrs., since they accounted (72.0%), “Marital Status” showed that (97%) are accounted married status, and only two of them has divorced status, and only one has recorded widowed, “Educational Levels” showed that studied women are characterized by a low educational level, as the levels of education were distributed at the highest level at the intermediate graduate school achievement , “Income”, showed that about half of the studied respondents has recorded a sufficient income, and accounted (48%) , “Residency” showed that most of studied respondents has recorded urban residence, and they are accounted (73%), and finally “Occupation” showed that all studied respondents has recorded housekeeper.

**Discussion**

The results showed that the age groups of third and fourth mothers (i.e. $\geq 30$) years are the respondents, where the percentage of them reached about 72%. According to a report by Kamal, A. (2017) on the characteristics of a thoughtful caregiver, the vast majority of caregivers are mothers. Almost two-thirds of them were between the ages of 30 and 40, with the remainder being under 40. Caregivers did not exist, and more than half of them did. They can read and write, and their parents can as well they were not related to the majority of the people. The
educational level was found to be low for educated mothers. In a related survey, it was discovered that 97 % of mothers with education had adequate awareness and activities for their children when it came to the steps of chemotherapy for children with leukemia [1]. As for the monthly income, it reached about half of the studied sample, and they recorded that they had sufficient income at about 48%. As for residency, the results indicated that most of the mothers who participated in the study were urban residence by 73%, and the occupation made it clear the results are that all mothers are housekeepers. The findings of this study were close to those of [8] who conducted research on childhood cancer in the United States cases from the pediatric cooperative clinical trials were analyzed geographically. Several organizations have stated that mother’s work allows them to spend more time with their children, her to devote more time to studying and caring for their offspring. Following statistical analysis of the results, the research sample discovered that mothers’ awareness of the direction of home care for children with Wilms’ tumor in Baghdad children’s hospitals was not at the necessary level to achieve the study’s objective. As most of the responses relevant to the field were observed in the majority of the results the “disease definition” sub-field received a moderate rating and was assigned four (80%) objects, while the remaining portion was given a high rating. The second sub-field “causes, signs, and symptoms” follows after that. The assessment was moderate for three (60%) objects, while the remaining elements received high ratings. The third sub-domain, “disease spreading phases,” received a low rating for two (66.67%) components, while the remaining component received a medium rating. Following that, the fourth sub-domain of “treatment process” was evaluated. The remaining portion received a moderate rating, followed by a fifth sub-domain of ‘Multiples,’ which received a low rating for 3 (100%) objects. In a recent Conducted by [1] it was discovered that knowing the gender of the parents makes a major difference in the treatment of children, and that knowledge among mothers differed significantly from that of fathers, despite the fact that mothers were less educated. Most caregivers lacked information about sickness, diet, home treatment, and preventive steps, according to [3]. Illiteracy and a low socioeconomic status are the causes of this lack of education. Of this study showed that there is a significant gap in these mothers’ awareness about cancer, complications, and risk factors, as well as symptoms and different aspects of chemotherapy. This lack of information may jeopardize their ability to help. Similar but less information gaps were found by [10] in their analysis. In order to encourage parents’ and inspire their children to engage in frequent follow-up, the writers stressed the importance of improving parents’ awareness about cancer, care, possible late effects, and the importance of regular follow-up.

**Conclusion**

Mothers’ knowledge towards homecare for “Children with Wilms Tumours” at pediatric hospital’s items was not assigned at the established level in which that achieving the goal of this study.

**Recommendation**

Providing a permanent program in hospitals for mothers who have children with Wilms’ tumours to keep them informed of health education about the care of their children.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** “All experimental protocols were approved and carried out in accordance with approved guidelines”.

**Reference**


2- American Cancer Society. Cancer Facts & FiguresAtlanta, Ga: American Cancer Anticancer


Morphological Description of the Esophagus in the Mallard Duck (*Anas platyrhynchos*)

Hanan Raad Dheyaa Hussain¹, Wijdan Basheer Abed², Dalia H. Al-Hamawandy³

¹Assistant Lecturer, Directorate General of Education Al-Karkh first, Ministry of Education, ²Assistant Prof. Dr., Department of Biology, College of Education for pure Science (Ibn Al-Haitham), University of Baghdad, ³Lecturer, Directorate General of Education Al-Karkh first, Ministry of Education

Abstract

The esophageal morphology of Mallard Duck (*Anas platyrhynchos*) was investigated to fill the dearth of information on the morphology of esophagus from available literature and help in understanding its digestive tract biology. The esophagus is long and highly distensible, musculomembranous tubular shape, it began from oropharyngeal cavity and terminated on the proventriculus. The esophagus was divided into two regions are represented by cervical part and Thoracic part, the crop was absent. Internal lining of the esophagus consisted of un-branched straight longitudinal folds the mean length of esophagus was about 25 cm; it was wider in the anterior part than the posterior part.

Key words: Avian, Mallard duck, esophagus, Anas platyrhynchos.

Introduction

Domesticated forms of ducks descend from two different species, i.e. from Cairina moschata and *Anasplatyrhynchos*. The mallard, *Anasplatyrhynchos*, originated in the northern hemisphere and gave rise to many duck breeds and varieties. The Mallard is widely distributed across the Northern Hemisphere, North America from southern and central Alaska to Mexico, the Hawaian Islands, and across Eurasia, from Iceland and southern Greenland and parts of Morocco (North Africa) in the west, Scandinavia to the north, and to Siberia, Japan, and China in the east. The distinctive anatomy and physiology of the avian gastrointestinal tract reflects the constraints of flight, in that most of the tract’s weight is centralized within the body cavity to optimize aerial maneuverability. The avian gastrointestinal tract has a larger number of organs, which have greater interorgan cooperation than their mammalian counterparts. The precise anatomic plan of the digestive tract of companion birds varies somewhat, depending on their typical diet.

Mallards is omnivorous and very flexible in its foods choice. Its diet may vary based on several factors, including the stage of the breeding cycle, short term variations in available food, nutrient availability, and inter- and intraspecific competition.

Birds have relatively long necks because their beak must serve the function of hands or paws in food gathering. Their esophagus is, therefore, also relatively long. It has numerous mucous glands to help lubricate the passage of food. The crop is an enlargement of the esophagus and it serves to store food for subsequent passage to the stomach. (This is analogous to the cardia in the mammalian stomach) The size and shape of crops vary between species.

Materials and Methods

The animals under investigation were collected from Babylon Governarate. Five birds were used to study the different aspects of the present study. The studied pigeons have two large lateral pouches, gallinaceous birds have a single pouch, hawks have a spindle-shaped enlargement of the esophagus and...
owls have no crop. Birds were anesthetized using chloroform. the length of esophagus and its different parts were measured.

**Results**

The esophagus of Mallard Duck (*Anas platyrhynchos*) (Fig. 1) was muscular membranous, tubular shape, which began from oropharyngeal cavity and terminated on the proventerculus, the esophagus divided into two regions represented by cervical part and thoracic part, the crop was absent (Fig. 2). The cervical part of esophagus was located in the right side of neck and began with the end of the oropharynx and intertwined on the trachea, the mean length of esophagus was about 25 cm, the mean width of esophagus was about 2.5 cm in cervical part and about 1.5 cm in the thoracic part. The lining of esophagus in Mallard Duck seems white color, the mucous membrane contain longitudinal folds varies in depth (height) and number, between them folds less tall, the cervical part of esophagus was composed of about 13 un-branched straight longitudinal folds, the thoracic part has contained about 7 un-branched straight longitudinal folds and the region in between contains about 10 folds (Fig. 3). There was a cardiac sphincter separating the esophagus and proventriculus (Fig. 4).
Figure 4: Inner view of esophagus showing ES, Esophagus; PV, proventriculus; SPh, cardiac Sphincter.

Discussion

Mallard (Anas platyrhynchos) anatomy was extensively studied in Iraq, studied anatomical and histological features of the kidney, studied Histomorphological and Histochemical structure of the stomach, studied Morpho-histological comparative study of the liver and studied Histomorphological and histochemical study of the small intestine of the mallard (Anas platyrhynchos) in south Iraq. The digestive tract of birds shows a great diversity according to their respective feeding and dietary habits, results of the present study showed that the esophagus in the mallard duck under investigation was an organ located in right side of neck and situated between the pharynx and stomach glandular this result agrees with Sisson et al. (1986). Esophagus was divided into two anatomical regions these are cervical oesophagus and thoracic oesophagus, the crop was absent this description agree with AL-Jumaily and Al-Borznge (2016) in their study of esophagus in white breasted kingfisher (Halcyon smyrnensis) (Piscivorous); Rajabi and Nabipour (2009) in Passeriformes birds and Bailey et al. (1997) in captive bustards (opportunistic omnivores) and disagree with Rasha et al. (2015); Ivey et al. (2005) and Shehan (2012) they found that esophagus was divided into three anatomical regions, cervical part, crop and thoracic part.

The results of the current study showed the presence of longitudinal folds in the mucous membrane varies in depth (height) and number, it turns out that the cervical part form longitudinal folds between them folds less tall and alternately, the diameter of the esophagus and number of folds gradually narrows towards the thoracic part this result agree with Al-Jumaily and Al-Borznge (2016).

The reason for the variation in numbers and lengths of folds maybe back to the feeding pattern as the esophagus in this study has many folds that increase widening when swallowing prey as well as narrowing of the lumen of the posterior part of the esophagus allows it to store food between its folds for a time because the crop was absent in this bird (Nickle, 1977), Although food may be stored throughout the length of the esophagus of avian species which have no crop (Ziswiler and Farner, 1972; McLelland, 1979a), In ratites, the crop is also absent and the large proventriculus and ventriculus of these birds is considered to help with food storage (Angel et al., 1996). Birds eating high-protein diets generally have less complicated digestive systems than those eating complex carbohydrates. In mallard, there was a strong sphincter between esophagus and proventriculus while it was absent in pigeon (Hassan and Moussa, 2012).

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the Ministry of Education and
carried out in accordance with approved guidelines”.

References


Effect of Nanoparticles and Ionization Radiation on Same Oxidant and Antioxidant Enzymes

Rafal Dawood Ali¹, Baydaa T. Sih², Dakhel Ghani Omran³

¹M.Sc., Student, ²Assistance Prof. Ph. D / Department of Physics / College of Science / University of Baghdad, Iraq., ³Prof. D / Department of Biology / College of Science for Women / Babylon University / Iraq

Abstract

Ionizing radiation effect on atoms and molecules of living organisms by formation of free radicals because it has high energy radiation which causes gain or loss one electron or more. In last year’s there are a biggest interest for using nanoparticles in medicine as a drag delivers or cancer treatments , as its will knew. The present research was focused to evaluate the effects of X- ray and nanoparticles (alone or together) on level of Malondialdehyde (MDA), it is the final product of oxidative reactions, and, on the other hand, alleviate these effects by addition of vitamin – E (as anti-oxidant) in blood samples in-vitro. Results which were yield from this research indicated that level of MDA had been significantly increased when blood samples exposed to X-rays but its values were significantly dropped in the presence of nanoparticles of titanium dioxide (TiO₂) in a comparison with those non-treated blood samples (control samples). It was well found that levels of MDA were markedly decrease in the samples treated with V-E after exposure them with both X-ray and nanoparticles. From those observations, it appear that exposure of living tissues to X-rays and nanoparticles causing production of free radicles (oxidative stress) and these adverse effects can be limited and ameliorated by treatment with antioxidant vitamins.

Keywords: TiO₂NPS (titanium dioxide nanoparticles), ROS (Reaction Oxygen Spaces), MDA (Malondialdialdehyde enzyme)

Introduction

Free radicals are naturally considered to be intracellular interactions from the metabolic processes of the cell to produce energy after Glucose or Cholesterol oxidation reactions, rapid free radicals enable to form other compounds mostly toxins or they interact with particles in cells. Free radicals are unstable atoms or molecules with free outer electrons. This makes them highly reactive because free electrons always strive to form a stable bond. This stabilization involves gaining an electron from another molecule, triggering a chain reaction. Such reactions are omnipresent in the human body, but under certain circumstances can damage biomolecules. There are many different sources of free radicals within cells and the environment. In aerobic organisms, free radicals are produced during and through normal metabolic processes. Various in vitro and in vivo studies show that free radical formation can be triggered by nanoparticles (fullerenes, carbon nanotubes, quantum dots, emission particles). Nanoparticles can be taken up actively (phagocytosis) by certain cells (macrophages) and initiate ROS formation. Passive cellular uptake of particles has also been documented. X-Ray is one of the most common electromagnetic radiation that characterised with high frequency and short wavelength, these radiation are employed to be applicator in wide range of science fields such as medicine, biology and industries. Radiotherapy has been used in treatment of many disease in particular cancerous diseases and oxidative
stress (OS) [2]. The Oxidative stress is an imbalance between production of reactive oxygen spaces (ROS) and antioxidant components within living cell that can mediate the damage of biological macromolecules including lipids, proteins and nucleic acids [3,4]. ROS are commonly called oxidants that are harmful for living tissues, among of the ROS are superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and the hydrogen radical (OH$^-$) as well as nitrogen reactive spaces (NRS) [5]. Reactive oxygen species can be removed and inhibited by antioxidant that are enzymatic and non-enzymatic, such as catalase and vitamins [6], such as hypertension, hypercholesterolemia, and diabetes, are associated with oxidative stress. These observations and further data derived from a plethora of investigations provided accumulating evidence that oxidative stress is decisively involved in the pathogenesis of endothelial dysfunction and atherosclerosis. Several enzymes expressed in vascular tissue contribute to production and efficient degradation of reactive oxygen species, and enhanced activity of oxidant enzymes and/or reduced activity of antioxidant enzymes may cause oxidative stress. Various agonists, pathological conditions, and therapeutic interventions lead to modulated expression and function of oxidant and antioxidant enzymes, including NAD(P Molondialdialdehyde (MDA) is the most common product of lipid peroxidation because of ROS reaction [7]. Nanoparticles were synthesized and employed in different fields of since, such as medical, biological and clean energy, these nanoparticles have been documented to induce oxidative stress because the production reactive oxygen species. [8]

**Materials and Methods**

Method of preparing and adding nanoparticles (Tio$_2$):

1. Titanium dioxide particles Tio$_2$ in a synthesized as in [9]

2. Normal Saline with concentration 20 micrograms per 10 ml (it is dissolved and placed in a water bath for 15 seconds to ensure that the nanoparticles in normal saline are distributed correctly)

**Sampling and addition method:**

1. Collecting 20-30 venous blood samples from healthy donors, with an amount of 15 ml of blood per person

2. Samples are placed in tubes containing anticoagulant

3. Blood samples are placed in the centrifugation apparatus to separate the plasma from the blood for a of 15 minutes at 3000 cycles per minutes

4. Blood plasma samples sprated to 7 test tubes (1 ml each tube), the samples classified as follows:

   a. The first sample is considered control sample (without any addition).

   b. The second sample we add 40 μl of NPSTio2 in plasma of blood to study the effect of NPSTio2

   c. The third part is left without additives for irradiation, but it is also treated like the rest of the samples

   d. the fourth part, put NP$_3$Tio$_2$ of 40 μl and radiate it to study the effect of NPSTio2 and irradiation together

   e. The fifth part, added 20 μl of therapeutic vitamin E to be used as antioxidant, not pure vitamin E

   f. The sixth part, add 20 μg of vitamin E and 40 microliters of NPSTio2 to study the effect of vitamin and nano together.

   g. The seventh part, add 20μl of the NPSTio2 and 40μg of Vitamin.E and left it around 24h to rest and after that irradiated with X-rays about 10 Gy to study the effect of radiation and the vitamin together

A chemical solution is applied to all parts of the samples except for the control sample, and this chemical solution works as a kit to measure
molondialdialdehyde, as it has been manually prepared in a chemical manner as described in [10] hydrogen peroxide (H2O2) and this kit is placed in studied and appropriate quantities for the purpose of the tests and measurements that are made on all the samples above.

* After the process of preparing the samples and left for 24 hours in appropriate conditions, away from moisture and kept at 4° C and far from the sun’s rays. The samples were examined by using a UV-visible spectroscopy device to found the absorbance for each one separately and study the differences between the different sample treatments described for the purpose applying absorbance value to the (MDA) equation and measurement.

Results and Discussion

MDA is considered to be a measure of free radicals in living cells as the time period for formation and dissolution of free radicals is very short, MDA is used as a measure of the percentage of free radicals in the body in the following research. MDA and increased enzyme measurements are the result of an increase in free radicals resulting from metabolic processes in the living cells of the human body.

Note that ionizing X-ray works to greatly increase Malondialdehyde (MDA) activity and that the MDA enzyme is responsible for creating a free radicals in the human body, which means that the radiation increases free radicals. Also noted that the samples are reduced by the presence of Tio2NPS, but by the presence of Tio2NPS and ionizing X-rays together, we notice that there is a slight increase in the value of enzyme activity.

Table (1) the activity of Malondialdehyde at (control) and Measurement the effect of NPs-Tio2, vitamin and x-ray each one at time and both to together.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2.169±0.353</td>
<td>0.654±0.196</td>
<td>0.165±0.059</td>
<td>1.75±0.197</td>
<td>3.15±0.27</td>
<td>0.69±0.221</td>
<td>1.45±0.177</td>
</tr>
<tr>
<td>1.501</td>
<td>0.83507</td>
<td>0.253</td>
<td>0.839</td>
<td>1.163</td>
<td>0.939</td>
<td>0.752</td>
</tr>
<tr>
<td>-50%</td>
<td>55%</td>
<td>-25%</td>
<td>-21%</td>
<td>-117%</td>
<td>52%</td>
<td>Change from control</td>
</tr>
</tbody>
</table>

Note that the MDA enzyme decreased in the presence of nanoparticles.
Fig1 (change of control)

Fig2: Average of changes
But it increased a lot when it was irradiated with 10Gy of x-rays because the x-rays increase the rate of production of free radicals, meaning that (MDA is an enzyme that results from the increase of free radicals). A slight increase in the presence of titanium dioxide and X-ray particles. We note that it decreased in the presence of vitamin E because vitamin E works to soothe and reduce free radicals. And it decreased slightly with the presence of nano-dioxide titanium + vitamin E (because the presence of nanoparticles reduces the effect of vitamin E). It also increased with the presence of (vitamin E and X-rays). Significant increase in (Nano-dioxide titanium, vitamin E and x-ray), in the cells of the human body, the use of ionization radiation (IR) was witnessed a wide applications in different fields of science such as medicine, diagnostic, methods and therapeutic options [11]. Recent studies was conducted by [12] confirmed that decrease or minimize the adverse stress by addition of vitamin D and melatonin hormone whose functions are ameliorating of oxidative stress and they have protective effect to minimize side effects of ionizing radiation. It is well documented the deterioration effects of (IR) are induce oxidative stress when attack living tissue, since, the (IR) causes ionization chemical structures including organic and non-organic structures of living cells [13]. Previous experimental study was conducted on rat animal in this study 1000 GY does of ionization radiation was exposed to head of animals the obtaining results inducted results of lipid peroxidation which manifest itself by increment of carbonyl and methylene group of lipid component as well as decrease member fluidity, strength of hydrogen bonds located in phosphate groups [14].

In addition, another previous experimental research investigate the effects of X-rays of antioxidant system of rabbits, the study involved in poseur of exposure of animals to 550-rad- X-rays with administration

Antioxidant vitamin (E and C) and antioxidant minerals (Manganese, Zinc, and copper) the results of this study showed increase of Malondialdehyde (MDA) and decrease of glutathione peroxidase in irradiated group without antioxidant (vitamin and minerals) but those group which administered with antioxidants do not affected with oxidative stress after irradiation because reinforcement of antioxidant system [15]. In conclusion, the results which are recorded above, it obviously appear that ionizing radiation and nanoparticles, alone or combined, can increase levels of free radicals but the application of synthetic antioxidant vitamins have ability to ameliorating and alleviate these effects

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the College of science and carried out in accordance with approved guidelines”.

References


Preparing Mixes of High Dietary Fiber Backed and Studying their Effect on the Storage Properties of Resulting Bread

Abdulbasit F. Al Ali¹, Nawfal A. AlHelfi², Roudha M. Al Ali³
¹Assist. Lecturer, M.Sc., ²Prof. Dr., ³Assist. Prof. Dr., Department of Food Science - University of Basra, Iraq

Abstract

Five types of grain available in local markets have been selected, viz: Barleys, Oat, Sorghum millet and wheat bran. They were milled in the form whole grain and replaced with proportions 5, 10, 15, and 20% of wheat flour extraction 72% with the control mixture 100% Wheat flour stored at laboratory temperature and Cooling for 24-72 hours and studied the effect of dietary fiber on the changes that occur during storage and Staling. It was found that the moisture of the crust increase when storing at laboratory temperature for a period of 24 hours and the increase continued after 72 hours until the equilibrium state was reached. As for the Crumb moisture, sediment value, Swelling Power, pH, Electrical conductivity and turbidity decreased rapidly after 24 hours at both temperature and continued to decrease slowly after 72 hour. This is due to the occurrence of starch retrogradation, the transformation of the starch from the amorphous state to the Crystalline state with loss of moisture in the bread crumb accompany by increase in the moisture content of the crust.

Keywords: Effect, Dietary fiber, storage properties, bread.

Introduction

Bread is one of the staple and important foods in developed and developing countries. Because it is an important component of the human diets a sources of Carbohydrate, Proteins, minerals and vitamins, bread is an important staple food made of wheat flour, salt and Yeasts. Bread Contains a wide range of important nutritional components which provide a positive effect on human health. The most important factor that was considered by researchers and manufactures was Producing bread high quality and long shelf life. Bread quality may undergo physical and chemical changes which called Staling. These changes caused decrease of bread firmens, flavor losses, and resulting in product determination. This phenomenon is frequently attributed to starch retrogradation, a tern used denot partial recrystallization. These changes differ from those that can occur to bread by the action of microorganisms.

Whole grain are used in the bakery industry to add dietary fiber which is important in reducing the risk of developing some chronic diseases such as diabetes and stroke (Wetlon et al., 2005; Montonen et al., 2003) Colon Cancer, Obesity.

Dietary fiber increases the absorption of the bread dough with water, which in turn helps preserve the thirst of bread for a long period of time during storage and reduce the occurrence of Staling.

Materials and Method

The whole grain was ground into five types of available in the local market have been selected viz: Barley, oat, sorghum millet and wheat bran, replaced by the ratio, 15, 10, 5 and 20% from wheat flour 72% extraction. It is used to make four types of bread:

First mixture: 80% Wheat flour, 10% Wheat bran and 10% Barley flour (WWbB).
Second mixture: 75 % Wheat flour, 5% Wheat bran, 5% Barley, and 15 % Oat flour. (WWbBO).

Third mixture: 80% Wheat flour, 5% Wheat bran, 10 % Oat flour and 5 % Sorghum. (WWbOS).

Fourth mixture: 75% Wheat flour, 5% Wheat bran, 5% Barley, 5% Oat, 5% Sorghum, and 5% Millet, (WWbBOSM).

Storage Test

Staling tests

Moisture in the crumb and crust

Moisture in the crumb and crust estimated according to the method mentioned in ACCC (2000).

Estimation the Swelling Power in the crumbs

The swelling power in bread crumb was determined to the method mentioned by AACC (1998) (20-50) with a weight of 2 gram in 50 ml centrifuge tubes, added 20 ml distilled water, mix the contents well for 30 minutes, centrifuged the tube with sediment and determined this swelling Power from the equation:

\[
\text{Swelling Power} = \frac{(A + B) - D}{B}
\]

A: weight centrifuge tube.

B: weight of the sample.

D: weight the sediment with the tube.

Determinations of pH

pH values of the Crumb was measured according the method of AOAC (2007).

Electrical conductivity

Electrical conductivity according to the methods described in Bales et al. (2011). Weight 10 gm of the selected backed and added 75ml of distilled water, mix well for 15 minutes and left 60 minutes, The filtrate was drained in to a glass cylinder and electrode of the apparatus was immersed in the solution the reading was recorded in mili Moz, The final was taken from the product of the device reading in the correction factor in special tables.

Turbidity

Turbidity according to the method described in Sobolewska and Fortuna (2010), added 75 distilled water to 10 gm from backed Crumb, Mix well for 15 minutes, left for one hour, the filtrate was drained into glass cylinder, take 5 ml from filtrate and put in tube for the device with the additions of 5 ml of distilled water, the tubes were closed tightly, the device reading recorded after the tubes were placed in NTU unite.

Statistical Analysis

The experiments were performed in a completely randomized design. All experiments were conducted in triplicates and the mean values and standard deviations were reported. Analysis of variance) ANOVA) was performed and results were Separated using the Multiple Ranger Duncan’s test (p > 0.05) using Statistical Software of Statistical Package for the Social Science (SPSS), Version 16 (SPSS Inc. New Jersey, USA).

Results and Discussions

The effect of adding dietary fiber on the bread staling

staling tests

The effect of adding dietary fiber on the moisture content of the crust and Crumb of bread stored at different temperatures and time periods

The tables (1) (2) showed the moisture of the crust and the crumb stored for a period of 24 and 72 hours at a room temperature and in a refrigerator at 5°C. Table (4-15) shows that there was significant differences (p < 0.05) in the percentage of moisture between the baked crust and control treatment. The results revealed an increase in the percentage of crumb moisture in the bakery products (WWbB, WWbBO, WWbOS, and WWbBOSM). The
The percentage of crumb moisture was 17.1 - 20.3 and 18.7 - 21.5% for WWbB, WWbBO, WWbOS and WWbBOSM respectively for a period of 24-72 hours at a room temperature and in a refrigerator at 5°C. The results also found an increase in the percentage of control sample compare to the bakery products. The moisture of the crust in in the bakery products increased faster within 24 hours of storage due to the migration of moisture from the crumb to the crust. In Contrast, the increase in the moisture became slower after 72 hours of storage.

Table (1) moisture of crust in room temperature and cooling period 24-72 hours.

<table>
<thead>
<tr>
<th>mixtures</th>
<th>moisture crust room 24 hour %</th>
<th>moisture crust room 72 hour %</th>
<th>moisture crust cooling 42 hour %</th>
<th>moisture crust cooling 72 hour %</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>20.6</td>
<td>22.8</td>
<td>23.7</td>
<td>24.1</td>
</tr>
<tr>
<td>1</td>
<td>16.4</td>
<td>17.1</td>
<td>18.2</td>
<td>18.7</td>
</tr>
<tr>
<td>2</td>
<td>16.7</td>
<td>18.8</td>
<td>19.4</td>
<td>19.8</td>
</tr>
<tr>
<td>3</td>
<td>19.7</td>
<td>20.3</td>
<td>21.0</td>
<td>21.5</td>
</tr>
<tr>
<td>4</td>
<td>17.1</td>
<td>18.6</td>
<td>19.1</td>
<td>20.6</td>
</tr>
<tr>
<td>LSD</td>
<td>0.4</td>
<td>0.2</td>
<td>0.3</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Control : 100% Wheat flour.

1-: 80% Wheat flour, 10 % Wheat bran and 10 % Barley flour. (WWbB).

2- 75 % Wheat flour, 5% Wheat bran, 5% Barley, and 15 % Oat flour. (WWbBO).

3- 80% Wheat flour, 5% Wheat bran, 10 % Oat flour and 5 % Sorghum. (WWbOS).

4- 75% Wheat flour, 5%Wheat bran, 5% Barley, 5% Oat, 5% Sorghum, and 5% Millet, (WWbBOSM).

Table (1-2) shows significant differences in the moisture of the crumb of the bakery products stored for a period of 24 and 72 hours at a room temperature and in a refrigerator at 5°C. The results noticed that the percentage of moisture of the crumb in the bakery products was decreased after 24-72 hours at a room temperature and in a refrigerator at 5°. The highest value of moisture was in the first sample 24.8%, which decreased to the 23.6 % after 24 hours at a room temperature. It continued to decline from 23.3% to 23.1% after 72 hours in a refrigerator at 5°C. It was also noticed that all the bakery products was better than the control sample of 23.5 and 22.1% after 24 hours at the room temperature to reach 21.6 and 21.2% in a refrigerator at 5°C after 72 hours of storage. The results also exhibited that the water content of the stored bakery products was decreased faster at room temperature for a period of 24 hours than the products stored in refrigeration after 72 hours.

The Tables (1) and (2) show an increase in the percentage of crust water content in the manufactured bakery products in the laboratory. Thereafter, the water content was decreased during the storage due to the addition of dietary fiber in different proportions that contain groups of hydroxyl that have the ability to The connection of water by forming the hydrogen bonds between the food fiber and the starch. This leads to a delay in the starch retrogradation, thus the bread retains its freshness for a longer period of time.
The process of amylopectin retrogradation in the starch is mainly responsible for the hardening of the bread crumb.

The phenomenon of staling is a major cause of spoilage of bread during storage, due to the transfer of moisture content from the crumb of bakery products to its crust until reaching a state of equilibrium in the moisture of the crust and crumb during the storage. Then, the transfer of moisture content stopped, which leads to a decrease in the moisture content of the baked crumb.  

Table (2) Moisture of Crumb in room temperature and cooling period 24-72 hours.

<table>
<thead>
<tr>
<th>mixtures</th>
<th>crumb moisture% room 24 hour</th>
<th>crumb moisture% room 72 hour</th>
<th>crumb moisture% cooling,24 hour</th>
<th>crumb moisture% cooling,72 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>22.5</td>
<td>22.1</td>
<td>21.6</td>
<td>21.1</td>
</tr>
<tr>
<td>1</td>
<td>24.8</td>
<td>23.6</td>
<td>23.3</td>
<td>23.2</td>
</tr>
<tr>
<td>2</td>
<td>24.2</td>
<td>23.3</td>
<td>23.9</td>
<td>22.6</td>
</tr>
<tr>
<td>3</td>
<td>23.8</td>
<td>22.6</td>
<td>22.3</td>
<td>22.0</td>
</tr>
<tr>
<td>4</td>
<td>22.9</td>
<td>22.7</td>
<td>22.6</td>
<td>22.4</td>
</tr>
<tr>
<td>LSD</td>
<td>0.6</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Control : 100% Wheat flour.

1- 80% Wheat flour, 10% Wheat bran and 10% Barley flour. (WWbB).

2- 75% Wheat flour, 5% Wheat bran, 5% Barley, and 15% Oat flour. (WWbBO).

3- 80% Wheat flour, 5% Wheat bran, 10% Oat flour and 5% Sorghum. (WWbOS).

4- 75% Wheat flour, 5% Wheat bran, 5% Barley, 5% Oat, 5% Sorghum, and 5% Millet, (WWbBOSM).

Effect of adding dietary fiber on the sediment volume and the pH to mixtures stored at different temperatures and for different time periods

The results in Table (1-3) showed that there were significant differences in the sediment volume of the water crumb for the manufactured bakery products. The values of the sediment volume in the watery crumb for all manufactured bakery products decreased after 24-72 hours at a room temperature and in a refrigerator at 5°C. The bakery products of the first mixture significantly better than the rest of the bakery mixes regarding the sediment volume after 24-72 hours at a room temperature and in a refrigerator at 5°C, followed by the bakery products of the second mixture. The results also indicated that the bakery products of all mixtures were significantly better that the control mixture after 24-72 hours at a room temperature and in a refrigerator at 5°C. Whereas, the results showed no significant differences between the bakery products of the first mixture and second mixture at room temperature for a 24 hours due to the high volume of the water crumb sediment. The decrease in the storage capacity to retain the moisture content during the storage period led to the possibility of the phenomenon of starch retrogradation and the shift from the amorphous state to the crystalline state with the possibility of water migration from the bread crumb to its crust.
<table>
<thead>
<tr>
<th>mixtures</th>
<th>sediment Value room 24 hour- ml</th>
<th>sediment Value room 72 hour- ml</th>
<th>sediment Value cooling,24 hour- ml</th>
<th>sediment Value cooling,72 hour- ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>72</td>
<td>70.3</td>
<td>68.4</td>
<td>66.7</td>
</tr>
<tr>
<td>1</td>
<td>77.5</td>
<td>75.2</td>
<td>73.8</td>
<td>72.1</td>
</tr>
<tr>
<td>2</td>
<td>77.0</td>
<td>75.0</td>
<td>73.5</td>
<td>71.4</td>
</tr>
<tr>
<td>3</td>
<td>72.0</td>
<td>71.2</td>
<td>69.7</td>
<td>68.1</td>
</tr>
<tr>
<td>4</td>
<td>74.0</td>
<td>74.8</td>
<td>72.0</td>
<td>69.3</td>
</tr>
<tr>
<td>LSD</td>
<td>1.21</td>
<td>0.64</td>
<td>1.33</td>
<td>1.57</td>
</tr>
</tbody>
</table>

Control: 100% Wheat flour.

1: -80% Wheat flour, 10% Wheat bran and 10% Barley flour. (WWbB).

2- 75% Wheat flour, 5% Wheat bran, 5% Barley, and 15% Oat flour. (WWbBO).

3- 80% Wheat flour, 5% Wheat bran, 10% Oat flour and 5% Sorghum. (WWbOS).

4- 75% Wheat flour, 5% Wheat bran, 5% Barley, 5% Oat, 5% Sorghum, and 5% Millet, (WWbBOSM).

The results of Table (1-4) show that there were significant differences in the pH values of the manufactured bakery products in the laboratory. These products showed a slight decrease in the pH values after 24-72 hours at a room temperature and in a refrigerator at 5°C. The pH of the bakery products of the first mixture was significantly higher among all mixtures. The values of all the bakery products increased compared to the control mixture. The results also were found that the pH of the bakery products mixtures was decline after storage for 72 hours. The pH was used to know what happens during the occurrence of the phenomenon of starch retrogradation and the shift from the amorphous state to the crystallized state. The phenomenon of bread flaking can be followed, as it is possible to follow up on the changes that occur in the starch by studying the effect of acidity on the properties of gels in starch.
Table (4) pH of mixture in room temperature and cooling period 24-72 hour.

<table>
<thead>
<tr>
<th>mixtures</th>
<th>pH - room 24 hour</th>
<th>pH - room 72 hour</th>
<th>pH - cooling 24 hour</th>
<th>pH - cooling 72 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>6.15</td>
<td>6.11</td>
<td>6.09</td>
<td>6.06</td>
</tr>
<tr>
<td>1</td>
<td>6.28</td>
<td>6.24</td>
<td>6.23</td>
<td>6.21</td>
</tr>
<tr>
<td>3</td>
<td>6.19</td>
<td>6.18</td>
<td>6.16</td>
<td>6.15</td>
</tr>
<tr>
<td>4</td>
<td>6.22</td>
<td>6.21</td>
<td>6.20</td>
<td>6.19</td>
</tr>
<tr>
<td>LSD</td>
<td>0.03</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Control: 100% Wheat flour.

1: 80% Wheat flour, 10% Wheat bran and 10% Barley flour (WWbB).
2: 75% Wheat flour, 5% Wheat bran, 5% Barley, and 15% Oat flour (WWbBO).
3: 80% Wheat flour, 5% Wheat bran, 10% Oat flour and 5% Sorghum (WWbOS).
4: 75% Wheat flour, 5% Wheat bran, 5% Barley, 5% Oat, 5% Sorghum, and 5% Millet (WWbBOSM).

The effect adding dietary fiber on electrical conductivity and turbidity of mixture stored at different temperatures and time periods

Table (5) shows significant differences in the electrical conductivity of the manufactured bakery products in the laboratory. The electrical conductivity of the manufactured bakery products was decreased after 24-72 hours at a room temperature and in a refrigerator at 5°C. The results showed that the highest electrical conductivity was the bakery products of the first mixture of 3.81 dS/cm, which then decreased to reach 3.73 dS/cm after 24 hours of storage at room temperature, while the electrical conductivity of the bakery products of the first mixture was declined from 3.64 dS/cm to 3.61 dS/cm in a refrigerator at 5°C for a period of 72 hours. In contrast, the value of electrical conductivity of bakery products for all mixtures was higher than the control sample at the same storage conditions. It is also noted that the electrical conductivity of bakery products for all mixtures decreased significantly after 24 hours at a room temperature and in a refrigerator at 5°C, while the decrease was slow after 72 hours. The electrical conductivity test is one of the main modern tests used to monitor the occurrence of the Staling of bread, as the electrical conductivity is a measure of the material’s ability to pass the electric current. This test was used often to know the moisture content in the materials. Therefore, measuring the electrical conductivity in the crumb of the bread is an accurate measure of the shelf life of the bread (Saleh et al., 2016). This result was in agreement with Bales et al. (2011) who found a clear decrease in the electrical conductivity in bagged and unpacked of bread during storage. Chintan and Nagaraju (2010) mentioned that the electrical conductivity decrease was greater during a 24-hour due to the moisture content and its movement between the crumb and the crust until reaching equilibrium.
Table (5) Average electrical conductivity of mixtures stored at room temperature and cooling period 24-72 hours

<table>
<thead>
<tr>
<th>mixtures</th>
<th>Electrical conductivity - room 24 hour dS / cm</th>
<th>Electrical conductivity - room 72 hour dS / cm</th>
<th>Electrical conductivity - cooling 24 hour dS / cm</th>
<th>Electrical conductivity - cooling 72 hour dS / cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>3.50</td>
<td>3.10</td>
<td>2.90</td>
<td>3.71</td>
</tr>
<tr>
<td>1</td>
<td>3.81</td>
<td>3.72</td>
<td>3.64</td>
<td>3.62</td>
</tr>
<tr>
<td>2</td>
<td>3.69</td>
<td>3.64</td>
<td>3.60</td>
<td>3.59</td>
</tr>
<tr>
<td>3</td>
<td>3.62</td>
<td>3.58</td>
<td>3.47</td>
<td>3.43</td>
</tr>
<tr>
<td>4</td>
<td>3.61</td>
<td>3.57</td>
<td>3.52</td>
<td>3.45</td>
</tr>
<tr>
<td>LSD</td>
<td>0.04</td>
<td>0.11</td>
<td>0.12</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Control : 100% Wheat flour.

1: 80% Wheat flour, 10% Wheat bran and 10% Barley flour (WWbB).

2- 75% Wheat flour, 5% Wheat bran, 5% Barley, and 15% Oat flour (WWbBO).

3- 80% Wheat flour, 5% Wheat bran, 10% Oat flour and 5% Sorghum (WWbOS).

4- 75% Wheat flour, 5% Wheat bran, 5% Barley, 5% Oat, 5% Sorghum, and 5% Millet (WWbBOSM).

Table (6) shows the significant differences in the percentage of crumb swelling power in the manufactured bakery products in the laboratory. The results observed that swelling power decreased with increasing the time of storage at a room temperature and in a refrigerator at 5°C. It also found that the swelling power of all bakery products at the room temperature for a period of 24 was 2.32 - 2.65%, which decreased to reach 2.24 - 1.38 after 24 hours at the same temperature. Whereas, the swelling power of the crumb after 72 hours in a refrigerator at 5°C reached 2.20 - 2.41 and 2.18 - 2.38%, respectively. The results showed that all mixtures of the bakery products had higher values compared to the control sample. The results were consistent with 8 who studied the swelling power of crumb in bakery products after storage for four days. They stated that the swelling power was decreased by 13.3% on the second day, while it decreases by 7% on the fourth day due to the occurrence of the staling of starch retrogradation and the shift from the amorphous state to the crystalline state.

Table (6) Average Swelling Power of mixtures stored at room temperature and cooling period 24-72 hours

<table>
<thead>
<tr>
<th>mixtures</th>
<th>Swelling Power room 24 hour</th>
<th>Swelling Power room 72 hour</th>
<th>Swelling Power cooling 24 hour</th>
<th>Swelling Power cooling 72 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>2.18</td>
<td>2.16</td>
<td>2.12</td>
<td>2.10</td>
</tr>
<tr>
<td>1</td>
<td>2.65</td>
<td>2.58</td>
<td>2.41</td>
<td>2.33</td>
</tr>
<tr>
<td>2</td>
<td>2.45</td>
<td>2.34</td>
<td>2.39</td>
<td>2.35</td>
</tr>
<tr>
<td>3</td>
<td>2.32</td>
<td>2.24</td>
<td>2.20</td>
<td>2.18</td>
</tr>
<tr>
<td>4</td>
<td>2.57</td>
<td>2.46</td>
<td>2.38</td>
<td>2.32</td>
</tr>
<tr>
<td>LSD</td>
<td>0.12</td>
<td>0.11</td>
<td>0.07</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Control : 100% Wheat flour.
1. 80% Wheat flour, 10% Wheat bran and 10% Barley flour. (WWbB).

2. 75% Wheat flour, 5% Wheat bran, 5% Barley, and 15% Oat flour. (WWbBO).

3. 80% Wheat flour, 5% Wheat bran, 10% Oat flour and 5% Sorghum. (WWbOS).

4. 75% Wheat flour, 5% Wheat bran, 5% Barley, 5% Oat, 5% Sorghum, and 5% Millet. (WWbBOSM).

Table (1-7) shows the significant differences in the average values of turbidity of the solution of the crumb of the manufactured bakery products in the laboratory. The results showed that the manufactured bakery products for mixtures were significantly superior to the control sample. The turbidity values for manufactured bakery products were 293-794 NTU and 234-701 NTU at a room temperature and in a refrigerator at 5°C after 24-72 hours. The results also found that manufactured bakery products for all prepared mixtures had higher values than the control sample of 307, 277, 246 and 227 NTU respectively at the same storage conditions. A significant decrease in the turbidity value of all bakery products was observed in the first 24 hours of storage, while the decline was slower after 72 hours of storage. The turbidity test can give an idea of the qualitative description of the Staling of retrogradation in the bread, and this test can also be used to know the effects of storage on this Staling and the extent of the effect of the Staling bread on stored bakery products, and this test was also used to find out the effect of adding Maltodextrins on the Staling of starch retrogradation.

Conclusion

In conclusion, the results were consistent with those of Abdul Aziz N, Bhat R, Azahari B. Storage studies of bread prepared by incorporation of the banana pseudo-stem flour and the composite breads containing hydrocolloids. CyTA-Journal of Food. 2014; 12(2): 141-149. who confirmed an increase in the percentage of moisture in the crust after 72 hours of storage, whereas the percentage of crumb moisture, the volume of the watery crumb sediment, swelling power, pH, electrical conductivity, and turbidity were gradually decreased during the storage period. The reason for the decrease in the ability of the crumb to retain water was due to the occurrence of the staling of starch retrogradation from the amorphous state to the crystallized state and the transfer of water from the crumb of the bread to its crust, therefore, the occurrence of the Staling of bread increases when the swelling power, the size of the sediment in the crumb, pH, electrical conductivity, and turbidity decreases.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the Department of food science and carried out in accordance with approved guidelines”.

References


The Level of Anxiety and Depression in Patients with Lung Cancer in Al-Diwaniyah Teaching Hospital

Mortada Abdel Reda Eajal1, Kareem R. Sachit2
1M.Sc., Student, 2 Assist. Prof. Dr., Department of Psychiatric and Mental Health Nursing, College of Nursing, University of Baghdad

Abstract

Objectives: To assess level of anxiety and depression among Patients with lung cancer. To find out the relationship between level of anxiety and depression with age, gender, educational level, occupation, residence, frequency of admission, marital status, and monthly income.

Methodology: Descriptive analysis designs are used to conduct the study. The study period started from March 1th, 2021 to April 15th 2021 at the Department of Oncology in Al-Diwaniyah Teaching Hospital. Total of (110) patients selected from oncology department in Al-Diwaniyah Teaching Hospital. ten patients were excluded from the study for the pilot study. So the total number of patients participating in the study was (100) patients. The depression and anxiety Scale are consisting from 14 items self-rated questionnaire that involves two dimensions. These are depression (7 items) and anxiety (7 items). These items are measured on a (4) point Likert-type scale of 1 (Not applicable), 2 (Somewhat applicable), 3 (Often remarkable) and 4 (Very often applicable). The total score is computed by summing scores of all items. The score of each dimension is calculated by summing the scores of the items and dividing by the number of items.

Results: The demographic and clinical characteristics of the samples the vast majority of the patients were (73%) were all males, (85%) are married couple, the participants (48%) were housewives and unemployed, (62%) from lung cancer patients are urban, (46.8%). Monthly income, Concerning the significant differences between depression and anxiety with patients’ age, the study showed that there is high significant difference in depression symptoms with regard to older age patients at p-value= 0.001 respectively.

Key words : lung cancer, anxiety, depression.

Introduction

Lung cancer is a serious disease that puts people’s lives in jeopardy. Lung cancer in charge of more than a quarter (27%) both cancer-related deaths. The diagnosis and treatment of cancer will bring up a wide range of negative emotions, including anxiety and depression. Patients with malignant diseases, especially lung cancer, suffer from a high rate of depression and anxiety. Anxiety, depression, and other forms of emotional distress have been shown in studies to have a detrimental impact on lung cancer patients’ standard of life and even survival 1. Small cell lung cancer (SCLC) and non–small cell lung cancer (NSCLC) are two of the most common forms of lung cancer. The most prevalent form of lung cancer is non-small cell lung cancer (NSCLC), which accounts for 85 to 90 percent of all cases. Squamous cell carcinoma, adenocarcinoma, and large cell carcinoma are the three subtypes of NSCLC, which account for 85 to 90 percent of all cases. Adenocarcinoma and squamous
cell carcinoma are the most prevalent histologic subtypes; NSCLC cases account for 50% and 30% of all cancer cases, respectively. Small cell lung cancer affects ten to twenty percent of lung cancer patients. Its aggressive genetics, proclivity for early metastatic disease, and poor prognosis set it apart from other forms of cancer. In addition, Lung cancer is the most common cancer-related death in the world. Despite advancements in treatment effectiveness, affected patients also suffer from debilitating disease-linked signs and symptoms such as dyspnea, cough, nausea, anxiety, depression, insomnia, and pain. Lung cancer patients can suffer a wide range of health problems, including psychological distress as a consequence of cancer or its therapy unfavorable impact. Physical activity and exercise can help to reduce these symptoms. Comorbid depression and other psychological disorders are common in lung cancer patients, also a wide range of physical symptoms. For example, Progressive illness, pulmonary venothrombosis is a complication of lung cancer, or until therapy lung cancer may cause anemia and anorexia. Progression of disability exacerbates distress, anxiety, and depression in people with metastatic lung cancer, a lack of autonomy, unrelenting physical symptoms like pain, or underlying mechanistic triggers like systemic inflammation are all possible causes. Anxiety and depression are both severe and chronic mental illnesses. Anxiety is described as an uncomfortable feeling of danger or possible harm that lasts for a long time, which is frequently accompanied by fear, discreet, lack of concentration, Muscle pain, palpitations, and Mood swings, lack of enjoyment or interest in hobbies and sports, reduced motivation, difficulty focusing or making choices, Depression manifests itself in a variety of ways, including disturbed appetite and sleep, psychomotor like irritable bowel syndrome, insomnia, psychomotor agitation, and attempted suicide. Patients’ capacity to withstand the disease burden and aggressive treatment regimens can be harmed by anxiety and depression, this lengthens hospital stays, lowers quality of life, and raises mortality rates.

Methodology

Design of the Study: Descriptive analysis designs are used to conduct the study. The study period started from February 14th, 2021 to April 15th 2021.

Setting of the Study: at the Department of Oncology in Al- Diwaniyah Teaching Hospital.

A Sample of the Study: Total of (110) patients selected from oncology department in Al-Diwaniyah Teaching Hospital. ten patients were excluded from the study for the pilot study. So the total number of patients participating in the study was (100) patients.

Instrument: The study instrument includes the patients’ socio-demographic sheet and the Depression, Anxiety and Stress Scales

Reliability of the practice Items: The reliability of the instrument was determined through the computation of Alpha Cronbach’s test (Alpha Correlation Coefficient); internal consistency method was used for determining the reliability. The Alpha Correlation Coefficient was applied to determine the reliability of the present study instrument by application of Statistical Package for Social Science Program (IBM SPSS) version 24.0.

Statistical Methods: The data were analyzed using SPSS (Statistical Package for Social Sciences) version 25 application of statistical analysis system. The following statistical data analysis approaches were used for analyzing and assessing the results of the study.
## Result

Table (1): Distribution of Patients According to their Socio-demographic Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>f</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>Female</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 – 28 year</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>29 – 38 year</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>39 – 48 year</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>49 – 58 year</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>59 ≤ year</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Residency</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>Rural</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Level of education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doesn’t read &amp; write</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Read &amp; write</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Primary school</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Intermediate school</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Secondary school</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Institute/college +</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unmarried</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Married</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Widowed/er</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Divorced</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Separated</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employee</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Free works</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Retired</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Jobless/ housewife</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Monthly income</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insufficient</td>
<td>68</td>
<td>68</td>
</tr>
<tr>
<td>Barely sufficient</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Sufficient</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>House ownership</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Owned</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>Rented</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Shared</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
This table shows that more of patients are males (73%) and remaining are females (27%). The variable of age refers that they are older patients in which the highest percentage is 58% for those with age 59 years or more. Regarding residency, 62% of them are resident in urban areas and 38% are resident in rural areas. The level of education among patients refers to “read and write” as recorded with high percentage of 44% among them. The marital status indicates that more than half of patients are married (52%) and 14% are still unmarried.

The occupational status refers that 48% of them are jobless or housewife and 22% of them are retired while only 14% are still working as employee.

The patients are perceived insufficient monthly income as reported among 68% while 23% are perceived barely sufficient monthly income. 51% of them are having their own house while 36% are live in rented houses.

### Table (2): Assessment of Depression Levels among Patients with Lung Cancer

<table>
<thead>
<tr>
<th>Level of depression</th>
<th>f</th>
<th>%</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>36</td>
<td>36</td>
<td>14.87</td>
<td>2.665</td>
</tr>
<tr>
<td>Severe</td>
<td>63</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**f**: Frequency, **%**: Percentage, **M**: Mean, **SD**: Standard deviation

Mild= 0 – 7, Moderate= 8 – 14, Severe= 15 – 21

This table indicates that 63% of patients are associated with severe symptoms of depression and 36% are associated with moderate symptoms of depression.

### Table (3): Assessment the Level of Depression Symptoms among Patients with Lung Cancer (N=100)

<table>
<thead>
<tr>
<th>Items</th>
<th>Responses</th>
<th>f (%)</th>
<th>M.S</th>
<th>Assess.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I could not seem to experience any positive feeling at all</td>
<td>Not applicable</td>
<td>6(6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Somewhat applicable</td>
<td>31(31)</td>
<td>1.89</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>Often remarkable</td>
<td>31(31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very often applicable</td>
<td>32(32)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Cont... Table (3): Assessment the Level of Depression Symptoms among Patients with Lung Cancer (N=100)**

<table>
<thead>
<tr>
<th>Statement</th>
<th>Not applicable</th>
<th>Somewhat applicable</th>
<th>Often remarkable</th>
<th>Very often applicable</th>
<th>Assess</th>
</tr>
</thead>
<tbody>
<tr>
<td>I found it difficult to work up the initiative to do things</td>
<td>5(5)</td>
<td>32(32)</td>
<td>39(39)</td>
<td>24(24)</td>
<td>1.82</td>
</tr>
<tr>
<td>I felt that I had nothing to look forward to</td>
<td>3(3)</td>
<td>22(22)</td>
<td>34(34)</td>
<td>41(41)</td>
<td>2.13</td>
</tr>
<tr>
<td>I felt down-hearted and blue</td>
<td>5(5)</td>
<td>17(17)</td>
<td>34(34)</td>
<td>44(44)</td>
<td>2.17</td>
</tr>
<tr>
<td>I was unable to become enthusiastic about anything</td>
<td>4(4)</td>
<td>23(23)</td>
<td>33(33)</td>
<td>40(40)</td>
<td>2.09</td>
</tr>
<tr>
<td>I felt I was not worth much as a person</td>
<td>1(1)</td>
<td>6(6)</td>
<td>26(26)</td>
<td>36(36)</td>
<td>1.98</td>
</tr>
<tr>
<td>I felt that life was meaningless</td>
<td>1(1)</td>
<td>3(3)</td>
<td>12(12)</td>
<td>84(84)</td>
<td>2.79</td>
</tr>
</tbody>
</table>

M.S: Mean of Score, Assess: Assessment

Low= 0 – 1, Moderate = 1.1 – 2, High= 2.1– 3
This table presents the mean scores for assessing the depression symptoms among patients with lung cancer; the findings indicate that patients are with moderate to severe symptoms of depression in which the mean scores is seen moderate among the items of 1, 2, and 6 while seen high among items 3, 4, 5, and 7.

**Table (4): Assessment of Anxiety Levels among Patients with Lung Cancer**

<table>
<thead>
<tr>
<th>Level of Anxiety</th>
<th>f</th>
<th>%</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>54</td>
<td>54</td>
<td>13.56</td>
<td>3.205</td>
</tr>
<tr>
<td>Severe</td>
<td>43</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

f: Frequency, %: Percentage
M: Mean for total score, SD: Standard Deviation for total score

Mild= 0 – 7, Moderate= 8 – 14, Severe= 15 – 21

This table exhibits that patients are associated with moderate to high anxiety level; in which 54% are associated with moderate level and 43% are associated with severe level of anxiety.

This chapter presents a systematically designed interpretation and reasonably derived discussion of the results with the support of the available related studies.

**Part I: The Discussion of the patients’ Demographic Characteristics and clinical characteristics of the Study Sample, as Shown in Table (4.1) and Figure (4.1) and (4.2):**

According to the findings of the study, the vast majority of the patients were (73%) were all males, as showed in (Table 4-1). This finding supported by Khue et al. (2019) and Hu et al. (2018) who discovered that the vast majority of the study participants were men.

Concerning educational levels, the majority of the sample patients (44%) was read and writes, as showed in (Table 4-1). Patient’s level of education could control detecting of the symptoms in the earlier stages of the cancer, and decreasing the effect of the...
risk factors as well as the complications by enhancing their awareness toward the disease and also treatment modalities. So, most of persons who suffer from is poor of education have higher risks of cancer. The educational level gives an indicate about patients awareness toward illness so, a high level of education means a high level in their awareness toward the disease, and low level of education means less awareness with increasing stress levels in their lives. This finding comes along with Hu et al. (2018) who discovered that the bulk of the study subjects came from elementary school and lower (50 percent).

In terms of employment status, the current study’s results show that the majority of the participants (48%) were housewives and unemployed, as showed in (Table 4-1). This result is supported by Hu et al. (2018) found that 85.1 percent of the people in the study were unemployed. The higher the educational standard, the higher the number of people who can read and write.

In terms of home ownership, the majority of the study patients (51%) are homeowners, as shown in Figure 1. (Table 4-1). This contradicts the findings of Ni et al. (2018), who found that the majority of the study participants were share (85 percent).

Regarding residency, the bulk of the lung cancer cases in the sample, (62%) from lung cancer patients are urban, as mentioned, one of the most important causes in increasing risk of cancer is the environment. In Iraq, highly increment of environmental pollution as a result of the chemical factors affecting air like industrial pollution and explosions also the remnants of War, participates in being the most important risk leading to cancer factors especially in big cities. This finding disagree with Choenyi et al., (2016) in Uttarakhand shown the area of living most of the cancer patients 35 (58%) belonged to rural area, 25 (42%) were from urban area.

Related to monthly income, the majority of the sample patients are insufficient monthly income (46.8%). Monthly income may be play a role in patient information about self-care, poverty is considered as an important indicator that may raise the risk of developing the cancer and delay the early detection due to money shortage of the family. This result is supported by Cancer Research UK (2012) in their reports, The study found that as the prevalence of cancer rises in low and middle-income countries, the global risk of cancer and cancer-related death rises.

It has been shown out of the analysis that 69% of patients are with 3-4 years duration of illness and 16% are diagnosed since 1-2 years. This mean that are recently diagnosed with cancer and this duration may be a source of stress for these patients as diagnosed with such illness. This finding is slightly different from findings of Polanski and others who report that patient with limited stage are between 1 and 1.5 year while those with end stage are between half year to one year (Polańska J, Rosinczuk J, Chabowski M, and Szymanska-Chabowska A, 2015).

The number of admission referred that 66% of patients are admitted more than three times to hospitals and 32% are admitted only twice. This finding indicates that patients are on regular treatment which may contribute to their stress and markedly increase their depression and anxiety. Skaug and his colleagues found in their study and stated “Median survival time was 170 days. Mean age at the first admission was 67.4 years (range 21–89 years). Median number (inter quartile range) of admissions was 3 (2, 5) and total hospitalization days 35 (18, 58)”.

Part II Assessment of Depression Levels among Patients with Lung Cancer:

According to the findings, patients with lung cancer experience mild depression as a result of their illness. These findings are along with study performed by, who revealed the lung cancer patients experience a lot of anxiety and depression. Hospitalization is linked to higher rates of anxiety and depression, necessitating effective interventions. Hu et al. (2018) published another study that found that
the diagnosis and treatment of lung cancer patients has a significant impact on their mood.

**Part III Assessment of Depression Levels among Patients with Lung Cancer:**

Patients with lung cancer experience mild anxiety as a result of their illness, according to the findings of the report. In most of the questions, anxiety levels were higher. Patients with lung cancer may be increase sensitive and feel the pressure, which can impact their recovery and make them more frustrated. These findings are along with study performed by Khue et al., (2019). Anxiety and depression were found to have the highest proportion of respondents having any issues (92.8 percent).

**Conclusions**

1. Most of lung cancer patients have severe levels of anxiety.
2. Most of lung cancer patients have severe levels of depression.
3. Most socio-demographic characteristics of the sample are: age (59 and more) years old, males, married, insufficient monthly income, reads and writes, housewives, live in urban area, owned house, duration of lung cancer (3-5) and admitting frequently three times through month.
4. There is no significant association between level of anxiety and level of depression with the patient’s age, gender, educational level, marital status, monthly income, occupation, residence, frequency of admission, duration of lung cancer.
5. There is significant difference in depression symptoms with the patient’s age.
6. There is significant difference in anxiety symptoms with the patient’s level of education and occupation.

**Recommendations:**

The present study recommended that:

1. Increase the level of awareness for lung cancer patients and their families about the nature and stage of lung cancer and how to minimize their distress which caused by the disease.
2. Improve the psychological and emotional status of lung cancer patients; especially by nursing staff after taking chemotherapy treatment use psychological treatment reduce anxiety and depression for cancer patient.
3. By using educational programs and posters about lung cancer, development stages, treatment and side effects of treatment and how to prevent anxiety and depression for cancer patient.
4. Coordinate between oncology center and the Department of Psychiatry in the hospital for giving treatment to reduce anxiety and depression levels.
5. Support for lung cancer patients through the government economically and provide the required treatment.
6. Conducting similar studies at the national level on the largest sample to assess anxiety and depression among lung cancer patients.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** “All experimental protocols were approved under the Department of Psychiatric and Mental Health Nursing and carried out in accordance with approved guidelines”.

**References**

1. Hu, T, Xiao, J, Peng, J, Kuang, X, He, B. Relationship between resilience, social support as well as anxiety/depression of lung cancer patients: A cross-sectional observation study. Journal of


Self-esteem of Children Living with their Parents for Secondary Schools in AL-Rusafa: Comparative Study to the Children Living in Orphanage

Rehab K. Habsi¹, Zaid W. Ajil²

¹M.Sc., Student, Department of Nursing, Ministry of Health, Baghdad, Iraq, ²Lecturer, Dr., Department of Nursing, College of Nursing, University of Baghdad, Iraq

Abstract

Objectives: to find out differences in the level of self-esteem between orphanage children and children who live with their Parents.

Methods: A descriptive studies that use comparative design. The method of A Purposive sampling is used which is located in Rusafa, Baghdad. The sample size was 200 students (male and female) from all secondary schools in Baghdad al-Rusafa who lived with their parents, and another 200 adolescent who lived in an orphanage. The data was collected during January 11th to March 2nd, 2021. Short form of (coopersmith,1967) scales for assessing self-esteem level.

Results: The result of the study shows that highly significant differences are accounted between studied groups at P<0.01 regarding of studied “Negative’s Items Domain”, and “ Overall Evaluation”, while no significant different was accounted with reference to “Positive’s Items Domain”. Through the results of the significant comparisons, it becomes clear that the trends of the negative items of the scale are the distinguishing evidence between the two studied groups at the time when the outcomes of the positive trend items didn’t formed the presence of significant differences between the two groups.

Keywords: Self-esteem, Children, Orphanage, Secondary School

Introduction

Self-esteem is a term related to the concepts of self-image and self-consciousness. There are many attempts used to describe self-esteem, the best of them admitted that it represents the manner in which each individual evaluates himself related to the group. William James was the first who puts the term self-esteem in 1890, making it one of psychology’s oldest concepts. The development of the phase of self-esteem is critical especially during the age of adolescence. The development of self-esteem may it encourages by both teachers and parents. Self-esteem level is expressed in the attitude of adolescent and behaviour at home as well as at school. Adolescence is a critical period for developing self-esteem and self-identity. When self-esteem is low may threaten adolescent emotional control.

Adolescence is a time of transition, both physical and Psychological, and often a time when they develop a sense of their own identity in which interactions between families and peers play a crucial role. Some adolescents do not receive the individual affection and love that others of their age receive from both parents. Some are left to fend on their own, while others are packed off to live in institutions or with relatives. While an alternative choice is given for the child to be in a foster family or an orphanage home.

Material and Methods

Descriptive study was use comparative design.
The method of sampling was Purposive sampling is used. The research design was used and carried out in order to achieve the study’s goals that evaluate the self-esteem of children living with their parents of secondary schools in al-Rusafa comparative to children living in orphanages which is located in Rusafa, baghdad. The research instrument consists of three axes: 1-a list of common items for the participating students’ demographic data assessment, II-a list of common items for the Orphan demographic data assessment, III Coopersmith self-esteem scale. The study instrument is then revised by a variety of experts From inside and outside the college of nursing. The research was conducted in baghdad, in forty-one secondary schools. It was carried out in Rusafa for secondary schools, which includes (Rusafa I, Rusafa II, Rusafa III Sadr City), Baghdad governorate general directorate of education and orphanages in baghdad governorate in rusafa. The data was collected during January 11th to March 2nd, 2021. The content validity and reliability was performed to the tool of study before used, a pilot study was conducted before data collected with (10) children living with their parents and (10) children living in orphanage and they were excluded from study sample. The score are consists 25 items of short form introduced by (Coopersmith, 1967) using (Likert) score of the fifth categories scales, such that (Strongly Disagree, Disagree, Neutral, Agree, and Strongly Agree) by the integer numbers (1, 2, 3, 4, and 5) respectively, and The data was analysed by (SPSS) ver. (21.0), frequencies and percentages, mean of score, standard deviation, relative sufficiency, as well as evaluation for observed responding through using differentiated intervals: [(20.00 – 46.66) for Low (L) evaluation; (46.67 – 73.33) for Moderate (M) evaluation; and (73.34– 100) for High (H) evaluation].

Results

Table (1): Testing different of studied groups regarding (Negative, and Positive) item’s direction domains and an overall evaluation

<table>
<thead>
<tr>
<th>Test Statistic</th>
<th>Sub &amp; Main Domains</th>
<th>Negative’s Items Domain</th>
<th>Positive’s Items Domain</th>
<th>Overall Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mann-Whitney</td>
<td>Z-Statistic</td>
<td>-6.445</td>
<td>-0.406</td>
<td>-4.030</td>
</tr>
<tr>
<td></td>
<td>Asymptotic Sig.</td>
<td>0.000</td>
<td>0.685</td>
<td>0.000</td>
</tr>
<tr>
<td>C.S. (*)</td>
<td>HS</td>
<td>NS</td>
<td>HS</td>
<td></td>
</tr>
</tbody>
</table>

(*) H S: Highly Sig. at P<0.05; NS: Non Sig. at P>0.05; Testing statistic are based on the Mann-Whitney test.
The present study results showed that highly significant differences in self-esteem between orphan children and children living with their parents regarding overall evaluation where the negative items of the scale are the distinguishing evidence between the two groups.

Table (2): Summary Statistics of “Self-Esteem” score’ evaluation for Children living with their parents for secondary schools in AL-Rusafa (The Control Sample) and Children living in orphanage home (The Study Sample)

<table>
<thead>
<tr>
<th>Self-Esteem Score</th>
<th>Groups</th>
<th>Study</th>
<th>Control</th>
<th>C.S. P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>MS</td>
</tr>
<tr>
<td></td>
<td>Response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I often wish I</td>
<td>Strongly</td>
<td>15</td>
<td>7.5</td>
<td>3.71</td>
</tr>
<tr>
<td>were someone else</td>
<td>Disagree</td>
<td>31</td>
<td>15.5</td>
<td>3.08</td>
</tr>
<tr>
<td>(-)</td>
<td>Neutral</td>
<td>35</td>
<td>17.5</td>
<td>3.96</td>
</tr>
<tr>
<td></td>
<td>Strongly</td>
<td>7</td>
<td>3.5</td>
<td>3.4</td>
</tr>
<tr>
<td>There are lots of</td>
<td>Disagree</td>
<td>13</td>
<td>6.5</td>
<td>3.5</td>
</tr>
<tr>
<td>things about</td>
<td>Neutral</td>
<td>37</td>
<td>18.5</td>
<td>3.96</td>
</tr>
<tr>
<td>myself I’d change</td>
<td>Agree</td>
<td>67</td>
<td>33.5</td>
<td>3.4</td>
</tr>
<tr>
<td>if I could (-)</td>
<td>Strongly</td>
<td>76</td>
<td>38</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Strongly</td>
<td>12</td>
<td>6</td>
<td>3.4</td>
</tr>
<tr>
<td>I make decisions</td>
<td>Disagree</td>
<td>35</td>
<td>17.5</td>
<td>3.5</td>
</tr>
<tr>
<td>in any situation</td>
<td>Neutral</td>
<td>54</td>
<td>27</td>
<td>3.4</td>
</tr>
<tr>
<td>without trouble</td>
<td>Agree</td>
<td>59</td>
<td>29.5</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Strongly</td>
<td>40</td>
<td>20</td>
<td>3.4</td>
</tr>
</tbody>
</table>
Cont... Table (2): Summary Statistics of “Self-Esteem” score’ evaluation for Children living with their parents for secondary schools in AL-Rusafa (The Control Sample) and Children living in orphanage home (The Study Sample)

<table>
<thead>
<tr>
<th></th>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Others are happy when I be with them</td>
<td>12</td>
<td>6</td>
<td>3.93</td>
<td>1.15</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36</td>
</tr>
</tbody>
</table>

Testing based on Mann-Whitney test.

In the table (2) most of children living with their parents from secondary schools has a better responses to the phrases of coopersmith scale, this findings mean that children living in orphanage had low self-esteem compare to children living with their parents for secondary school, from the researcher point of view may be related to school environment, teacher-student interaction, and activity structure, such as debate competitions, are likely to aid in the promotion of self-esteem and serve as a nurturing factor in the development of confidence in all school-aged children. In Pakistan clarified that students showed high self-esteem.

Figure (1): Negative and Positive Percentile (Grand/Global) mean of score for “Self-Esteem” Score in the studied groups

Results shows that highly significant differences are accounted between studied groups at P<0.01 regarding of studied “Negative’s Items Domain”, and “ Overall Evaluation”, while no significant different was accounted with reference to “Positive’s Items Domain”.

Through the results of the significant comparisons, it becomes clear that the trends of the negative items of the scale are the distinguishing evidence between the two studied groups at the time when the outcomes of the positive trend items didn’t formed the presence of significant differences between the two groups.
Discussion

Self-Esteem of Children living with their parents for secondary schools at Al-Rusafa in comparative with the study group of Children living in orphanage home through the short form of (Coopersmith, 1967) scales shows that most of Children living with their parents has a better responses due to their self-esteem than Children living in orphanage home. The trends of the negative items of the scale are the distinguishing evidence between the two studied groups, and orphan who are living in the orphanage are more reliable for this study.

The present study results showed that highly significant differences in self-esteem between orphan children and children living with their parents regarding overall evaluation where the negative items of the scale are the distinguishing evidence between the two groups. This finding is supported by study conducted in Anantnag district of Jammu and Kashmir by 6, in their study on self-esteem of institutionalized orphan children and family reared. Stated that orphans children have a lower degree of self-esteem than children living with both parents.

Agree with study conducted by 1, in Dubai-UAE (United Arab Emirates) in titled orphan and non-orphan children’s self-esteem and depression who reported that non-orphan children received a high self-esteem score relative to their orphan children’s counterparts. Also, agree with study conducted in India done by 9, in their study A comparison of self-esteem and depression levels in adolescents living in orphanages with those living with their parents. They reported that low self-esteem and high depression in orphan children. Furthermore, Mr. Siyad and Mr. Muneer, (2016) consistent with the findings of the current study, they mention that lower self-esteem was reported by orphan children than by children living with their parents, and Farooqi and Intezar (2009) supported this results and, they reported that Children have lower levels of self-esteem in orphanages than children living with their parent’s

Conclusion

Self-Esteem of children living with their parents for secondary schools at Al-Rusafa in comparative with the study group of Children living in orphanage home through the short form of (Coopersmith, 1967) scales shows that most of children living with their parents for secondary schools has a better responses due to their self-esteem than children living in orphanage home in light of studied items, and especially at the negative trend items.

Recommendation

The study recommend that future studies need to apply a wide and diverse sample of participants from various schools and orphanage in Iraq’s provinces will be repeated to obtain more externally valid results.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved and carried out in accordance with approved guidelines”.

References

5. Lin, H, Tang, T, Yen, J-Y. Depression and its association with self-esteem, family, peer and


Knowledge, Attitudes and Practices towards Coronavirus Disease 2019 (COVID-19) among the Public in Baghdad City

Ayad M. Mousa1, Tahseen R. Mohamed2, Bassima A. Naji3
1Instructor, 2Assistant Professor, 3Assistant Instructor, Fundamentals of Nursing Department, College of Nursing-University of Baghdad

Abstract

Background: Coronavirus disease (COVID-19) is a contagious disease caused by a newly discovered coronavirus. Coronaviruses transmits through personal contact and infection severity ranges from mild to severe one. Methods: Descriptive cross-sectional design study to assess Knowledge, Attitudes and Practices towards Coronavirus Disease 2019 (COVID-19) Among the Public in Baghdad city. The study started from 10th April until 15th August 2020 during pandemic of COVID19 in order to complete the study The data collection has been achieved by internet (online) through Google forms. Results: the finding of the study demonstrated moderate level for knowledge, moderate level for attitudes, and high practice level of study participants related to Coronavirus Disease 2019 in addition knowledge and attitudes variables have significant impact upon practices of the study participants. Conclusions: the study has concluded that practices of population were influenced by age, living place, educational level, living place and presence of COVID19 cases in residence area finally knowledge and attitudes of community has impact on practices regarding COVID19.

Keywords: Knowledge, Attitudes, Practices, COVID-19, Baghdad city

Introduction

Coronavirus disease (COVID-19) is a contagious disease caused by a newly discovered coronavirus. Many patients with pneumonia in Wuhan city were found and many causative agents were rule out like influenza and adenovirus, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV). After that Chinese center for disease control and prevention (China CDC) notified World Health Organization (WHO) about the situation. The virus was identified and named (COVID-19) which is the new name for novel coronavirus on January 7 2020. COVID-19 is more infectious than both SARS-CoV and MERS-CoV.

COVID-19 affects sinuses, nose, and throat as upper respiratory tract or lungs as lower respiratory tract. It transmit the same way in other Coronaviruses through personal contact furthermore infection severity ranges from mild to severe one. The major symptoms are low-grade fever, shortness of breath, and fatigue despite the virus can last in the human body from two days to fourteen days without any symptoms. Some risk factors should be taken in considerations that make some people more susceptible for COVID-19 infection which are cancer, diabetes mellitus type II, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), obesity, coronary heart disease (CAD), and heart failure (HF).

The COVID-19 has fatal complications ends with death in some patients. The major causes of complications is by condition known as “cytokine release syndrome or a cytokine storm” this happens when infection attacks immune systems by triggering it leading to saturation of blood stream with cytokines as inflammatory proteins which can
damage internal organs especially kidney, lungs and heart. More importantly, these complications occur as one out of six persons with COVID-19. The spread of COVID-19, which is affected by people’s knowledge, attitudes, and practices (KAP) towards COVID-19 hence to control this disease population should be educated toward prevention measures to control the situation. The knowledge, attitudes and practices (KAP) is important tool to estimate that community is ready or not toward promoting behavioral changes to control the virus also KAP studies give us feedback about KAP level of community to tailor appropriate interventional programs to raise prevention and awareness level for the public. The objectives of the present study to assess (KAP) of public toward Coronavirus Disease 2019 (COVID-19) in Baghdad city during pandemic of the virus and to compare KAP toward COVID19 by demographic characteristics of studied samples.

Methodology

Descriptive cross-sectional design study to assess Knowledge, Attitudes and Practices towards Coronavirus Disease 2019 (COVID-19) Among the Public in Baghdad city. The study started from 10th April until 15th August 2020 during pandemic of COVID19 in order to complete the study. Official request and Ethical approval were taken from Deanship of the college of nursing for approval of the study and permission for data collection. The study was conducted by google form online. Convenient sample of (278) participants. Instrument of the study was questionnaire form consisted of two parts the first part consists of socio-demographic characteristics, which are (agreement of study participation, age, gender, marital status, educational level, occupation, monthly income, living place, and Have you seen or heard previously about anyone in your place infected COVID19 ). Second part consist of eleven questions related to knowledge variable, four questions about attitudes, and six questions regarding practices which were built, based on extensive and related literature review. Content validity of the instrument was established through a panel of six experts they were faculty members from college of nursing/University of Baghdad. The purpose of reviewing the questionnaire validate the items of questionnaire in terms of clarity, relevance, and understandability. A pilot study is conducted on a convenient sample of fifteen samples. They were excluded from original sample of the study to achieve to estimate the average time to fill the entire questionnaire, and to validate understanding of items. The data collection has been achieved by internet (online) through Google forms. The form was sent to respondent to fill it by self-reported method via social media which are Facebook channel, WhatsApp, and telegram. Statistical Analysis was applied by statistical package for social sciences (SPSS version 26). Frequency, mean, and standard deviation for descriptive analysis and independent two-sample t test and analysis of Variance (ANOVA test) for inferential analysis.

Results

The majority of the study were female who accounted for (50.4%) of the total participants. More than of one-third (44.6 %) of the study participants within age group (16-26) years old. More than half of studied samples (51.4%) were married. Preparatory graduates (33.8 %) were high percent in regard educational level of studied samples. High percent of study participants were student and their percent was (32.7 %). More than half of samples (51.4 %) had sufficient monthly income and most of samples were from Al Rasafa (55.8%). Finally, nearly two thirds of study participants (72.7%) had seen COVID19 people in their living place.
### Table (1). The Descriptive Statistics For Studied Samples’ Knowledge Concerning COVID19

<table>
<thead>
<tr>
<th>Items</th>
<th>Resp.</th>
<th>F</th>
<th>%</th>
<th>MS</th>
<th>SD</th>
<th>A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>The main clinical symptoms of COVID-19 are fever, fatigue, dry cough, and myalgia</td>
<td>Incorrect</td>
<td>22</td>
<td>7.9</td>
<td>0.92</td>
<td>0.270</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Correct</td>
<td>256</td>
<td>92.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>There currently is no effective cure for COVID-19, but early symptomatic and supportive treatment can help most patients recover from the infection</td>
<td>Incorrect</td>
<td>19</td>
<td>6.8</td>
<td>0.93</td>
<td>0.253</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Correct</td>
<td>259</td>
<td>93.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only elderly and chronic disease patients will have severe stages of COVID19</td>
<td>Incorrect</td>
<td>213</td>
<td>76.6</td>
<td>0.23</td>
<td>0.424</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>Correct</td>
<td>65</td>
<td>23.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80% of people with COVID19 will cure without treatment in the hospital</td>
<td>Incorrect</td>
<td>159</td>
<td>57.2</td>
<td>0.43</td>
<td>0.496</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>Correct</td>
<td>119</td>
<td>42.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asymptomatic Persons with COVID-19 cannot infect the virus to others, due to the virus is weak</td>
<td>Incorrect</td>
<td>113</td>
<td>40.6</td>
<td>0.59</td>
<td>0.492</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>Correct</td>
<td>165</td>
<td>59.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The COVID-19 virus spreads mainly via respiratory droplets during speaking and sneezing of infected individuals</td>
<td>Incorrect</td>
<td>15</td>
<td>5.4</td>
<td>0.95</td>
<td>0.226</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Correct</td>
<td>263</td>
<td>94.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>children and young adults cannot be infected with the virus because they have strong immunity</td>
<td>Incorrect</td>
<td>60</td>
<td>21.6</td>
<td>0.78</td>
<td>0.412</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Correct</td>
<td>218</td>
<td>78.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>To prevent the infection by COVID-19, individuals should avoid going to crowded places such as avoid taking public transportations</td>
<td>Incorrect</td>
<td>3</td>
<td>1.1</td>
<td>0.99</td>
<td>0.104</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Correct</td>
<td>275</td>
<td>98.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The clinical manifestations can occur within 1-14 days after infection the virus</td>
<td>Incorrect</td>
<td>26</td>
<td>9.4</td>
<td>0.91</td>
<td>0.292</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>Correct</td>
<td>252</td>
<td>90.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics are an effective treatment for COVID-19.</td>
<td>Incorrect</td>
<td>180</td>
<td>64.7</td>
<td>0.35</td>
<td>0.479</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>Correct</td>
<td>98</td>
<td>35.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The virus can be live on plastic surfaces for 24 hours</td>
<td>Incorrect</td>
<td>247</td>
<td>88.8</td>
<td>0.11</td>
<td>0.315</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>Correct</td>
<td>31</td>
<td>11.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Assessment</td>
<td></td>
<td></td>
<td></td>
<td>0.65</td>
<td>0.324</td>
<td>M</td>
</tr>
</tbody>
</table>

(A.): Assessment, M.s=mean of score, SD= standard deviation, MS = ≤ 0.49 = Low (L), MS =0.50- 0.75 Moderate (M), MS = 0.76-1= high (H)
This table shows the total assessment of samples’ knowledge concerning COVID19 was moderate with respect to their mean of score (0.65)

**Table (2) The Descriptive Statistics For Studied Samples’ Attitudes Concerning COVID19**

<table>
<thead>
<tr>
<th>NO.</th>
<th>Items</th>
<th>Agree</th>
<th>Does not agree</th>
<th>Not sure</th>
<th>MS.</th>
<th>SD.</th>
<th>Ass.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F.</td>
<td>%</td>
<td>F.</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Iraq can win the battle against the COVID-19 virus successfully</td>
<td>108</td>
<td>38.8</td>
<td>0</td>
<td>0.0</td>
<td>169</td>
<td>60.8</td>
</tr>
<tr>
<td>2.</td>
<td>Compliance with the Iraqi Ministry of Health instruction and precautions will prevent the spread of COVID-19</td>
<td>246</td>
<td>88.5</td>
<td>0</td>
<td>0.0</td>
<td>32</td>
<td>11.5</td>
</tr>
<tr>
<td>3.</td>
<td>Comply with necessary precautions such as Washing hands and wearing gloves to protect myself from COVID-19.</td>
<td>270</td>
<td>97.1</td>
<td>0</td>
<td>0.0</td>
<td>7</td>
<td>2.5</td>
</tr>
<tr>
<td>4.</td>
<td>Comprehensive curfew can contribute toward prevention of COVID19 transmission and win the battle.</td>
<td>230</td>
<td>82.7</td>
<td>0</td>
<td>0.0</td>
<td>48</td>
<td>17.3</td>
</tr>
</tbody>
</table>

Total Assessment: 2.53 0.519 M

MS= mean of score, SD= standard deviation, Ass. = assessment level < 2= low(L), 2-2.59 = Moderate(M), 2.6-3 = high (H)

This table showed that high percentage (60.8%) of studied sample had negative attitudes concerning item “Iraq can win the battle against the COVID-19 virus successfully”, while vast majority of them (88.5%;97.1%) respectively they have (agree answer) related to following items “Compliance with the Iraqi Ministry of Health instruction and precautions will prevent the spread of COVID-19” and “Comply with necessary precautions such as washing hands and wearing gloves to protect myself from COVID-19” in addition studied samples have positive attitudes related to “Comprehensive curfew can contribute toward prevention of COVID19 transmission and win the battle” . total assessment of studied samples regarding their attitudes was moderate.
Table (3) The Descriptive Statistics For Studied Samples’ Practices Concerning COVID19

<table>
<thead>
<tr>
<th>NO.</th>
<th>Items</th>
<th>Always</th>
<th>Sometimes</th>
<th>Never</th>
<th>MS.</th>
<th>SD.</th>
<th>Ass.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Washing hands frequently or disinfecting them with hand alcohol rub</td>
<td>215</td>
<td>77.3</td>
<td>62</td>
<td>22.3</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>Avoid touch eyes, mouth, and nose with your hands</td>
<td>156</td>
<td>56.1</td>
<td>113</td>
<td>40.6</td>
<td>9</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>Cover your mouth and nose when sneezing and coughing</td>
<td>233</td>
<td>83.8</td>
<td>45</td>
<td>16.2</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>Avoid crowded places such as supermarkets</td>
<td>227</td>
<td>81.7</td>
<td>50</td>
<td>18.0</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>Wear mask and gloves when you are outdoor</td>
<td>198</td>
<td>71.2</td>
<td>73</td>
<td>26.3</td>
<td>7</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>Keep distance 1 meter from others to prevent infection.</td>
<td>166</td>
<td>59.7</td>
<td>105</td>
<td>37.8</td>
<td>7</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Total Assessment 2.70 0.128 H

MS= mean of score, SD= standard deviation, Ass. = assessment level < 2= low (L), 2-2.59 = Moderate (M), 2.6-3 = high (H)

It can be seen that high percent of studied samples practice all the measures that are listed in the studied questionnaire such as washing hands and wearing gloves and masks, this can be indicated from total assessment, which had high mean score.
Table (4) Comparison of Dependent Variables by Demographic Variables

<table>
<thead>
<tr>
<th>Demographic variables</th>
<th>Knowledge Statistic value</th>
<th>Knowledge P value</th>
<th>Attitudes Statistic value</th>
<th>Attitudes P value</th>
<th>Practices Statistic value</th>
<th>Practices P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>-.972</td>
<td>0.332</td>
<td>1.111</td>
<td>0.267</td>
<td>-.384</td>
<td>0.701</td>
</tr>
<tr>
<td>Age groups</td>
<td>2.518</td>
<td>0.042</td>
<td>1.493</td>
<td>0.205</td>
<td>2.476</td>
<td>0.045</td>
</tr>
<tr>
<td>Marital status</td>
<td>1.207</td>
<td>.308</td>
<td>0.864</td>
<td>0.486</td>
<td>2.324</td>
<td>0.057</td>
</tr>
<tr>
<td>Educational level</td>
<td>6.669</td>
<td>0.000</td>
<td>1.256</td>
<td>0.278</td>
<td>4.093</td>
<td>0.001</td>
</tr>
<tr>
<td>Occupation</td>
<td>1.834</td>
<td>0.093</td>
<td>1.399</td>
<td>0.215</td>
<td>2.096</td>
<td>0.054</td>
</tr>
<tr>
<td>Monthly income</td>
<td>8.572</td>
<td>0.000</td>
<td>0.184</td>
<td>0.832</td>
<td>1.245</td>
<td>0.290</td>
</tr>
<tr>
<td>Living place</td>
<td>-.930</td>
<td>0.353</td>
<td>0.754</td>
<td>0.451</td>
<td>2.158</td>
<td>0.032</td>
</tr>
<tr>
<td>Have you seen or heard previously about anyone in your place infected COVID19</td>
<td>2.121</td>
<td>0.036</td>
<td>2.060</td>
<td>0.040</td>
<td>2.328</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Results of this table demonstrated that there are significant differences between knowledge variable and following demographic variables which are (age groups, educational level, monthly income and Have you seen or heard previously about anyone in your place infected COVID19). Also attitudes has significant difference only with (Have you seen or heard previously about anyone in your place infected COVID19), finally practice variable has significant differences with Age groups, Marital status, Educational level, Living place, and Have you seen or heard previously about anyone in your place infected COVID19.
Table (5) Multiple Linear Regression Analysis for Effect of Knowledge and Attitudes on Practices toward COVID19

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>Knowledge</td>
<td>.251</td>
<td>.065</td>
<td>.227</td>
<td>3.851</td>
</tr>
<tr>
<td>Attitudes</td>
<td>.137</td>
<td>.069</td>
<td>.117</td>
<td>1.992</td>
</tr>
</tbody>
</table>

The results indicate that knowledge and attitudes variables have significant effects on practices of studied samples at p value < 0.05.

**Discussion**

This cross-sectional study is one of the first studies regarding COVID19 in Iraq specifically in Baghdad city. Hence, the disease is infectious as it can transmit through communication between human beings especially in crowded areas. The people are to protect themselves they should be knowledgeable toward disease in terms of incubation period, transmission routes, signs and symptoms and most importantly the prevention methods, and treatment strategies. The study’s findings revealed that participants had moderate level of knowledge toward COVID19 indicated by mean of score (0.65±0.324) although vast majority of study participants were highly educated ;This Finding disagrees with results from cross-sectional study was done in china to assess knowledge, attitudes, and practices (KAP) regarding COVID19 where high mean score was found toward knowledge related to COVID19 \(^{(8)}\).

Study participants had high mean score regarding the main clinical symptoms of COVID-19 and they can recognize that there is no cure for COVID19 now. High educational level may induce them to be curious about COVID19 to know how disease symptoms starts and what the best cure method to be free from disease. However, the study samples have low level of knowledge toward items related to “Antibiotics are an effective treatment for COVID-19 and the virus can be live on plastic surfaces for 24 hours”. It is a reasonable some people may believe that antibiotics had powerful effect toward viruses. The belief might come from news published in social media and some malpractices of health care professional, which make people, think that antibiotic is an effective solution against the virus, So that the community should be educated about role of antibiotics during pandemic of COVID19 to avoid improper use of antibiotics that can end with unhealthy complications. Findings from cross-sectional study done in (Tanzanian residents) stated that high mean scores regarding major clinical symptoms and cure from COVID19 had been showed. \(^{(10)}\)

COVID19 is a new virus so most people have no idea toward hours of virus life on plastic and other surfaces this is considered a new concept that should orient community during pandemic of COVID19 by health education campaign such as posters ,social media regarding duration of coronaviruses on surfaces to avoid the disease and protect them.

The results of the current study revealed low level of attitudes regarding population confidence toward Iraq to win against pandemic of COVID19. This may be due to the population in Baghdad city had negative attitudes regarding the health system in Iraq based on
their experiences with it. However, they had positive attitudes concerning precautions, and instructions that are targeted to control COVID19 pandemic. In contrast to, Outcomes from study conducted in Malaysia that reported 95.9% from study participants were confident toward winning of battle against COVID19 that can reflect positive attitudes of population toward the health system in Malaysia. (7)

The current findings demonstrated high mean score for study participants regarding practices toward COVID19. Similarly, high practice level of the study participants in Saudi Arabia to assess their KAP (9). The curfew in Baghdad city may make perception about the risk of COVID19 in people residing in Baghdad furthermore high percent of the current study were highly educated which may considered cause for high practice level rather than our study revealed knowledge and attitudes variables impact significantly on practice variable.

The present study’s outcomes revealed that significant differences between educational level and knowledge and practices variables toward COVID19. These findings come in total agreement with results from study conducted in Saudi Arabia (9). It may be seem that individuals who have high educational level search information and practice instructions and precautions toward COVID19 more than individuals with low educational level. In addition, results demonstrated age groups has significant difference with knowledge and practice variables toward COVID19. This result is consistent with results from Chinese study, which reported age considered significant factor regarding knowledge of studied sample (11). The results showed that Living place has significant difference with practices of the studied samples. This may be due to there is geographical differences between territories in Baghdad concerning population crowd, services available furthermore in onset of pandemic the incidence of COVID19 was fluctuated where most cases were more in Rasafa than AlKarkh after that incidence rate become equal approximately between two sectors after that Baghdad as whole become pandemic in most its territories .The study has some limitations which are , convenient sample may place results of the study with limited generalizability. In addition, questions of the questionnaire were built on review so maybe there are questions important lost in this questionnaire. The collection of samples of the study have been collected by online google form, this may let study participants mandatory to answer all questions even poorly understood questions due to conditions of the pandemic .

Conclusions

The study has concluded that study participants from Baghdad city had moderate knowledge, moderate attitudes, and good practice levels toward COVID19. Factors that affect knowledge were age, educational level, and monthly income and presence of COVID19 cases in residence of population, in addition to attitudes are affected by hearing or seeing cases of COVID19. In addition, the study has concluded that practices of population were influenced by age, living place, educational level, living place and presence of COVID19 cases in residence area finally knowledge and attitudes of community has impact on practices regarding COVID19.

Recommendations

The study recommends involving the community in health education program to raise their knowledge, attitudes, and practices toward COVID19 .also especially practicing preventive measures to prevent infection of COVID19 especially in crowded areas. Giving high attention in low socioeconomic areas and low educational level individuals in Baghdad city to increase their knowledge level toward COVID19 thereby attitudes and practices levels will raise positively.

Acknowledgements: The author acknowledge all participants of the study and their efforts to complete this study that help as tool to control the pandemic in Iraq in addition author acknowledge all efforts of experts who reviewed the questionnaire of the study .
**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Nursing-University of Baghdad and all experiments were carried out in accordance with approved guidelines.

**References**

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Molecular Detection of BK Polyomavirus in Patients Under Hemodialysis

Eman Yousuf Rasheed1, Fidan Ghanim Reshad2, Muhannad Abdullah Allazzawy3

1Bacteriologist, HD in Hospital Management, Department of Microbiology, Al-Karkh, Health Directorate, Baghdad, Iraq, 2M. Sc. Microbiology, 3Ph.D. Medical Microbiology/Virology, Department of Microbiology, Kirkuk Health Directorate, Iraq

Abstract

The study was conducted in Kirkuk city-Iraq from February 2018 to September 2018. The number of chronic kidney disease patients under study were 60 patients whose ages were between 40-70 years old. These patients admitted to Kirkuk general hospital (hemodialysis unit). The control group who were matched to the patients studied, included 30 healthy blood donor and their ages were between 30-75 years old. Blood samples were collected for detection of BK polyomavirus DNA by real time-PCR. The study showed that the highest rate of BK polyomavirus DNA occurrence were recorded in patients with chronic kidney disease when compared with control group (51.66% versus 3.33%) (P: <0.01). The study showed that the highest mean level of IL-6 was recorded in patients with chronic kidney disease comparing with the control group (78.42 v.s. 3.40 ng/ml). The result was highly significant. The present study demonstrated that the highest mean level of IL-6 was found in prostates cancer patients who were positive to BK polyomavirus DNA comparing with BK polyomavirus DNA negative (52.54 versus 25.88 ng/ml)

It was concluded that BK polyomavirus was frequently detected in chronic kidney disease patients and could play a relevant role in the development and progression of human hemodialysis

Keyword: Hemodialysis; BK polyomavirus, CKD

Introduction

BK virus (BKV) belongs to genus Polyomavirus within the Polyomaviridae, a family of small, nonenveloped, double-stranded DNA viruses (1). Its genome is divided into 3 regions: the early, late and transcriptional control region (TCR). Early genes code the regulatory small and large T proteins and late genes code the viral capsid proteins (VP1, VP2, and VP3) and agnoprotein [1-3]. The major prognostic factor of BK virus nephropathy is the stage of the disease at the time of diagnosis. When documented in advanced stage with associated chronicity changes, it has worse prognosis with a renal survival at 12 months from 10 to 40%. In contrast, an early stage diagnosis with only viral cytopathic changes, survival is over 80% at 12 months [5-6]. The primary infection with BKV usually occurs in early childhood. It is estimated that 35-90% of the general population will acquire the primary infection during infancy and its seroprevalence reaches 46%-94% in adults depending on the studied regions [7-9]. Primary infection is usually asymptomatic, but after initial infection, BKV may persist lifelong in the kidney and genitourinary tract epithelium and possibly peripheral blood mononuclear cells, tonsils and other hematopoietic tissues [10,11]. Since the pre-transplant status affects the renal transplantation success and ultimately the survival rate, identifying the probable risk factors that increase the chance of BK virus replication in end-stage renal disease patients can be included in
proposing proper surveillance guidelines during pre and post-transplantation \cite{12,13}. The aim of the study was to study the relation of BK polyomavirus (BKPyV) in hemodialysis

**Material and Method**

The study was conducted in Kirkuk city-Iraq from of February 2018 to September 2018. The number of chronic kidney disease patients under study were 60 patients whose ages were between 40-70 years old. These patients admitted to Kirkuk general hospital/ Hemodialysis unit. The control group who were matched to the patients studied, included 30 healthy blood donor and their ages were between 30-75 years old. Blood samples were collected (3ml EDTA and 2ml for serology) for detection of BKPyV DNA by real-time-PCR and the level of IL-6 by immunefluorescent technique.

**DNA extraction**

DNA was extracted from all prostate samples by lysis and proteinase K digestion in EDTA , and 0.1% sodium dodecyl sulfate (SDS), the samples were extracted with phenol chloroform and precipitated with ethanol. DNA concentration and purity was determined by spectrophotometry at $\lambda=260/280$nm. The extraction process was performed in an area that was BKV free while great care was taken during the tissue sectioning procedure in order to avoid any contamination. Sectioning of the tissues was carried out using a clean microtome and a separate new blade in each case, as well as clean gloves and forceps. The sections were placed in autoclaved DNAse-free microtubes for the DNA isolation procedure.

**Real time PCR**

The BKPyV Real-TM Quant kit (SaCycler Biotechnologies) is a Real-Time test for the Qualitative and Quantitative detection of BKPyV in the biological materials. DNA is extracted from samples, amplified and detected using fluorescent reporter dye probes specific for BKPyV DNA.

![Figure 1: Real-time curves of BKPyV DNA detection](image-url)
Statistical analysis: Computerized statistically analysis was performed using Minitab ver 18.0 statistic program. Comparison was carried out using Chi-square ($X^2$) for determination of the $P$ value.

Findings

The study showed that the highest rate of BKPyV DNA occurrence were recorded in patients with chronic kidney disease when compared with control group (51.66% versus 3.33%) ($P: <0.01$), Table 1.

Table 1: Detection of BKPyV DNA in chronic kidney disease patients and healthy control

<table>
<thead>
<tr>
<th>BK viral DNA (RT-PCR)</th>
<th>HD patients</th>
<th>Control group</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>31</td>
<td>51.66</td>
<td>7</td>
</tr>
<tr>
<td>Negative</td>
<td>29</td>
<td>48.33</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100</td>
<td>30</td>
</tr>
</tbody>
</table>

The study showed that the highest mean level of IL-6 was recorded in patients with chronic kidney disease comparing with the control group (78.42 v.s. 3.40 ng/ml). The result was highly significant, Table 2.

Table 2: Serum IL-6 levels in patients with chronic kidney disease and the control group.

<table>
<thead>
<tr>
<th>IL-6 level (pg/ml)*</th>
<th>HD patients</th>
<th>Control group</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>60</td>
<td>30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean</td>
<td>78.42</td>
<td>3.40</td>
<td></td>
</tr>
<tr>
<td>SD.</td>
<td>12.745</td>
<td>1.59</td>
<td></td>
</tr>
</tbody>
</table>

The present study demonstrated that the highest mean level of IL-6 was found in prostates cancer patients who were positive to BKPyV DNA comparing with BKPyV DNA negative (52.54 versus 25.88 ng/ml), Tale 3.

Table 3: Frequency of BKPyV DNA in chronic kidney disease patients according to IL-6 level

<table>
<thead>
<tr>
<th>IL-6 level (pg/ml)</th>
<th>BKPyV DNA (RT-PCR)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>Mean</td>
<td>52.54</td>
<td>25.88</td>
</tr>
<tr>
<td>SD.</td>
<td>12.745</td>
<td>1.59</td>
</tr>
</tbody>
</table>
Discussion

Recent studies have confirmed that chronic hemodialysis could raise the chance of BK virus replication in ESRD patients as compared to control group [11-15]. Nevertheless, we previously demonstrated that prolonged pre-transplant dialysis could be a potential risk factor for shedding of BK virus into urine in renal transplant recipients during post-transplantation [16,17]. Therefore, it can be concluded that chronic hemodialysis is a risk factor for reactivation of BK virus pre and post-transplantation. In present study we assessed the potential predictive factors for development of BK virus viremia during hemodialysis period among end-stage renal disease patients. Overall, 192 ESRD patients who were under hemodialysis were enrolled without preliminary screening. The number of patients enrolled in our study was higher than previous studies, which can be helpful to gain more accurate and reliable results. The prevalence of BK viremia was 7.3% among our patients. The prevalence of BKV infection in patients with ESRD under hemodialysis or peritoneal dialysis has been reported from 0 to 33.3% in various studies [18,19]. These differences can be due to the difference in the number of studied patients, the duration and frequency of dialysis and the difference in the sensitivity of the PCR method used to identify BK virus. In general, deliberate or non-deliberate immunosuppression can cause the reactivation of BK virus. Hence, high incidence rate of BK virus infection among patients who received corticosteroid drugs is not surprising [20,21]. There are contradictory reports on the presence of BKV DNA in urinary tract tumors: the authors of one study detected BKV DNA using PCR in 31 of 52 samples, whereas other authors were unable to find it [17]. An other study detected BKV in 4 of 30 fresh tissue prostate samples and were unsuccessful with archival specimens, and observed the virus in atrophic lesions in prostate tumors. One of the most important proteins for BKV is the replicational regulatory protein, the large T-antigen that binds onto the tumor suppressor proteins p53 and pRb1, inhibiting their functions and leading to a variety of transforming effects. Given the low frequency of either TP53 or RB1 mutations in hemodialysis, it was intriguing to investigate the prevalence of BKV in prostate tumor samples [23].

Conclusions: BKV was frequently detected and could play a relevant role in the development and progression of human hemodialysis

Conflict of Interest: non

Source of Findings: self findings.

Ethical Clearance: This research was carried out with the patient’s verbal and analytical approval before the sample was taken. According to this approval, all the samples were collected and the tests were carried out. A copy of the results of the tests was then given to the patients

References

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The Relationship of Aerobic Voltage to the Most Critical Physiological Variables Related to Health for Women Players of Some Group Games in the Faculty of Education for Girls

Ivan Nemeh Kazem¹, Rana Hussein Abdul Sada²

¹Assistant Prof., ²Assistant Lecturer, University of Kufa/Faculty of Education for Girls / Department of Physical Education and Sports Sciences, Iraq

Abstract

The research aims to identify the most critical physiological variables for the players of some group games in the College of Education for Girls (basketball - futsal - volleyball) and to compare the essential physiological variables for the players among group games (basketball - futsal - volleyball) in the College of Education for Girls. The research community consisted of the players of some group games in the College of Education for Girls / the University of Kufa. Their number was (30), by (10 basketball players, ten futsal players, ten volleyball players) and the researcher used the descriptive approach to suit the research problem and the most important conclusions were, was Aerobic effort has an apparent effect on physiological variables compared to rest time, showing apparent significant differences in blood pressure variable, especially systolic pressure.

Keywords: Women, Critical physiological variables of some group, Faculty of Education for Girls

Introduction

Sports physiology is concerned with identifying the various functional responses of the organs and systems of the body and the reactions of different exercises on the physiological and chemical aspects. Significantly since the functions of the human body’s organs and their responses are constantly changing throughout the day, the week and the month, whether at rest or when making physical effort, which leads to identifying the various responses to benefit from them when planning training, the players need aerobic effort to complete the match. In the performance practiced to reach the required level and through the foregoing highlights the importance of the research in the researchers’ attempt to identify the relationship of air effort between some team games (basketball - futsal - volleyball) in the physiological variables and health-related fitness variables for female players in the College of Education for Girls, University of Kufa for the academic year 2020-2021 and to identify the difference between the games under the influence of Aerobic effort (movement belt) and the objectives of the research were to identify the air voltage and its relationship to the most essential physiological variables for female players in some team games (Cr Basketball - Futsal - Volleyball) in the College of Education for Girls.(1)

Practical Part

The researchers chose the descriptive approach in a one-group style, the pre-test and the post-test for suitability in solving the nature of the research problem on female players (basketball - and futsal - volleyball) in the College of Education for Girls / University of Kufa for the season 2020-2021 and
their number was (30 players), and a sample chose the number of basketball players (10 players), futsal (10 players) and volleyball (10 players) randomly among the students facing the work, the researchers conducted the homogeneity of the research sample, if the researchers used the skew coefficient as shown in table (1)

**Sample homogeneity**

To avoid the problem of individual differences

**Table (1): It shows the mean, standard deviation, and skewness coefficient of the research sample**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Unit of measure</th>
<th>mean</th>
<th>Std. deviation</th>
<th>Mode</th>
<th>skewness</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>years</td>
<td>22.5</td>
<td>14.8</td>
<td>22</td>
<td>0.03</td>
<td>homogeneity</td>
</tr>
<tr>
<td>Length</td>
<td>cm</td>
<td>163</td>
<td>10.9</td>
<td>160</td>
<td>0.27</td>
<td>homogeneity</td>
</tr>
<tr>
<td>Weight</td>
<td>kg</td>
<td>60.33</td>
<td>6</td>
<td>66</td>
<td>0.94</td>
<td>homogeneity</td>
</tr>
<tr>
<td>Training age</td>
<td>years</td>
<td>3.4</td>
<td>1.14</td>
<td>3</td>
<td>0.04</td>
<td>homogeneity</td>
</tr>
</tbody>
</table>

It shows through the skew coefficient limited between (+-1), where the research sample was homogeneous and distributed normally, and this indicates that the members of the research sample are homogeneous.

**Field Research Procedures:**

**Determine the physiological variables used in the research:**

After seeking the opinions of experts and specialists in the physiological field to determine the variables most relevant to the title of the research, the researchers presented a set of physiological variables in a questionnaire form to the experts in the field of physiology to select the most appropriate variables. After collecting and unloading the questionnaire, the researchers extracted the relative importance and accepted the variable that obtains a more percentage (60). %) as shown in table (2).

**Table (2): It shows the degree of the relative importance of the physiological research variables**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Physiological</th>
<th>Relative Importance</th>
<th>Mark</th>
</tr>
</thead>
<tbody>
<tr>
<td>lactic acid</td>
<td></td>
<td>25.03%</td>
<td>X</td>
</tr>
<tr>
<td>Vo2Max</td>
<td></td>
<td>30.02 %</td>
<td>X</td>
</tr>
<tr>
<td>systolic pressure</td>
<td></td>
<td>95.50%</td>
<td>/</td>
</tr>
<tr>
<td>diastolic pressure</td>
<td></td>
<td>80%</td>
<td>/</td>
</tr>
<tr>
<td>the number of breaths</td>
<td></td>
<td>72.04 %</td>
<td>/</td>
</tr>
<tr>
<td>heart rate</td>
<td></td>
<td>90.07%</td>
<td>/</td>
</tr>
<tr>
<td>Red blood cells</td>
<td></td>
<td>17.10%</td>
<td>X</td>
</tr>
</tbody>
</table>
The scientific basis for research tests:

The scientific foundations extract from the exploratory experiment tests on the treadmill.

The stability of the tests: The researchers tested a sample of the exploratory experiment (6) players from the research community. The researchers worked to provide the atmosphere in which the first measurements take; then, the test results extract using the correlation coefficient (Pearson) between the first and second tests. (2)

Description of the tests used in the research:

First: the number of breathing times: the number of respiratory chest movements during one-minute call breathing speed. Breathing occurs by respiratory movements that renew the air of the lungs continuously. The speed of breathing decreases in the state of rest so that it becomes sufficient to provide the amount of oxygen that secures the basic need of the body, and the speed of breathing varies for people. According to age and gender, it increases in children and ranges between 30-40 times/minute, in adults 16-22 times/minute, and in females 18 times per minute during rest. The respiratory movement occurs every 3-4 seconds, and the movement takes place in two stages: inhalation and exhalation, and air enters the lungs in the process of inhalation and exits from them.

In the exhalation process, the number of breathing times, the percentage of oxygen in the blood and the pressure measure simultaneously. (3)

Second: Measure the percentage of oxygen in the blood:

The researchers used the direct method to measure the percentage of oxygen in tissues using an oximeter, as the device installed on the index finger of the left hand, where the device works automatically as soon as the index finger place inside it, and the result appears on the device’s screen with the pulse, and then the reading is taken and fixed in the form. (4)

Third: - Measuring blood pressure and heart rate:

1- Systolic blood pressure measurement:-

2- Diastolic blood pressure measurement:-

The Rossmax measured the heart rate and blood pressure, and this device contains two parts, one of which wraps around the arm above the elbow joint area. The other is the measuring part and is placed on the table and gives measurements of the pulse and pressure so that the measurement is done immediately after the end of the physical effort and quickly. It should be taken into account when taking a blood pressure measurement that the person for whom the
measurement take should be seated. (5)

Pre-test

The Pre-test conduct on the primary research sample of players (basketball - futsal - volleyball), which numbered (30 female students) representing the players of the Girls’ College of Education team for the season (2020-2021), then tests were conducted in the fitness hall, and tennis court at the College Physical Education and Sports Sciences / the University of Kufa On Sunday and Monday, 10-11/2021, tests of physiological variables (number of breaths - number of heartbeats - systolic pressure - diastolic pressure - blood oxygen percentage) were conducted on the research sample for each game on the side in order to reach Objectives and solution to the research problem. (6)

Main experience:

After the scientific foundations of the research were confirmed, the students’ air effort was applied, which is a moving treadmill, after collecting data for the tribal tests:

pneumatic tension (treadmill)

After completing the tribal tests and measurements, the research sample subject to a pneumatic effort through performance on a moving treadmill, where the researchers conducted personal interviews with experts and specialists. The experts were approved as it is suitable for performing aerobic effort. Then the warm-up was conducted by the research sample for a period of (5) - (10) minutes; the player climbs on the Treadmill device, as it starts operating the device within the specified speed (5.1) km / h, the device starts quickly gradually up to the prescribed speed, and this allows the player to work on the device in a consistent and compatible manner for safety and security from the occurrence of injury. (7) When the prescribed speed reach, the time specified for performance begins, and after the time expires, the device gradually stops to avoid the player falling. The angle of inclination of the device is (1) degrees (20%) and the time specified for the air effort (15 minutes) With the help of the work team, taking measurements after the effort, as was done in the pre-measurements, to obtain data for the research variables. (8)

Statistical means:

Statistical manipulations perform using the SPSS program

Results

Presentation and analysis of the results of the variable number of breathing times of the research sample members:

<table>
<thead>
<tr>
<th>variables</th>
<th>Contrast source</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Average of squares</th>
<th>F value</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>The number of times breathes</td>
<td>between groups</td>
<td>92.47</td>
<td>2</td>
<td>46.23</td>
<td>2.997</td>
<td>moral</td>
</tr>
<tr>
<td></td>
<td>within groups</td>
<td>416.5</td>
<td>27</td>
<td>15.43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Two degrees of freedom (2-27) and a level of significance (0.05)
Table (4): It shows the differences of the arithmetic means for the variable number of breath times and the value of (L.S.D), and the statistical significance of the research sample

<table>
<thead>
<tr>
<th>Games</th>
<th>Differences in arithmetic means</th>
<th>Product of differences</th>
<th>L.S.D</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basketball - Futsal</td>
<td>37.84-35.61</td>
<td>2.23</td>
<td></td>
<td>insignificant</td>
</tr>
<tr>
<td>Basketball - Volleyball</td>
<td>37.84-32.53</td>
<td>5.31</td>
<td>4.75</td>
<td>significant</td>
</tr>
<tr>
<td>Futsal - Volleyball</td>
<td>35.61-32.53</td>
<td>3.08</td>
<td></td>
<td>insignificant</td>
</tr>
</tbody>
</table>

Presentation of the results of the variable blood oxygen content of the subjects of the research sample and their analysis:

**Discussing the results of the physiological variables of the research sample members**

The statistical results indicated significant and statistically significant differences between team games (basketball - futsal - volleyball) in some physiological variables after aerobic effort (walking). Moreover, the variables (number of breaths - blood oxygen percentage - systolic blood pressure - diastolic blood pressure - number of heartbeats), the researchers attribute the presence of these significant differences to the air effort (moving walking), where the difference in the variable number of breaths between the game of basketball, volleyball and in favour of basketball in order for the players to move in rapid attack and running during an attack, defence and volleyball. A lack of running and fast running, as the air effort (moving belt) is related to the number of breaths, the need for oxygen increases to produce energy to perform the activity. (9) The need for oxygen increases in abundance to get rid of Carbon dioxide and thus cause an increase in the number of breathing times to compensate the body for the energy consumed and an attempt to reduce acids in the case of moderate-intensity physical activity, such as brisk walking for a long time, the speed of breathing increases, which was the reason for the difference. (10) We intend between the basketball and volleyball games in the table (6). Where the number of breathing times varies according to the sporting specialities, related to the player’s level in terms of training and according to the type of game where primary variables occur for the process of inhalation and exhalation; hence, it notices that it increases and increases the number of times of breathing. (11)

As for the presence of significant differences for the players in the variable percentage of oxygen in the blood between basketball and futsal and in favour of basketball, the researchers attribute the reason for these significant differences in the performance of the aerobic effort (moving treadmill) exerted. Basketball players use oxygen in a more significant proportion than futsal due to the frequent movement and also due to the lack of Resisting breathing and increasing breathing muscles, the lungs’ capacity increases, and thus the volume of breathing air increases. Regular exercise leads to positive functional changes in the respiratory system. (12)

As for the systolic blood pressure variable, the differences were significant after performing the aerobic effort (moving walking), so the difference occurred between the two games of futsal and volleyball, favouring the game of futsal and volleyball, and in favour of the game of futsal football. As for the diastolic blood pressure, significant differences between the two ball games occurred. Basketball and
futsal and between basketball and volleyball, and the difference favoured basketball between the two teams. The researchers attributed this difference to the body’s need for oxygen during aerobic effort.\(^{13}\) It leads to an increase in blood pressure on the walls of blood vessels as well as an increase in the body’s need for energy through blood, which in turn leads to Increasing the process of sending blood pressure receptors by giving orders to the Cardiovascular Control Center to reorganize the blood flow to the working muscles according to the body’s need for blood.\(^ {14}\) This process leads to an increase in systolic blood pressure, and this linear increase in heart rate and the cardiac output shows the need to increase blood pressure for flow blood flow to the muscles to supply them with nutrients, and this result is consistent with what was mentioned by (Fletcher) and others that systolic blood pressure increases when there is an increase in blood pressure. For muscular contraction due to increased cardiac output.\(^ {15}\)

As for the diastolic blood pressure, there is a slight increase because the vascular expansion in vessels in the vessels of the working muscles occurs little in the diastolic blood pressure if the differences in diastolic blood pressure are minor if they are small.\(^ {16}\)

As for the number of heartbeats, the significant differences after performing aerobic effort (moving treadmill) between basketball and futsal and between basketball and volleyball, and this difference favoured basketball, as the researchers attributed the difference to a direct rise in the number of heartbeats—the heart rate increases at the beginning of an exercise.\(^ {17}\) Due to the retaliation of the heart centre, the medulla region with a signal from the cerebral cortex and the signal reflected from the muscular movement; the heart, and this increase follow the flow of the heart parasympathetic nerve signals towards heart and blood vessels.\(^ {18}\) The increase in heart rate during the performance of the inotropic effort is of a linear nature commensurate with the workload on the body systems and the amount of oxygen consumed during the effort.\(^ {19}\)  

Conclusions

1. Aerobic effort (mobile walking) has great importance and a positive relationship in the occurrence of changes in physiological variables (number of breaths - blood oxygen percentage - systolic blood pressure - diastolic blood pressure - heart rate) and health-related fitness variables (cardiorespiratory fitness - muscular fitness Structural (abdominal muscle strength)

2. Significant differences appeared for the female players between some team games in some physiological and health-related fitness variables for the research sample.

3. Effort had an apparent effect on physiological heart variables compared to rest time

4- In general, there were no appreciable differences in the body temperature variable among the female athletes.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the Faculty of Education for Girls and carried out in accordance with approved guidelines”.

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Effect of Clinical Guideline Concerning Cardiopulmonary Bypass on Nurses’ Knowledge in Open Heart Surgery Center.

Mustafa Amjed Majeed¹, Rajha Abdul-Hassan Hamza²

¹M.Sc., Student, ²Professor Dr., College of nursing, University of Kufa, Iraq

Abstract
Open-heart surgery is considered a major surgery with potential risks and complications, but it is a lifesaving operation that can treat a variety of diseases such as heart valve disease, heart birth defects, and coronary artery disease. A Quasi-experimental design has been used in the existing study to evaluate the effect of clinical guideline concerning cardiopulmonary bypass on nurses’ knowledge in open heart surgery center in AL-Najaf AL-Ashraf city at Al-Najaf center for cardiac surgery and trans-catheter therapy through the period October 1th, 2020 to, April 19th, 2021. The results of the study show there is a high significant effect of the educational program, it also, show that high significant mean difference among study group concerning pre-test and post-test scores at p-value (0.0001). Also, in this study there is a significant association between post-test knowledge and year of experience as a perfusionists and number of training courses at p-value (0.02) and (0.01). The study concluded that educational program can improve in nurses’ knowledge and to produce perfusionists who are able to successfully apply their knowledge and skills to improve patients’ outcome. The study recommended a written program for all new nurses working in heart-lung machine department, and improving the expertise of nurses and medical staff through use the finding of the present study to give care for the patient.

Keyword: Guideline, Cardiopulmonary bypass, Nurse, Knowledge, Perfusions.

Introduction
Open-heart surgery is considered a major surgery with potential risks and complications, but it is a lifesaving operation that can treat a variety of diseases [1]. Surgical care has become an increasingly important part of global health in recent years due to its ability to save lives, prevent disability, and the need to promote economic growth [2]. The price of such a procedure varies greatly across the globe. The United States had the highest cardiac bypass prices of any country in 2019. In contrast, (India) had the lowest cost for a heart bypass [3].

Study Objectives:
1. To assess nurses’ knowledge regarding cardiopulmonary bypass machine pre and post-exposure to guideline.
2. To determine the effect of clinical guidelines regarding cardiopulmonary bypass on nurses’ knowledge about cardiopulmonary bypass machine.
3. To find out the relationship between Sociodemographic data and nursing knowledge regarding cardiopulmonary bypass machine.

Methodology
Study Design
The present study was using a quasi-experimental design to evaluation the effect of clinical guidelines on nurses’ knowledge of cardiopulmonary bypass in the open heart surgery center in AL-Najaf AL-Ashraf city at Al-Najaf center for cardiac surgery and trans-catheter therapy from October 1st, 2020 to April 19th, 2021.
Setting of the study:

The study is conducted in Al-Najaf City/Al-Najaf Al-Ashraf Health Directorate / Al-Najaf center for cardiac surgery and trans-catheter therapy.

Study Sample:

The researcher study is being conducted directly in the Al-Najaf center for cardiac surgery and trans-catheter therapy. Probability (purposive) study sample consist of 15 nurses. All samples are exposed to an educational program and there are no control groups are found because the 15 nurses represent all available samples that work on the cardiopulmonary bypass machine.

Statistical Analysis

Through the use of excel and statistical package of social sciences (SPSS) version 25 the data of present study are analyzed.

Result

Table (1): Socio-Demographic Characteristic of the Study Sample

<table>
<thead>
<tr>
<th>Demographic Data</th>
<th>Rating and Intervals</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age / Years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-24</td>
<td>2</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>25-29</td>
<td>7</td>
<td>46.7</td>
<td></td>
</tr>
<tr>
<td>30-34</td>
<td>5</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>35-39</td>
<td>1</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>46.7</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>53.3</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td><strong>Levels of Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nursing college</td>
<td>15</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td><strong>Year of Experience in Hospital</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5</td>
<td>8</td>
<td>53.3</td>
<td></td>
</tr>
<tr>
<td>6-10</td>
<td>5</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>11-15</td>
<td>1</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>16-20</td>
<td>1</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td><strong>Years of Experience in Perfusion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5</td>
<td>14</td>
<td>93.3</td>
<td></td>
</tr>
<tr>
<td>6-10</td>
<td>1</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td><strong>Training Course</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>12</td>
<td>80.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td><strong>Number of Training Courses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3</td>
<td>20.0</td>
<td></td>
</tr>
</tbody>
</table>
Table (1) reveals that the high percentage of nurse that participant in this study is at age group (25-29) years, (46.7%). In addition, the table shows that the high percentages of male participant in the study was (53.3%). Regarding the level of education, the highest percentage is (100%) of the sample in study graduated from nursing college. In regards to years of experience in hospital, the table shows that (53.3%) of the sample in the study is at group of (1-5) year. In regards to years of experience in perfusion, the results show that the majority of study sample (93.3%) has (1-5) years. while (80%) of the sample in the study have no training session regarding cardiopulmonary bypass machine. In relation to number about cardiopulmonary bypass machine there is only one person who have two session of training at (13.3).

Table (2) Mean Difference (paired t-test) in the Nurses’ knowledge Between Different Periods of Measurements (Pre-test and Post-test)

<table>
<thead>
<tr>
<th>Main studied domain</th>
<th>Periods of measurements</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>t-value</th>
<th>d.f.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Nurses’ Knowledge</td>
<td>Pre-test</td>
<td>1.37</td>
<td>15</td>
<td>.167</td>
<td>16.387</td>
<td>14</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td>Post-test</td>
<td>1.88</td>
<td>15</td>
<td>.059</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results of this table show that high Significant mean difference among study group concerning to their pre-test and post-test scores at p-value (0.0001)

Table (3) Analysis of Variance (ANOVA) in the Nurses’ Knowledge (post-test) according to their Age, Experience in Hospital, and Number of Training Courses

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Rating and intervals</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age / Years</td>
<td>20-24</td>
<td>2</td>
<td>1.84</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25-29</td>
<td>7</td>
<td>1.85</td>
<td>0.05</td>
<td>4.117</td>
<td>.035</td>
</tr>
<tr>
<td></td>
<td>30-34</td>
<td>5</td>
<td>1.91</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35-39</td>
<td>1</td>
<td>2.00</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>15</td>
<td>1.88</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year of Experience in Hospital</td>
<td>1-5</td>
<td>8</td>
<td>1.85</td>
<td>0.05</td>
<td>5.602</td>
<td>.014</td>
</tr>
<tr>
<td></td>
<td>6-10</td>
<td>5</td>
<td>1.91</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11-15</td>
<td>1</td>
<td>1.84</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16-20</td>
<td>1</td>
<td>2.00</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>15</td>
<td>1.88</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Number of Training Courses</td>
<td>No training Courses</td>
<td>12</td>
<td>1.86</td>
<td>0.05</td>
<td>6.974</td>
<td>.010</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>1.94</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>2.00</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>15</td>
<td>1.88</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (3) shows that there is significant association between post-test knowledge and some variables in demographic data of the study. Regarding age there is a significant association with knowledge at p-value (0.035) was at rating of (35-39). In addition this table show at rating (16-20) year of experience in hospital there is a significant association with nurses’ knowledge at p-value (0.014). While the number of training courses explain a significant association with nurses’ knowledge at p-value (0.010).

Table (4) Mean Difference (Independent Sample t-test) in the Nurses’ Knowledge (post-test) according to their Gender and Experience as a nursing perfusionist

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Rating</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>t-value</th>
<th>d.f</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>7</td>
<td>1.88</td>
<td>0.03</td>
<td>.283</td>
<td>13</td>
<td>.782 NS</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>8</td>
<td>1.88</td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experience as a perfusionist</td>
<td>1-5</td>
<td>14</td>
<td>1.87</td>
<td>0.05</td>
<td>2.485</td>
<td>13</td>
<td>.027 S</td>
</tr>
</tbody>
</table>

Table (4) shows that there is no significant difference in nurses’ knowledge at post-test regarding gender. While regarding to year of experience as a perfusionist there is a significant difference at p-value (0.027).

**Discussion**

According to (table 4.1) in the results, the study shows some variation difference regarding the Socio-demographic data of the study sample.

Concerning their age, the majority of the study sample are in the age group of (25-29) years. This result matches with the result of [4] who finds in his study that the largest group of age was between (24-30) years. While our result disagree with [5] which found that the highest percentage of age was (55%) above (50). This difference, according to researchers, occurs due to the Iraqi cardiac centers being newly established centers and the number of staff in these centers was at a young age, while cardiac centers in Europe and America were old centers and staff were at middle age.

About the gender of the study subjects, the highest percentage were males in the study sample, which is in consistency with [5] in his thesis about “The relationship between self-efficacy and employee commitment among perfusionists “, he mentioned that males are the dominant gender in his study (63%). While our findings contradict [6]. This difference, according to researchers, occurs due to the Iraqi ministry of health suffering from a shortage of female sex because an old Iraqi family refused any female members to participate in the nursing field, while in other countries there is no idea like this.

Regarding the level of education, the largest group sample of the study graduated from the nursing college for the study sample. Many previous studies were in agreement with this result. They found that the majority of the nurses working in the heart-
Concerning the year of experience, the result of the present study revealed that the majority of nurses in the study sample are between (1-5) years of experience. This result is supported by [8,1], as their results indicated that the higher percentage of the study groups for years of experience in nursing is less than (5) years.

Regarding years of experience in the cardiopulmonary bypass department, the present study shows that the majority of the study sample are between (1-5) years. These results disagree with another study done by [9] discovered in his study “Fundamental clinical skills of adult cardiopulmonary bypass” that the majority of the study sample had more than (25) years of experience in cardiopulmonary bypass machine, and (Long & Matthews, 2016) discovered that the majority of the nurses in the study groups had more than (30) years. This variation, I think as a researcher, occurred due to the oldest building of these centers with staff at the same time as these centers while our centers were assembled later.

About training sessions toward cardiopulmonary bypass machine, the study show that few number of the sample in this study had training sessions. This result disagrees with several studies that indicate in their results that the majority of the study sample had good numbers of training sessions [10]. These differences, I think, are related to the weak interest of the health governorate and the low budget of these centers.

Concerning the number of training courses, the study shows that a very small study sample has training courses, while other studies have results that disagree with this study as the sample has more than 1000 hours of training course [4].

The current study’s findings indicated that the study sample’s knowledge is lacking in the pre-test, with approximately 53% of the study sample failing the pre-test and approximately 40% of the study sample having a fair level of knowledge. Many studies support this result where they found pre-test knowledge was poor [11].

[12] also shows that in her study on knowledge of nurses’ care for patients connected to extracorporeal membrane, they discovered that nurses’ knowledge of educational programs improved compared to before the educational program.

[13] In their study “impact of an evidence-based educational program about pediatric open-heart surgery care on both nurses and patients’ outcomes” they concluded there a deficit knowledge of nurses about cardiopulmonary bypass machine before participation in the educational intervention.

In regarding to the result in the above tables show that nurses knowledge regarding management of cardiopulmonary bypass machine has been improved after exposure to educational program on guideline. This indicates by the significant difference between pre-test and post-test result, which is supported by a previous study, which indicated that there is a high significance difference between pre-posttest [12].

Present study is supported by many studies that mentioned there are highly significant differences for nurses’ knowledge in pre and post-test of the study sample [13].

This result is in agreement [14,15], who indicates that providing postoperative education classes to nurses can be successful in increasing nurses’ knowledge. Therefore, the implemented educational program is effective and has an impact on nurse’s knowledge about cardiopulmonary bypass machine. Concerning the result related to associations between post-test and demographical data in

Regarding to the age, the result of the present study show that a significant association between age and nurses’ knowledge at p-value (0.035). This result is agreed with [5] who stated in their study that there
is a significant association between age and nurses’ knowledge toward cardiopulmonary bypass machine.

According to year of experience in hospital, the study explains that is a significant association with nurses’ knowledge about clinical guide line of cardiopulmonary bypass machine as the study explain that highest mean was (2) at rating (16-20) at P-value (0.014).

Regarding years of experience in heart lung machine department, the present study show that a significant association between years of experience as a perfusionist and nurses’ knowledge at p-value (0.027). This result is agreed with [9]

The study reveals that there is no significant association between post-test and gender. The results of the present study are supported by other studies that indicated no significant association between gender and knowledge. [16]

Conclusion

1. The level of nurses’ knowledge towards cardiopulmonary bypass machines in open-heart surgery was a deficit in different domains. Also, there was a significant relationship between nurses’ knowledge and experience in the hospital as well as nurses as a perfusionist in Al-Najaf center for cardiac surgery and trans-catheter therapy.

2. Because patients are exposed to several complications undergoing extracorporeal circulation, therefore the improving in nurses’ perfusionist knowledge that occur during educational program can have favorable effect on the incidence of these complications..

3. The study concluded that educational program can improve in nurses’ knowledge and to produce perfusionists who are able to successfully apply their knowledge and skills to improve patients’ outcome.

Recommendations:

1. There is a need for a written program for all new nurses working in heart-lung machine department. Moreover, there is a need for implement an active updated educational activities through seminar and symposium to all nurses concerning cardiopulmonary bypass machine.

2. Further study with larger sample to include other Iraqi governorates is necessary in nurse’s knowledge concerning cardiopulmonary bypass machine in order to demonstrate more clearly

3. The study recommended a written program for all new nurses working in heart-lung machine department, and improving the expertise of nurses and medical staff through use the finding of the present study to give care for the patient.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the College of nursing and carried out in accordance with approved guidelines”.

References


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Effectiveness of an Instructional Program on Teachers’ Knowledge about Epilepsy in Elementary Schools at Al-Rusafa Third Education Directorate in Baghdad City/Iraq

Ahmed Jasim Mohammed Aldraje¹, Aqeel Habeeb Jasim²

¹M.Sc. Student, University of Baghdad, College of Nursing, Community Health Nursing Department, ²Assist Prof (PhD), University of Baghdad, College of Nursing, Fundamentals of Nursing Department

Abstract

Background: Teachers knowledge about epilepsy is an important issue since teachers have lots of effect when children spend epileptic seizure at the school, and plays a vital role on providing an educational support for epileptic child and to keep good communication between epileptic and normal child.

Objectives of the Study: The aim of this study is to determine the effectiveness of an instructional program on Elementary school teacher’s knowledge concerning epilepsy.

Methodology: A quasi-experimental design used in the present study with the application a (pre-posttest approach) for the study group and control group after implementation of instructional program. Data collection was done at two times: for both study and control group and (15) days after giving the instructional program in the study group.

Results: The results of the study shows that there is a highly significant differences related to teacher’s knowledge concerning epilepsy at Al-Rusafa Third Education Directorate in Baghdad City, There is a significant association between teacher’s knowledge with their socio-demographic characteristics in term of educational level, marital status, and family monthly income.

Keywords: Teachers, Knowledge, Epilepsy.

Introduction

Epilepsy is a continuum of disorders, in which a child, can experience often irregular seizures (fits). Most seizures are well managed by medications and other therapies, but epilepsy may cause problems in social, school and work conditions, making it difficult to live independently ¹¹. Students with seizures appear to have both physical problems (e.g. fractures, bruises and a significantly greater chance of sudden death) and psychological issues due to the stigma attached to the disorder. Epileptic students and their families may lack social support or experience social isolation, humiliation, fear and prejudice and some parents may feel guilty ⁹. Epilepsy self-management refers to, a broad variety of health habits, and practices which can be practiced and adjusted by epileptic students to monitor their seizures, and improve their wellbeing. This strategy includes a collaboration between its students and the service providers ¹¹.

Teachers play a critical role in, students with epilepsy becoming mentally, socially and academically well-rounded. Teachers who know how to respond to seizures can boost school safety levels and influence fellow students’ and school staff reactions. A teacher who calmly and supportively responds to the seizures will help others learn to do the same. In certain cases, the teachers are the first to note and understand a student’s signs of seizures. Teachers who recognize,
encourage and inspire epileptic students foster learning, confidence and self-esteem.

**Methodology**

A quasi-experimental design used in the present study with the application a (pre-posttest approach) for the study group and control group after implementation of instructional program. The study instrument and program were constructed by the researcher to measure the aim of the study. Data collection was done at two times: for both study and control group and (15) days after giving the instructional program in the study group. The period of the study was from (1st of October, 2020) to (1st of June, 2021) to. A non - probability (purposive) sample selected from Al-Rusafa Third Education Directorate schools in Baghdad City. The sample divided into two groups (30) teachers considered as study group, and another (30) teachers considered as control group. The study group was exposed to an instructional program, while the control group was not exposed to the program. Data collection performed through the use of the study instrument and the application of the instructional program both constructed and developed by the researcher through extensive review of previous literature. Validity is determined through panel of (14) experts in health care fields who have more than (10) years of experience. Reliability of instrument is determined through the use of the computation of Alpha Cronbach’s test (Alpha Correlation Coefficient); internal consistency method was used for determining the reliability, the results was (0.73), Data analysis is done through the use of descriptive statistics via SPSS version 20 with and excel application (frequency, percentage, mean of score, and standard deviation) and inferential analysis procedure (Cronbach’s alpha, Pearson Correlation Coefficient, and Paired sample T-test).

**Results**

**Table (1): Distribution of the Sample According to their Socio-demographic Characteristics**

<table>
<thead>
<tr>
<th>List</th>
<th>Characteristics</th>
<th>Study Group</th>
<th>Control Group</th>
<th>X2</th>
<th>df</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>f</td>
<td>%</td>
<td>f</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>20 – 29</td>
<td>12</td>
<td>39.9</td>
<td>12</td>
<td>40</td>
<td>22.643</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>30 – 39</td>
<td>11</td>
<td>36.7</td>
<td>10</td>
<td>33.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 ≤</td>
<td>7</td>
<td>23.4</td>
<td>8</td>
<td>26.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>13</td>
<td>43.3</td>
<td>14</td>
<td>46.7</td>
<td>.475</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>17</td>
<td>56.7</td>
<td>16</td>
<td>53.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Level of education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diploma</td>
<td>20</td>
<td>66.7</td>
<td>21</td>
<td>70</td>
<td>3.375</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Bachelor</td>
<td>8</td>
<td>26.6</td>
<td>7</td>
<td>23.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High diploma</td>
<td>2</td>
<td>6.7</td>
<td>2</td>
<td>6.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

f: Frequency, %: Percentage, X²: Chi-square, df: degree of freedom, p: Probability, Sig: Significance, S: Significant, N.S: Not significant
This table show that teachers in the study group are with age 20-29 years as referred by the highest percentage (39.9) and the highest percentages also refer for those in the control group refer to 40% for age 20-29 years.

Regarding gender variable, the finding indicates that more than half of teachers are females for those in study group (56.7%) and in control group (53.3%).

The level of education indicates that teachers are with diploma among the study and control group (study= 66.7% and control= 70%)

Table (2): Overall Evaluation of Teachers’ Knowledge about Epilepsy in Children at elementary Schools among Study and Control Group

<table>
<thead>
<tr>
<th>Levels of Knowledge</th>
<th>Study Group (N= 30)</th>
<th>Control Group (N= 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-test</td>
<td>Post-test</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>%</td>
</tr>
<tr>
<td>Poor</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2.13</td>
<td>1.106</td>
</tr>
<tr>
<td>Fair</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Good</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>3.3</td>
</tr>
</tbody>
</table>

f: Frequency, %: Percentage, M.S: Mean of score, SD Standard deviation

Poor= 0-10, Fair= 11 -20, Good= 21-30

This table presents the overall evaluation of teachers’ knowledge; the findings indicate that teachers in the study group are showing poor level of knowledge during the pre-test time (100%) while they are showing good level of knowledge during the post-test time (100%) that indicate the significant changes in their level of knowledge.

The teachers in the control group are showing poor level of knowledge over both times; pre-test and post-test (100%) that doesn’t reveal changes in their level of knowledge.

Table (1): Discussion of the socio-demographic characteristics.

Age

The study shows that the highest percentage (12; 39.9%) of study participants in both study and control groups were age (20-29 years).

This results agree with a cross sectional study was conducted in Kathmandu, Metropolitan City done by Khanal, Maharjan, Pkhairel, and Sanjel (2015) which stated that the median age of (165) teachers are (29 years). Additionally, comparable to Toudou-Daouda and Ibrahim-Mamadou (2020) which certain that a high percentage of participants were age less than 30
years old.

**Gender**

The finding of the present study indicate that more than half of teachers are females in study group and control group (56.7%) (53.3%) respectively.

This totally agree with 18 that it results showed a percentage (65.5%) of the study participants were female. Moreover, agree with Bhesania, Rehman, Savu, and Zehra (2013) which point out that the female and male percentage was (36.56 ± 10.92).

**Educational level**

The highest percentage of teachers are with diploma among the study and control group (66.7%), (70%) respectively.

This finding similar to a cross sectional study with analytic portion, conducted in Baghdad done by 3 which displayed that a percentage (63.4%) of study participants were institute graduate. Furthermore, agree with results obtained in a study done by Salih (2015) which presented that (54.0%) of participants were graduate from Institute.

Table (2): Discussion of the instructional program effectiveness on teacher’s knowledge (comparison significance of participant’s knowledge, pre and post the intervention between study and control group’s related to overall domains).

The findings indicate that teachers in the study group are showing poor level of knowledge during the pre-test time (100%) while they are showing good level of knowledge during the post-test time (100%). The teachers in the control group are showing poor level of knowledge over both times; pre-test and post-test (100%).

The findings reveals that educational program is highly effective on teachers’ knowledge regarding epilepsy in children as indicated by high significant difference in times of pre-test and post-test (at p-value=0.01) among the study group on the contrary of control group.

Poor knowledge at pretest for both groups were common and may be due to few serious conditions of epilepsy occurs in Iraqi schools or due to the absence of statistics related to confirm such conditions; as well supported by many relevance and recent international studies which revealed deficient knowledge about epilepsy among teachers both in developing countries such as the results obtained in a study done by Kadhim et al. (2021) supported the results of this study which mentioned that the majority of participants have a moderate level of knowledge. Others studies like Mohammed (2018), Elhassan et al. (2017), additionally in Goal, Singh, Lal, and Singh (2014).

As this topic is a public health condition, it appears that the educational program has a good impact in improving teacher’s knowledge toward epileptic children.

**Conclusion**

The study concluded that the majority of study sample has poor knowledge regarding epilepsy in elementary schools at Al-Rusafa third education directorate in Baghdad city. Teacher’s knowledge concerning epilepsy at Al-Rusafa Third Education Directorate in Baghdad City has been improved after application of the instructional program in the study group, which confirmed the effectiveness of the delivered program toward improving knowledge teacher’s knowledge concerning epilepsy.

**Recommendations**

The study recommended to applying this program as an instructional booklet in all the directorate of Ministry of Education in all Iraqi governorates and on a larger sample to increase teachers knowledge about epilepsy.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.
Ethical Clearance: “All experimental protocols were approved under the University of Baghdad and carried out in accordance with approved guidelines”.

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Effectiveness of an Educational Program on Nurses Knowledge toward Pre and Post Nursing Interventions Laparoscopic Cholecystectomy at Surgical Ward in AL-Imam AL-Hussein Teaching Hospital in Al Nasiriya City

Badr Kareem Ibrahim1, Suad Jasim Muhamad2

1Academic Nurse, Ministry of Health/ Dhi Qar Health Directorate, Iraq, 2Assist. Prof, University of Baghdad, College of Nursing, Adult Nursing Department, Iraq

Abstract

Background: Laparoscopic cholecystectomy is the gold standard treatment for gallstones. Because of the numerous advantages of open surgery, such as limited incisions, short hospital stay, no drainage tube and stomach tube use, fewer analgesic and fewer complications.

Aims: This study aimed at determine the effectiveness of an educational program on nurses knowledge toward pre and post-operative nursing intervention for patient with laparoscopic cholecystectomy; and identify the relationship between study findings and selected variables (age, level of education, and years of experience).

Methods: A pre-experimental design one group (pre-post test) is conducted for the periods of December 26th 2020 to Jun 1st 2021. The study is carried out at the at AL-Imam AL-Hussein Teaching Hospital in AL- Nasiriya city. By non-probability purposive sample is selected from nurses who in surgical wards before and after implementing educational program, data were collected and analyzed through the application of descriptive and inferential statistics.

Results: Findings indicate that nurses age at mean 24.2, (54%) females, (52%), nursing diploma graduated (46.0%) have 1-5 years of experience and no participated in training sessions. Knowledge statistically significance differences between pre and post -test for study group at (P < 0.05). There were statistically significances differences between (level of educational, years of experience and number of years of work in surgical wards) and effectiveness of educational program among the two period (Pre and Post-Test).

Conclusion: More years of experience in training the staff nursing in surgical wards by local officials help raising professionals’ nurses. Provide the health resources and exploiting young energies of nurses which indeed helps to develop their knowledge.

Key-words: Effectiveness, Knowledge, Nursing Interventions, Laparoscopic Cholecystectomy.

Introduction

Cholecystectomy is gallbladder surgical removal. A common therapy for symptomatic gallstones and other gallbladder disorders is cholecystectomy [1]. Laparoscopic cholecystectomy is recommended for the treatment of acute / chronic cholecystitis, symptomatic Cholelithiasis, biliary dyskinesia, calculus cholecystitis, pancreatitis of the gallstone and polyps / gallbladder mass [2]. Laparoscopic cholecystectomy (LC) is the most common abdominal surgery in the United States and has an annual
incidence of over 500,000 per year [3]. Around 66,000 cholecystectomies are performed annually in the UK, costing the National Health Service around £110 million [4]. Most of these cases, due to substantially lower morbidity and mortality rates relative to traditional open surgery, are now conducted laparoscopically. A national multicenter study recently reported that 96% of cases are conducted laparoscopically, establishing laparoscopic cholecystectomy as the central treatment of multiple benign gallbladder diseases [5]. Biliary colic or acute cholecystitis is responsible for > 70% of the cholecystectomy indications [6]. With an aging population, surgery requirements are projected to increase with associated growing frailty over the next decade [7]. In parallel, the numbers of elderly patients are projected to present with gallstone disease, and 28 percent and 42 percent of male and female patients are assumed to account for patients aged 80-89 years [8]. Developing nurses’ knowledge and practices will help to prepare a planned nursing care for improving patients’ health condition [9]. Preoperative and postoperative care is important to reduce hospital stays and contain costs and enhance the healing without complication. Has resulted in patients undergoing cholecystectomy preadmission testing and preoperative preparation before admission to the hospital many facilities have a pre-surgical services department to facilitate testing and to initiate the nursing assessment process, which may focus interventions laparoscopic cholecystectomy.

Methodology

A pre-experimental design one group (design of pretest and posttest) was performed on the effectiveness of an educational program on nurses’ knowledge of nursing interventions pre and post laparoscopic cholecystectomy in the surgical ward. It was conducted with the application of the pre-test approach to the study group in assessing their knowledge, and then the post-test was applied to the study group three weeks after they were given the educational program. It was implemented in order to achieve the stated initial goals. The study started from (21st December, 2020 to 15th April, 2021).

A non-probability sample was randomly selected from the nurses working in the surgical wards of Al-Imam Al-Hussein Teaching Hospital in Dhi/Qar. The sample consists of (50) nurses. It consisted of one group, which is the study group, and this sample was all exposed to the educational program to evaluate their knowledge. Random sample allocation was performed to avoid selection bias and control for potential confusion.

The educational program was arranged into three sessions for the study group. Each session, divided into two groups of nurses, each group lasted about (30-45) minutes over the course of one lecture for the morning staff and one lecture for the evening staff.

The data collection process uses the self report and questionnaire and analyzed through the descriptive and inferential statistic.

Results

Table 1: Distribution of the study sample by their demographic characteristics

<table>
<thead>
<tr>
<th>Basic Information</th>
<th>Groups</th>
<th>Frequency</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-25 years</td>
<td>10</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>26-30 years</td>
<td>20</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>31-35 years</td>
<td>12</td>
<td>24.0</td>
<td></td>
</tr>
<tr>
<td>36-40 years</td>
<td>5</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>41 and more</td>
<td>3</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>(\bar{x} \pm S.D.)</td>
<td></td>
<td></td>
<td>24.2± 1.108</td>
</tr>
</tbody>
</table>
This table indicates the socio-demographic distribution of nurses in terms of frequencies and percentage.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male</th>
<th>23</th>
<th>46.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>27</td>
<td>54.0</td>
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<table>
<thead>
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<th>Education level</th>
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<td>Nursing Institute</td>
<td>26</td>
<td>52.0</td>
</tr>
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<td></td>
<td>College of Nursing</td>
<td>7</td>
<td>14.0</td>
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<table>
<thead>
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<th>The number of years of Experience:</th>
<th>1- 5 years</th>
<th>13</th>
<th>26.0</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>6-10 years</td>
<td>16</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td>11-15 years</td>
<td>11</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>16-20 years</td>
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<tr>
<td></td>
<td>More 21 years</td>
<td>4</td>
<td>8.0</td>
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<table>
<thead>
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<th>Number of years of work in surgical wards:</th>
<th>1- 5 years</th>
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<th>46.0</th>
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<td>15</td>
<td>30.0</td>
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<td>11-15 years</td>
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<td>16-20 years</td>
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<table>
<thead>
<tr>
<th>Participation courses before and after the laparoscopic cholecystectomy</th>
<th>No</th>
<th>46</th>
<th>92.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>4</td>
<td>8.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Participation courses before and after the laparoscopic cholecystectomy</th>
<th>No</th>
<th>49</th>
<th>98.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>1</td>
<td>2.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The Number of Sessions</th>
<th>0</th>
<th>45</th>
<th>90.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>5</td>
<td>10.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration of the Course</th>
<th>None</th>
<th>45</th>
<th>90.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One Week</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Two week</td>
<td>2</td>
<td>4.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The Course Place:</th>
<th>None</th>
<th>45</th>
<th>90.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inside Iraq</td>
<td>5</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Outside Iraq</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Table 2: Effectiveness of Educational Program among the Two Period (Pre and Post-Test) for Nurse’s Knowledge toward the Pre and Post Nursing Interventions Laparoscopic Cholecystectomy for Study Sample

<table>
<thead>
<tr>
<th>Period</th>
<th>Mean ±S.D.</th>
<th>N</th>
<th>T</th>
<th>P. value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-test</td>
<td>1.4761± 0.15124</td>
<td>50</td>
<td>18.910</td>
<td>0.003</td>
<td>S</td>
</tr>
<tr>
<td>Post –test</td>
<td>1.8880± 0.05975</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

 means Arithmetic Mean ( ) and Std. Dev. (S.D.), F = Fisher test, d.f. = degree of freedom, P = probability value. NS: Non Significant at P ≥ 0.05 , S : Significant at P < 0.05.

Findings shows there is statistically significance differences between pre and post -test for study group at (P < 0.05) which mean effectiveness of educational program among the two period (Pre and Post-Test ) for nurse’s knowledge toward the Pre and Post nursing interventions laparoscopic cholecystectomy for study sample.

Table 3: Statistical Associations of the Study Group between the Demographic Variables of Nurses and Effectiveness of Educational Program among the Two Period (Pre and Post-Test)

<table>
<thead>
<tr>
<th>No</th>
<th>Demographic Variables</th>
<th>Nurse’s Knowledge</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean± S.D.</td>
</tr>
<tr>
<td>1</td>
<td>Age</td>
<td>24.2± 1.108</td>
<td>0.959</td>
</tr>
<tr>
<td>2</td>
<td>Gender</td>
<td>1.54±0.503</td>
<td>1.023</td>
</tr>
<tr>
<td>3</td>
<td>Educational Level</td>
<td>2.78±0.708</td>
<td>0.894</td>
</tr>
<tr>
<td>4</td>
<td>Years of Experience</td>
<td>2.44± 1.232</td>
<td>1.343</td>
</tr>
<tr>
<td>5</td>
<td>Number of years of work in surgical wards</td>
<td>1.80±0.857</td>
<td>2.174</td>
</tr>
<tr>
<td>6</td>
<td>Participation in courses that laparoscopic cholecystectomy</td>
<td>1.88±0.05975</td>
<td>2.166</td>
</tr>
<tr>
<td>7</td>
<td>Participation in Courses for laparoscopic cholecystectomy</td>
<td>1.8±0.05975</td>
<td>1.897</td>
</tr>
</tbody>
</table>

 means Arithmetic Mean ( ) and Std. Dev. (S.D.), F = Fisher test, d.f. = degree of freedom, P = probability value, , NS : Non Significant at P ≥ 0.05 , S : Significant at P < 0.05.
Findings show their no statistically significances differences between demographics variables (age, gender and participation in the Nursing intervention courses that laparoscopic cholecystectomy at the hospital, and participation in courses for nursing at the other hospital), while there is statistically significances differences between demographics variables (level of educational, Number of years of Experience and Number of years of work in surgical wards) and effectiveness of educational program among the two period (Pre and Post-Test) for nurse’s knowledge toward the Pre and Post nursing interventions laparoscopic cholecystectomy for study sample, when analyzed by ANOVA.

Discussion

The study sample consists of (50) nurses working in the surgical wards of Al-Imam Al-Hussein Teaching Hospital.

According to Table 1, findings indicated that 20 (40.0%) of the nurses are in the age group (26-30) years with an average of (24.2) years. This result was supported by findings of study investigated performance among nurses regarding laparoscopic cholecystectomy patients. Their findings showed that nurses (20-30 years) are (52%) with mean age (31.4±2.8) \[10\]. Nurses in surgical wards need to be young.

With respect gender, cooperative in study the majority of study sample were female 27 (54.0%) of all study sample. This result disagree with findings of study has been assessed postoperative nurses’ interventions in Baghdad Teaching Hospitals, which indicated that two-third of the nursing staff are male \[11\]. In fact, the surgical wards need to males nurses due to workload and covers all duties.

With regard to the educational qualifications of the studied sample, the current study indicated that almost half of them are from technical institutes and their percentage is 26 (52.0%) and less than half of them (6-10) years of experience 16 (32.0%). This result agreed with study of AbdElGhil et al. (2020), where it was found that less than half of the sample 24 (48%), have a technical institute of nursing, while less than half of them, 20 (40%), have an experience of 5 to less than 10 years in the hospital with the mean of (9.18±8.4) \[12\]. Hospitals insinuations were depends on nurses institute graduated due to the academic nurses were numbers are still small, unlike the institutions that graduate diplomas.

Regarding the number of years working in the surgical wards, the majority of the study sample ranges between (1-5 years) and represents 23 (46.0%) of the entire sample. This result is agreed with (Kadhim, 2014) that was conducted in Baghdad hospitals, which showed the results of the study sample in the surgical wards of (1-5) years, they represented 23 (46%). These results contrast with findings evaluated nurses’ practices toward postoperative wound dressing in surgical wards. It reported that most of the nurses had 24 (43.6%) of (6-10) years of experience in surgical wards \[13\]. This results come because the diploma graduated immediately after graduation, they are appointed.

Regarding the question (participation in nursing intervention courses before and after laparoscopic cholecystectomy, which the hospital conducts periodically), the majority of the study sample had answers with (No participation) and represented 46 (92.0%) of all the study sample.

Regarding the question (participation in courses of nursing interventions before and after laparoscopic cholecystectomy, which are conducted by other hospitals), the majority of the study sample had answers with (No participation) and represented 49 (98.0%) of the entire study sample.

Regarding the question (number of training sessions), the majority of the study sample was 45 (90.0%) of all study sample individuals who did not have a share in any training sessions. While only 5 (10%) of the course participants answered.
To the question (duration of the course), the majority of the study sample had answers with (No participation) and represented 45 (90.0%) of all the study sample individuals. While those who answered (yes participation), of them 3 (6%) for one week and 2 (4%) for two weeks and therefore did not have sufficient time or the duration of the training session.

These results are consistent with findings of Kadhim (2014), who mentioned that there was significant relationship between sharing in training sessions which established (by the hospital or by other hospitals), duration of the training session, number of training sessions and which reported that the majority of the results answered (No) 47 (94%) of the study sample did not have the opportunity to participate in the training sessions related to nurse surgical interventions for patients with (LC) established by the hospital. All study samples answered (No) 50 (100%) did not have the opportunity to participate in training sessions related to nursing interventions for (LC) established by other hospitals. Regarding the topics of “number of training sessions”, the majority of the sample, 47 (94%), did not have a share in any training sessions, while only 3 (6%) answered that their participation was less than a week and therefore they did not have sufficient time or duration for the training session [11].

With regard to the question (place of the session), the majority of the study sample had their answers (inside Iraq) and they represented 45 (90.0%) of the total study. This study is supported by study investigated the effectiveness of the educational program in the knowledge of nurses in relation to nursing management before and after surgery. Demonstrated findings that all the courses held inside Iraq, in addition to the absence of any of the sample members who had a training course outside Iraq [14].

There is statistically significance differences between pre and post -test for study group at P < 0.05, which mean effectiveness of educational program among the two period (Pre and Post-Test) for nurse’s knowledge toward the Pre and Post nursing interventions laparoscopic cholecystectomy for study sample. These results are supported by Kreem and Hamza (2019) as it shows that the educational program related to nursing management before and after surgery with an increased knowledge of the study group, where the p-value is (0.001) [14].

Our show their no statistically significances differences between demographics variables (age and gender and participation in the Nursing intervention courses about laparoscopic cholecystectomy at the hospital and participation in courses for nursing at the other hospital) ,while there is statistically significances differences between demographics variables (level of educational, Number of years of Experience and Number of years of work in surgical wards) and effectiveness of educational program among the two period (Pre and Post-Test ) for nurse’s knowledge toward the Pre and Post nursing interventions laparoscopic cholecystectomy for study sample, when analyzed by ANOVA. These results are inconsistent with the study conducted by (ElSayed et al. 2021) on knowledge of nurses and practices related to the care of patients undergoing cholecystectomy, which was studied in Zagazig University Hospitals, which showed that the result of the study there is a statistically significant relationship between the total knowledge of nurses regarding patients who have undergone for cholecystectomy and age and training course [15]. However, this study is in agreement with the results of the current study, as it showed that there is a positive statistically significant correlation between knowledge degrees and years of experience. This explained that the increase in years of experience in surgical units enhances the knowledge of nurses as a result of the increase in the number of follow-up cases and dealing with them. Also, nurses with years of experience become more experienced and knowledgeable. The results of the current study also showed a statistically significant relationship between the nurses knowledge the level of nursing qualification.
It is also supported by a study, which found that years of experience in the field of surgery are statistically related between nurses’ total knowledge of patients undergoing cholecystectomy and work in surgical wards.

Regarding participation in the training courses, the results of the current study are disagree with, who indicated that the nurses who participated in the surgical or perioperative training courses were exposed to their satisfaction with preoperative teaching.

This result also differs from the current study with (Arab et al. 2016) on the effects of training programs on the knowledge and attitudes of nurses after surgery, which stated that despite the increased knowledge of general surgery compared to other units, there is no statistically significant relationship between hospital units Increasing levels of knowledge and attitudes.

In general, the researcher suggests that to provide high-quality nursing interventions for patients with cholecystectomy before and after surgery in the surgical wards by nurses, the nurses’ knowledge should be further developed through their participation in training courses inside and outside Iraq, and the continuing education of nurses who were working in the surgery wards and the increase in the number of nurses Professionals graduating from the College of Nursing in the surgical wards. In addition, the use of guidelines for nursing interventions to improve the efficiency of nurses in the care of patients with laparoscopic cholecystectomy to avoid complications after the operation.

**Conclusion**

More years of experience in training the staff nursing in surgical wards by local officials help raising professionals’ nurses. Provide the health resources and exploiting young energies of nurses which indeed helps to develop their knowledge.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** “All experimental protocols were approved under the Dhi Qar Health Directorate and carried out in accordance with approved guidelines”.

**References**


Effectiveness of an Instructional Program on Gastric Carcinoma Patients Knowledge Undergoing Chemotherapy toward Self Care after Gastrectomy at Oncology Unit of AL-Haboby Teaching Hospital in AL-Naseria City

Hussein Ali Jabbar¹, Suad Jasim Muhamad²
¹Academic Nurse, Ministry of Health/ Dhi Qar Health Directorate, Iraq, ²Assist. Prof, University of Baghdad, College of Nursing, Adult Nursing Department, Iraq

Abstract

Aims: To determine the effectiveness of instructional program on self-care knowledge patients undergoing chemotherapy after gastrectomy. Methods: Pre-experimental pre-post test design was conducted in Al-Naseria City. The data collection process uses the interview technique and analyzed through the descriptive statistic. Results: There is statistically significance differences between pre-post test knowledge, which mean effectiveness of instructional program knowledge of self care after gastrectomy. Conclusion: In the post-test, there was a big difference in the patients’ knowledge level towards self-care. Establishing specialized centers to educate patients importance of early detection of cancer.

Key-wards: Effectiveness, Patients, Knowledge, Self Care, Gastrectomy.

Introduction

Gastric cancer, especially among older males, remains one of the most common and deadly cancers worldwide. Stomach cancer is the 5th most prevalent neoplasm and the 3rd most deadly cancer, based on GLOBOCAN 2018 results, with an estimated 783,000 deaths in 2018 [1]. Less than a century ago, in the United States and maybe all over the world, gastric cancer was the most prevalent cancer. While gastric cancer is no longer the world’s most common cancer, it remains the world’s second leading cause of cancer-related mortality and the most prevalent cancer in Eastern Asia [1]. Gastric cancer is a worldwide health epidemic, with over 1 million people hospitalized worldwide with gastric cancer every year. Gastric cancer is the leading third cause of cancer-related death, although it has fallen globally in prevalence and death in the past 5 decades [2]. One of the world’s most frequent causes for death is the growth of malignant cells in some region of the stomach, known as gastric cancer. It is important to develop the decision-making method to lead a greater and more effective range of treatment options to maximize the survival rate of patients with this disease [3]. Self-care exists even before traditional insurance services and contributes significantly to health outcomes. For certain individuals, self care may be the only healthcare they have access to under well defined cultural and social standards. Where there are healthcare systems, self-treatment is a critical element of health care [4]. Improve home self-care of patients with chemotherapy, which leads to a decrease in the complication of chemotherapy treatment. Patients undergoing chemotherapy without treatment knowledge and side effects can lead to a decline in the patient’s health. Patient self-care teaching helps to recognize the patient and family member’s learning needs since the cancer diagnosis and treatment crush the patient and family. Previous research has found that 50-90 percent of cancer patients want to die at home. There was a downward trend in home self-deaths,
dropping between 1974 and 2003 from 31 percent to 18 percent. If the pattern continues, by 2030, less than 10% of deaths will occur at home [5]. Therefore, the study aim to determine the effectiveness of an instructional program on knowledge of self care for gastric carcinoma patients undergoing chemotherapy after gastrectomy; and to identify the relationship between study findings and selected variables.

Methodology

A pre experimental (one-group pre-/posttest) design was carried out to assess Effectiveness of an Instructional Program on Gastric Carcinoma Patients Knowledge Undergoing Chemotherapy toward Self Care after Gastrectomy at Oncology Unit of Al-Haboby Teaching Hospital in Al-Naseria City, through application of Instructional Program and application of pre-test and post-test approach for the study group, the study lasted from 21th of December, 2020 to the 14th April 2021.

In patients with gastric cancer and undergo chemotherapy after gastrectomy at Al-Haboby Teaching Hospital Oncology Unit in Al-Naseria City, a non-probability deliberate sample was randomly picked. The study is consist of (40) patients. Whole the study group was exposed to an instructional program.

A questionnaire consists of the following parts including:

**Part 1**: Socio-demographic data of nurses includes age, gender, level of education, marital status, occupation, residents, and monthly income.

**Part 2**: Knowledge of gastric carcinoma patients undergoing chemotherapy after gastrectomy towards self-care. This domains divided into three sub-domains include

A. Knowledge of gastric carcinoma patients undergoing chemotherapy after gastrectomy about general information about stomach cancer and gastrectomy which include of (8) items.

B. Knowledge of gastric carcinoma patients undergoing chemotherapy after gastrectomy about general information of chemotherapy which include of (6) items.

C. Knowledge of gastric carcinoma patients undergoing chemotherapy after gastrectomy about self-care of side effects of chemotherapy and self-care of daily activities which include of (14) items.

A content validity were achieved through a (11) panel of experts and reliability were achieved through a pilot study. It conducted on (10) were selected among chemotherapy patients at Oncology Unit of AL-Haboby Teaching Hospital. Cronbach’s Alpha= 83 which indicate a pass questionnaire items. The data collection process uses the interview technique and questionnaire and analyzed through the descriptive statistic.
## Results

Table (1): Distribution of the Study by their demographic characteristics

<table>
<thead>
<tr>
<th>Basic Information</th>
<th>Groups</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age groups</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36-40</td>
<td>2</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>41-45</td>
<td>4</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>46-50</td>
<td>6</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>51-55</td>
<td>4</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>56-60</td>
<td>13</td>
<td>32.5</td>
<td></td>
</tr>
<tr>
<td>above 60 Y</td>
<td>11</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td>$\bar{x}\pm S.D.$</td>
<td></td>
<td>7.38± 1.531</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>26</td>
<td>65.0</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>35.0</td>
<td></td>
</tr>
<tr>
<td><strong>Level of Education of Patients</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>0</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Can Read &amp; Write</td>
<td>3</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Primary School Graduate</td>
<td>24</td>
<td>60.0</td>
<td></td>
</tr>
<tr>
<td>Secondary School Graduate</td>
<td>7</td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td>Institute Graduate</td>
<td>3</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Bachelor’s degree</td>
<td>3</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Postgraduate graduate</td>
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<td>0.00</td>
<td></td>
</tr>
<tr>
<td><strong>Marital status:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>35</td>
<td>87.5</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>2</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>2</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>1</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td><strong>Occupation Status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employee</td>
<td>6</td>
<td>15.0</td>
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</tr>
<tr>
<td>Free job</td>
<td>8</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Retired</td>
<td>2</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>House wife</td>
<td>13</td>
<td>32.5</td>
<td></td>
</tr>
<tr>
<td>Student</td>
<td>4</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>7</td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td><strong>Monthly Income</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sufficient</td>
<td>3</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Barely Sufficient</td>
<td>13</td>
<td>32.5</td>
<td></td>
</tr>
<tr>
<td>Insufficient</td>
<td>24</td>
<td>60.0</td>
<td></td>
</tr>
</tbody>
</table>

This table indicated that 13 (32.5%) of the patients within age group of (56-60) years with mean of (73.8)
years. Related to the gender cooperative in study the majority of study sample were male 26 (65.0%) of all study sample. Regarding to educational level of patients in study sample the majority with primary school graduation 24 (60.0%). Concerning to the social status, the greater number of study sample are 35 married and account for (87.5%). Regarding to the level of occupation status, the greater number of them with house wife and they are accounted for 13 (32.5%) of the sample. With respect to patients monthly income the majority of study sample with insufficient monthly income and account for 24 (60.0%) of the whole sample.

Table (2): Effectiveness of an Instructional Program on Gastric Carcinoma Patients Knowledge Undergoing Chemotherapy toward Self Care after Gastrectomy at Oncology Unit for Study Sample.

<table>
<thead>
<tr>
<th>Period</th>
<th>Mean ± S.D.</th>
<th>N</th>
<th>T</th>
<th>P. value</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-test</td>
<td>1.2446±0.06647</td>
<td>40</td>
<td>37.733</td>
<td>0.036</td>
<td>S</td>
</tr>
<tr>
<td>Post–test</td>
<td>1.9045±0.07899</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Findings shows there is statistically significance differences between pre and post test for study group at P < 0.05, which mean effectiveness of instructional program among the two period (Pre and Post-Test) on gastric carcinoma patients knowledge undergoing chemotherapy toward self care after gastrectomy at oncology unit for study sample.

Table (3): Statistical Associations of the Study Group between the Demographic Variables of Patients and Effectiveness of Instructional Program Among the Two Period (Pre and Post-Test) on Gastric Carcinoma Patients Knowledge Undergoing Chemotherapy toward Self Care after Gastrectomy at Oncology Unit for Study Sample by (ANOVA)

<table>
<thead>
<tr>
<th>No</th>
<th>Demographic Variables Patient’s Knowledge</th>
<th>Mean ± S.D.</th>
<th>F</th>
<th>d.f</th>
<th>P. value</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age of Patients</td>
<td>73.8±1.531</td>
<td>1.432</td>
<td>39</td>
<td>0.022</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>Gender of Patients</td>
<td>1.35±0.483</td>
<td>1.174</td>
<td>39</td>
<td>0.346</td>
<td>N.S</td>
</tr>
<tr>
<td>3</td>
<td>Educational Level of Patients</td>
<td>3.63±1.334</td>
<td>2.178</td>
<td>39</td>
<td>0.008</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>Marital status of Patients</td>
<td>1.22±0.660</td>
<td>5.095</td>
<td>39</td>
<td>0.004</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>Monthly Income of Patients</td>
<td>2.52±0.640</td>
<td>1.898</td>
<td>39</td>
<td>0.096</td>
<td>NS</td>
</tr>
</tbody>
</table>
$\bar{x} \pm S.D.$ = Arithmetic Mean ($\bar{x}$) and Std. Dev. (S.D.), $F$ = Fisher test, d.f. = degree of freedom, $P$ = probability value, NS : Non Significant at $P$ $\geq$ 0.05, S: Significant at $P$ $<$ 0.05.

Findings show their no statistically significances differences between demographics variables (patients gender, monthly income and family history of current disease), while there is statistically significances differences between demographics variables (patient age, educational level, marital status and duration of current disease) and effectiveness of instructional program among the two period (Pre and Post-Test) for patient’s knowledge toward the Pre and Post effectiveness of instructional program for study sample, when analyzed by ANOVA.

**Discussion**

The highest age group was 56-60 years old with a percentage of 13 (32.5%) with mean of (73.8) years, and this result was consistent with Chen et al. (2016) [6]. The average age of those living with stomach cancer was 60.6 ± 6.6 years.

The prevalence is increasing with age of gastric cancer. Aging is linked to increased injury susceptibility, delayed cure of gastric mucosa, and expression of cancer stem cell markers. Furthermore, H. pylori gastritis rises with age. It was not clear, however, whether the prediction of gastric cancer already known worsened with age [7].

Related to the gender cooperative in study the majority of study sample were male 26 (65.0%) of all study sample. There are no obvious explanations for these disparities. Exposures to the environment or work may take on a function. For example, men have traditionally been more likely to smoke tobacco products, including in countries where men and women have equal levels of smoking, but higher concentrations of men tend to exist. Alternatively, sex distinctions may represent variations in physiology. Estrogens can protect against gastric cancer growth [8].

Regarding to educational level of patients in study sample the majority with primary school graduation 24 (60.0%). Increased education of both sexes has been linked to a declining risk of esophagogastric cancer. Compared with elementary school graduation, higher education posed a lower risk to both sexes. This relationship was close for each of the 4 esophagogastric cancer subtypes and may be greater for gastric cancer [9].

Concerning to the social status , the greater number of study sample are 35 married and account for (87.5%). This result was consistent with findings investigated the prevalence and prognostic implications of psychological distress in patients with gastric cancer [10].

Regarding to the level of occupation status, the greater number of them with house wife and they are accounted for 13 (32.5%) of the sample. This finding disagrees with the outcome of the environmental factors in etiology of gastric cancer [11].

Rural people only see physicians as problems occur. This could lead to higher mortality in rural areas compared to urban areas through delayed diagnosis and treatment. Policymakers should propose improving the understanding of gastric cancer through education in health care and extending early detection screening and early therapy to minimize the disparity in gastric cancer mortality across urban and rural areas [12].

With respect to patients monthly income the majority of study sample with insufficient monthly income and account for 24 (60.0%) of the whole sample. This result was consistent with gastric cancer screening in low-income countries that reported; Gastric cancer shows significant geographical variations in and within regions with over 70% of the incidence cases clustered in countries that have low and mid-income [13].

Findings shows that information of study sample were presented low level of knowledge at all items
except (In a complete gastrectomy surgery the doctor is performed by) which presented as (Moderate) level of evaluation at pre-test for study group, So the results show high level of knowledge at post-test for study group. That there are statistically significance differences between two periods (pre and post-tests) of study group in all items of the patient’s knowledge of gastric carcinoma patients undergoing chemotherapy after gastrectomy about general information about stomach cancer and gastrectomy of the Study Sample (Study Group), This reflects that the patients’ knowledge was affected by educational program.

Findings shows that information of study sample were presented Moderate level of knowledge at items (Chemotherapy is a treatment that is used to eliminate, One of the factors that increase the chances of responding to chemotherapy? and As a side effect of chemotherapy?) at pre-test for study group. While presents low level of knowledge at items remaining at pre-test for study group , So the results show high level of knowledge at post-test for study group. That there are statistically significance differences between two periods (pre and post-tests) of study group in all items of the patients’ knowledge of gastric carcinoma patients undergoing chemotherapy after gastrectomy about general information of chemotherapy (Study Group), This reflects that the patients’ knowledge was affected by educational program.

Findings shows that information of study sample were presented low level of knowledge at all items except (Should you avoid high-fiber foods because they?) which presented as (Moderate) level of evaluation at pre-test for study group, So the results show high level of knowledge at post-test for study group. That there are statistically significance differences between two periods (pre and post-tests) of study group in all items of the knowledge of gastric carcinoma patients undergoing chemotherapy after gastrectomy about self-care of side effects of chemotherapy and self-care of daily activities of the study sample (Study Group), This reflects that the Patients’ knowledge was affected by educational program.

Kamil & Mohammed, (2020) show that statistically meaningful difference is seen in the plurality of items for the patient information community between the pretest and posttest sample analysis. The overall mean of expertise for patients in the field of guidance program has been seen to be mid-scale knowledge and posttest, fields of Patient cancer and chemotherapy know-how, self-care of chemotherapy side- effects and self-care awareness in day-to-day life [14].

Davoodi et al., (2015) it’s do not agree with this results an show After gastrectomy, a short self-care training program was not sufficient to substantially increase the quality of life of gastric cancer patients [15].

Show their no statistically significances differences between demographics variables (patients gender, monthly income and family history of current disease), while there is statistically significances differences between demographics variables (patient age , educational level , marital status and duration of current disease) and effectiveness of educational program among the two period (Pre and Post-Test) for patient’s knowledge toward the Pre and Post effectiveness of educational program for study sample.

Kamil & Mohammed, (2020) found that statistically relevant information was available at the post p-test at p value (0.010,043) between patient’s knowledge and (age and level of education) [14].

Choenyi et al., (2016) They stated that selected variables existed in their data. Height, degree of education, have no important connection with understanding and home control of harmful effects of chemotherapy [16]. The study findings indicate that there was no statistically significant difference between patients’ experience and their knowledge (marital status, job status, monthly revenues, when were you diagnosed with cancer in the past family, after diagnosis and type of care provided after diagnosis, and have chemotherapy information?).
Conclusion

In the post-test, there was a big difference in the patients’ knowledge level towards self-care, which indicates the smoothness of the program that was prepared for this purpose and the ease with which it was accepted by all participating patients. Establishing specialized centers to educate patients about self-care stomach cancer and disease-causing risk factors and importance of early detection of cancer.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the Dhi Qar Health Directorate and carried out in accordance with approved guidelines”.

References


Role of Antenatal Dexamethasone Administration on Fetal Doppler Velocimetry

Enas Mozer Abdalraheem¹, Zuhud M. Mustafa²
¹M.B.Ch.B. HD.G.O Stud. Diyala Health Directorate, Iraq, ²Assistant Prof Dr., Obstetric and Gynecology, College of Medicine, Tikrit University, Iraq

Abstract

Aim: The aim of this study was assess the change in fetal and uteroplacental circulation following antenatal dexamethasone administration to high-risk of preterm labor pregnant women.

Patients and Methods: Prospective study was conducted between January and May 2021 in Tikrit city at Salahadeen General Hospital /genealogy and obesterts department The study included 30 pregnant women with high risk of preterm labor with gestational age from 24 to 34 weeks. Doppler Study were performed just before dexamethasone administration and will be repeated 24 h after completion of the dexamethasone course.

Results: The study showed the highest means of Fetal MCA pulsitility index. levels were recorded in women who at risk of preterm uterine contractions, placenta previa and pre-eclampsia respectively before dexamethasone administration as compared with the same groups at 24 hour after dexamethasone administration (P<0.05). The study showed the highest means of Fetal MCA resistive index levels were recorded in women with preterm uterine contractions, placenta previa and pre-eclampsia respectively before dexamethasone administration as compared with the same groups 24 hour after dexamethasone administration (P<0.05). The study showed the highest means of Uterine artery pulsitility index. levels were observed in women with preterm uterine contractions, placenta previa and pre-eclampsia respectively before dexamethasone administration as compared with the same groups 24 hour after dexamethasone administration.

Keywords: dexamethasone, fetal Doppler velocimetry, antenatal administration

Introduction

Preterm birth is the delivery of a baby before 37 completed weeks’ gestation. Most mortality and morbidity affects “very preterm” infants (those born before 32 weeks’ gestation), and especially “extremely preterm” infants (those born before 28 weeks of gestation) (¹). Doppler assessment of the placental circulation plays an important role in screening for impaired placentation and its complications of pre-eclampsia, intrauterine growth restriction and perinatal death (²). Assessment of the fetal circulation is essential in the better understanding of the pathophysiology of a wide range of pathological pregnancies and their clinical management (³). Doppler ultrasound provides a non-invasive method for the study of fetal hemodynamics. Investigation of the uterine and umbilical arteries gives information on the perfusion of the uteroplacental and fetoplacental circulations, respectively, while Doppler studies of selected fetal organs are valuable in detecting the hemodynamic rearrangements that occur in response to fetal hypoxemia (⁴). Doppler velocimetry is used to assess small-for-gestational-age (SGA) fetuses at risk of adverse perinatal outcome. Doppler abnormalities in the umbilical artery (UA) are related closely to placental disease. On the other hand, changes in
the fetal middle cerebral artery (MCA) reflect fetal cardiovascular adaptations to hypoxia or blood flow redistribution\(^5\). Thus, decreased pulsatility index (PI) has been considered a compensatory phenomenon to protect the fetal brain in the context of intrapartum growth restriction (IUGR). Poor uterine artery blood flow is associated with biochemical and cellular evidence of impaired placental development and function\(^6\). Uterine artery Doppler assessment appears effective in screening populations at high-risk for preeclampsia.\(^1\) Poor uterine artery blood flow is also associated with adverse pregnancy outcome from other placental syndromes such as fetal growth restriction, abruption, and stillbirth\(^7\).

Maternal administration of synthetic corticosteroids (dexamethasone), by accelerating the maturity of the fetal lung, reduces neonatal mortality, respiratory distress syndrome, intraventricular hemorrhage and necrotising enterocolitis in preterm infants. Previous studies have shown that steroids have an effect on fetal behaviour and fetal heart rate variability\(^8,9\). In fact, conflicting results concerning the effects of betamethasone and dexamethasone on fetal heart pattern have been reported. Dexamethasone was associated with an increase in long term and short term variability and decreased fetal movements on the first day after steroid administration followed by a decline in fetal heart rate variability on the second day \(^10\). Different studies found that betamethasone treatment is associated with significant reduction of the middle cerebral pulsatility index, especially at gestation before 32 weeks\(^11-13\). The aim of this study was assess the change in fetal and uteroplacental circulation following antenatal dexamethasone administration to high-risk of preterm labor pregnant women.

**Patients and Methods**

Prospective study was conducted between January and May 2021 in Tikrit city at Salahadeen General Hospital /genecology and obstetrics department. The study included 30 pregnant women with high risk of preterm labor with gestational age from 24 to 34 weeks.

**Inclusion criteria :**

1) Women with singleton uncomplicated pregnancy.

2) Women with risk of preterm labor

3) Women with normal utero-placental vascular resistance at the time of initial scanning (umbilical artery flow-velocity waveforms values above the fifth centile according to the reference limits as published by Arduini and colleagues); and

**Exclusion criteria**

1- Women who were unfit for conservative management patient actually in labor and fetal demise.

2- women with infants with known major structural malformation, complicated pregnancy, preterm, rupture of the membranes (PROM), vaginal bleeding as in (placenta previa and abruption placentae), .

3- suspected chorioamnionitis

3- maternal medical conditions e.g. PET, autoimmune diseases, DM.

4- maternal obstetrical conditions e.g. Polyhydramnios and oligohydramnios

5- non-reassuring fetal wellbeing e.g. presence of fetal bradycardia (FHR<120bp) or tachycardia (FHR>160bp) detected by sonicaid.

Doses of 12 mg dexamethasone intramuscularly 12 hours apart

1-Standardized questionnaire: designed by researcher

2- Doppler Study were performed just before dexamethasone administration and will be repeated 24 h after completion of the dexamethasone course. Blood flow velocity waveforms were obtained from the umbilical artery, fetal middle cerebral artery (MCA), and maternal uterine arteries.
Eligible participants were evaluated through full history taking and detailed anatomical scan by level II sonographer before inclusion to confirm their gestational age and exclude any structural anomalies.

Each woman received the recommended course of corticosteroids to induce fetal lung maturity consisting of two doses of 12 mg dexamethasone (Dexamethazone 8 ml, Sigma Pharam, Egypt) intramuscularly 12 hours apart.

Doppler studies were performed just before dexamethasone administration and repeated 24 hours after completion of the dexamethasone course using a SonoAce X6 machine (Medison, Korea) with 3.75 MHz transabdominal probe. All patients underwent Doppler examination by 2 different level II sonographers.

Doppler examination was done with the fetus in a quiet state, in absent of fetal movements and fetal breathing movements. The angle of insonation was optimized to be as low as possible, never exceeding 45°. The sweep speed was 2.5 cm/s and the pulse repetition frequency ranged from 3.5 - 5.5 Khz. The Doppler spectrum was recorded during maternal voluntary apnea.

Blood flow velocity waveforms were obtained from the umbilical artery, fetal middle cerebral artery (MCA), fetal descending aorta and maternal uterine arteries. Spectral pulsed wave Doppler analysis was done after that; RI and PI were calculated for each vessel. The formulas used for PI and RI were PI = (S-D)/mean and RI = (S-D)/S respectively, when S is the peak Doppler frequency shift and D is the minimum. At least 5 uniform waves forms of the spectrum were recorded and analyzed. Blood flow velocity waveforms were recorded from the umbilical artery in the free floating mid-portion of the umbilical cord. Doppler signals registered from the fetal MCA in its proximal third. The MCA vessels were located with color Doppler ultrasound overlying the anterior wing of the sphenoid bone near the base of the skull. Doppler signals obtained from the uterine arteries in the region of the lower uterine segment. Insonation of the uterine artery was done at its crossover the iliac artery. Velocity waveforms from the fetal descending aorta were recorded at the lower thoracic level just above the diaphragm, keeping the angle of insonation of the Doppler beam below 45°.

**Results**

The study included 30 women with risk of preterm birth. We anticipated the risk of preterm birth on the basis of; preterm uterine contractions (n=17), placenta previa (n=9) and pre-eclampsia (n=4). The demographic data are presented in Table 4.1. The mean age of the study group was 28.4 ± 3.9 years. At the time of dexamethasone administration, mean gestational age was 30.6 ± 3.1 weeks.

<table>
<thead>
<tr>
<th>Table 1: Maternal and neonatal characteristics of the studied cases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal characteristics</strong></td>
</tr>
<tr>
<td>Age (years); mean ±SD</td>
</tr>
<tr>
<td>Parity; median (Range)</td>
</tr>
<tr>
<td>Gestational age (weeks+days)</td>
</tr>
<tr>
<td>At examination; mean ± SD (Range)</td>
</tr>
<tr>
<td>At delivery; mean ± SD (Range)</td>
</tr>
<tr>
<td><strong>Neonatal characteristics</strong></td>
</tr>
<tr>
<td>Birth weight (gm); mean ± SD</td>
</tr>
<tr>
<td>PCU recommended; n (%)</td>
</tr>
<tr>
<td>Apgar score (1 minutes) &lt; 7; n (%)</td>
</tr>
<tr>
<td>Apgar score (5 minutes) &lt; 7; n (%)</td>
</tr>
<tr>
<td>Gender (Female); n (%)</td>
</tr>
</tbody>
</table>
The study showed significant differences among studied women with risk of preterm uterine contractions, placenta previa and pre-eclampsia regarding the average of Umbilical artery pulsatility index, where the highest averages recorded in the three groups before dexamethasone administration as compared with the same groups 24 hour after dexamethasone administration (P<0.05), more details in Table 2 below.

### Table 2: Umbilical artery pulsatility index.

<table>
<thead>
<tr>
<th>Studied cases (Before and 24 after hours of Dexamethasone)</th>
<th>Umbilical artery pulsatility index.</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Threatened preterm labor (n:17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>1.24</td>
<td>0.23</td>
</tr>
<tr>
<td>24 hour after</td>
<td>1.02</td>
<td>0.16</td>
</tr>
<tr>
<td>Preeclampsia (n:8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>1.21</td>
<td>0.37</td>
</tr>
<tr>
<td>24 hour after</td>
<td>1.01</td>
<td>0.41</td>
</tr>
<tr>
<td>Placenta previa (n:5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>1.12</td>
<td>0.32</td>
</tr>
<tr>
<td>24 hour after</td>
<td>1.01</td>
<td>0.31</td>
</tr>
</tbody>
</table>

The study showed significant differences among studied women with risk of preterm uterine contractions, placenta previa and pre-eclampsia regarding the average of Umbilical artery resistive index, where the highest averages recorded in the three groups before dexamethasone administration as compared with the same groups 24 hour after dexamethasone administration (P<0.05), Table 3.

### Table 3: Umbilical artery resistive index

<table>
<thead>
<tr>
<th>Studied cases (Before and 24 after hours of Dexamethasone)</th>
<th>Umbilical artery resistive index</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Threatened preterm labor (n:17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.66</td>
<td>0.21</td>
</tr>
<tr>
<td>24 hour after</td>
<td>0.62</td>
<td>0.17</td>
</tr>
<tr>
<td>Preeclampsia (n:8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.71</td>
<td>0.13</td>
</tr>
<tr>
<td>24 hour after</td>
<td>0.68</td>
<td>0.11</td>
</tr>
<tr>
<td>Placenta previa (n:5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.65</td>
<td>0.14</td>
</tr>
<tr>
<td>24 hour after</td>
<td>0.61</td>
<td>0.21</td>
</tr>
</tbody>
</table>
The study showed the highest means of Fetal MCA pulsatility index levels were recorded in women who at risk of preterm uterine contractions, placenta previa and pre-eclampsia respectively before dexamethasone administration as compared with the same groups at 24 hour after dexamethasone administration (P<0.05), more details in Table 4 below.

### Table 4: Fetal MCA pulsatility index.

<table>
<thead>
<tr>
<th>Studied cases (Before and 24 after hours of Dexamethasone)</th>
<th>Fetal MCA pulsatility index.</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Threatened preterm labor (n:17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.21</td>
<td>0.71</td>
</tr>
<tr>
<td>24 hour after</td>
<td>2.17</td>
<td>0.51</td>
</tr>
<tr>
<td>Preeclampsia (n:8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.18</td>
<td>0.54</td>
</tr>
<tr>
<td>24 hour after</td>
<td>2.15</td>
<td>0.53</td>
</tr>
<tr>
<td>Placenta previa (n:5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.21</td>
<td>0.13</td>
</tr>
<tr>
<td>24 hour after</td>
<td>2.01</td>
<td>0.26</td>
</tr>
</tbody>
</table>

MCA: middle cerebral artery

The study showed the highest means of Fetal MCA resistive index levels were recorded in women with preterm uterine contractions, placenta previa and pre-eclampsia respectively before dexamethasone administration as compared with the same groups 24 hour after dexamethasone administration (P<0.05), more details in Table 5 below.

### Table 5: Fetal MCA resistive index

<table>
<thead>
<tr>
<th>Studied cases (Before and 24 after hours of Dexamethasone)</th>
<th>Fetal MCA resistive index</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Threatened preterm labor (n:17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.91</td>
<td>0.45</td>
</tr>
<tr>
<td>24 hour after</td>
<td>0.85</td>
<td>0.43</td>
</tr>
<tr>
<td>Preeclampsia (n:8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.88</td>
<td>0.34</td>
</tr>
<tr>
<td>24 hour after</td>
<td>0.82</td>
<td>0.55</td>
</tr>
<tr>
<td>Placenta previa (n:5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.86</td>
<td>0.25</td>
</tr>
<tr>
<td>24 hour after</td>
<td>0.80</td>
<td>0.31</td>
</tr>
</tbody>
</table>
The study showed the highest means of Uterine artery pulsitility index levels were observed in women with preterm uterine contractions, placenta previa and pre-eclampsia respectively before dexamethasone administration as compared with the same groups 24 hour after dexamethasone administration (P<0.05), more details in Table 6 below.

### Table 6: Uterine artery pulsitility index.

<table>
<thead>
<tr>
<th>Studied cases (Before and 24 after hours of Dexamethasone)</th>
<th>Uterine artery pulsitility index.</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Threatened preterm labor (n:17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.96</td>
<td>0.45</td>
</tr>
<tr>
<td>24 hour after</td>
<td>0.87</td>
<td>0.43</td>
</tr>
<tr>
<td>Preeclampsia (n:8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.94</td>
<td>0.34</td>
</tr>
<tr>
<td>24 hour after</td>
<td>0.86</td>
<td>0.55</td>
</tr>
<tr>
<td>Placenta previa (n:5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.90</td>
<td>0.25</td>
</tr>
<tr>
<td>24 hour after</td>
<td>0.85</td>
<td>0.23</td>
</tr>
</tbody>
</table>

The study showed significant differences among studied women with risk of of preterm uterine contractions, placenta previa and pre-eclampsia regarding the average of Uterine artery resistive index, before and 24 hour after dexamethasone administration (P>0.05), Table 4.7.

**Discussion**

Fetal biophysical profile and Doppler ultrasonography have been used to assess fetal well-being in utero. Studies have reported transient reduction of fetal body movements, fetal breathing and fetal heart rate variation after antenatal betamethasone administration (1,2). Likewise, Doppler velocimetry of the umbilical (UA) and middle cerebral arteries (MCA) has also been found to be affected by corticosteroid administration. Prior studies revealed that the maternal administration of betamethasone was associated with a transient decrease in the pulsatility index (PI) (3-5). However, the mentioned studies had methodological limitations in some aspects. First, precise timing of the onset of the steroid effect was not determined due to the retrospective review of pregnancies (6,7). Second, other components of fetomaternal vasculature such as ductus venosus and uterine arteries were not assessed in a majority of the studies (8-11). The study included 30 women at risk of preterm birth. We anticipated the risk of preterm birth on the basis of; preterm uterine contractions (n=17), placenta previa (n=8) and pre-eclampsia (n=45). In this study, dexamethasone administration was found to have a beneficial effect on fetuses at risk for preterm birth as evident by the decrease in the Doppler indices of umbilical artery, MCA and uterine artery. Umbilical artery Doppler indices showed statistically significant reduction 24 hours after dexamethasone administration. Our results agreed with Senat et al (12) study that reported an association between betamethasone treatment and decreased placental vascular resistance as reflected by waveforms obtained from umbilical artery. This is similarly agreed by Nozaki et al (13) who found a reduction in the umbilical artery PI within 24 hours following antenatal corticosteroid therapy. MCA Doppler indices decreased after the treatment. These findings are in agreement with the results of Chitrit et al (14) who observed a transient and significant decrease in fetal MCA (PI, RI) after maternal dexamethasone administration. However some studies disagree with
our results as Miracle et al (15) and Wijnberger et al (16), they were on growth-restricted preterm fetuses; no effect was found from betamethasone on PI in fetal MCA. In the latter study, circulation in fetuses was studied for up to 14 days, indicating that placental insufficiency was probably not severe enough to indicate early delivery(17). Moreover, Piazze et al (18) examined the effect of steroids on blood flow waveforms in IUGR fetuses and found no significant changes of PI, RI values in the different vessels after dexamethasone course. There was statistical significant reduction in the uterine artery Doppler indices before and 24 hours after dexamethasone administration in the present study. These findings are in agreement with data published by Chitrit et al who observed similar transient and significant decrease in uterine artery PI and RI after maternal dexamethasone administration in healthy fetus(19). However, other studies reported that there was no significant influence of betamethasone therapy on Doppler indices in uterine circulation in pregnancies with imminent preterm delivery(20,21). A study on human placentas by some authors showed that the mechanism behind dexamethasone-induced vasodilatation might be an endothelium independent mechanism, as they did not find any involvement of endothelium-derived products such as prostaglandin I and nitric oxide(22,23). In concordance with our findings, a previous retrospective cohort study showed that corticosteroids administration was associated with significant change in umbilical artery PI and a transient return of end-diastolic umbilical artery flow(24). Another study showed that maternal antenatal corticosteroids resulted in a significant transient change in the velocity waveform and a decrease in the PI in the umbilical artery and ductus venosus(25). Similarly, Elnsosy and colleagues(26), concluded that maternal dexamethasone administration to pregnant women at risk of preterm improves the blood flow of umbilical artery and MCA. In addition, a previous report showed that the MCA PI showed a trend to decrease 24–48 h and 4–7 days after steroids were given to the mother when compared to pretreatment values(27). In contrary, Yvon Chitrit and colleagues(17) showed that no significant change was documented on days 2 and 7 in umbilical artery PI after dexamethasone administration. Additionally, Salama et al (28) reported no significant variations were observed in the umbilical artery PI throughout dexamethasone therapy. In addition, a previous report showed no significant effects of corticosteroids were observed in the uteroplacental circulation(29). The exact causes of such discrepancies between our findings and the abovementioned studies is unclear, however, it can be attributed to many factors. Firstly, the dose and type of antenatal corticosteroids were different between our study and those reports; for example, Salama and colleagues administered 24 mg of dexamethasone intramuscularly in three divided doses 8 h apart), while Müller and colleagues administered betamethasone. Secondly, the sample size varied greatly among the abovementioned studies which may have attributed for such heterogeneity. The characteristics of the included women were apparently different which can be considered as another factor.

**Conclusion**

The administration of dexamethasone to pregnant women at risk of preterm birth could improve the blood flow in the uterine arteries, fetal MCA, and umbilical artery 24 hours after its administration.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** “All experimental protocols were approved under the Diyala Health directorate and carried out in accordance with approved guidelines”.

**References**


Advanced Glycation End Products and Malondialdehyde Serum Level Association with Nephropathy in Type Two Diabetic Patients

Alaa Abdullah Hassan¹, Sami A. Zbaar², Khedhair Abbas Khedhair²

¹M. Sc. Medical Biochemistry Stud, Erbil Health Directorate, Iraq.²Assistant Prof. Dr. Medical Biochemistry, College of Medicine, Tikrit University, Iraq

Abstract

Aim of the Study: Evaluate the relation of serum level of AGEs and MDA with the development of diabetic nephropathy in patient with type two diabetes mellitus and to evaluate the possible mechanism by which AGEs and MDA induce nephrotic complication in type two diabetic patients.

Patients and Methods: This case-control study was carried out in Erbil city from the period started from 1st of December 2020 to the end of March 2021. The study included 62 type 2 diabetic patients (31 patients nephropathy and 31 patients T2DM without nephropathy), their age between 40–69 years, these patients who attended Rizgari General Hospital. The study also included 28 healthy control individuals defended as subjects who apparently haven’t any chronic diseases. Blood was collected from each for determination of HbA1c level, AGE3 and MDA and RBS.

Results: The study revealed that, the highest mean of RBS and HbA1c was recorded in DN group followed by diabetic non-nephropathy and the control group. The study revealed that, the highest mean of UACR was recorded in DN group (258.6±28.56 mg/gm) followed by diabetic non-nephropathy (35.6±11.95 mg/gm) and the control group (20.39±1.72 mg/gm) (P<0.001). The study showed a significant positive of AGEs and MDA with each of duration of DM, blood sugar, HbA1c and UACR.

Conclusions: This study showed elevated of AGEs, MDA and Poor glycemic control were associated with diabetic nephropathy.

Keywords: AGES; MDA; Diabetic nephropathy; Type 2 Diabetes; HbA1c

Introduction

Diabetic nephropathy is the most common cause of end-stage renal disease in the world, and could account for disability and high mortality rate in patients with diabetes. According to the World Health Organization, it is expected that the number of patients with diabetes will rise to 370 million by 2030 in the world. Diabetic kidney disease is a major cause of morbidity and mortality in diabetes(1). Diabetic kidney disease is the single most common cause of ESRD in many parts of the world including Europe, Japan, and the United States, with diabetic patients accounting for 25% to 45% of all patients enrolled in ESRD programs (2,3). Reducing sugars can react non-enzymatically with the amino groups of proteins to form reversible Schiff bases, and then Amadori products(4,5). These early glycation products undergo further complex reactions such as rearrangement, dehydration and condensation to become irreversibly cross-linked, heterogeneous fluorescent derivatives termed AGEs(6). The formation and accumulation of AGEs have been reported to progress at an accelerated rate under diabetes. The pathological role
of the nonenzymatic glycation of proteins has become increasingly evident in various disorders including diabetic vascular complications\(^7\). Proteins are also susceptible to post-translational modifications that could alter their structure, function, and half-life during normal aging and pathological conditions such as diabetes. One such post-translational modification is non-enzymatic glycation (NEG, also called non-enzymatic glycosylation). NEG of client proteins ensue crosslinking and aggregation that may adversely affect the structural and functional properties of proteins\(^8\). Although NEG occurs at a lower rate over a lifetime, it occurs more rapidly in clinical conditions such as diabetes and is implicated in the pathophysiology of several complications of diabetes and other age-related disorders such as cataract, renal failure, cardiovascular complications, and Alzheimer’s disease. Oxidative stress has been defined as a loss of balance between reactive oxygen species (ROS) and protective antioxidant defense system. Increased oxidative stress induced by hyperglycemia may be due to multiple\(^9,10\). Experimental and clinical studies suggest an association between hyperglycaemia, malondialdehyde (MDA), and diabetic complications\(^8\). Malondialdehyde plays an important role in the pathogenesis of DN and its progression to end-stage renal disease (ESRD)\(^11\). The aim of this study was to evaluate the relation of serum level of AGEs and MDA with the development of diabetic nephropathy in patient with type two diabetes mellitus and to evaluate the possible mechanism by which AGEs and MDA induce nephrotic complication in type two diabetic patients.

**Patients and Methods**

This case-control study was carried out in Erbil city from the period started from 1\(^{st}\) of December 2020 to the end of March 2021. The study included 62 type 2 diabetic patients (31 patients nephropathy and 31 patients T2DM without nephropathy), their age between 40–69 years, these patients who attended Rizgari General Hospital. We collated all baseline clinical parameters including gender, age, body mass index, duration of DM, level of hemoglobin A1c, blood sugar, urine creatinine, urine albumin. The study also included 28 healthy control individuals defended as subjects who apparently haven’t any chronic diseases.

Urine have been collected from each subject in the study by using disposable urine cup for measuring urinary albumin / creatinine ratio UACR by testing micro-albumin and creatinine in urine (manual biochemical kits ) . Five ml of venous blood was collected from each subject by using sterile disposable syringe and transferred into two tubes, 1 ml added to EDTA tubes for determination of HbA1c level by immunofluorescence technique (i-chroma II ), the 2\(^{nd}\) part was 4 ml which transferred to sterile gell tubes, left to clot at room temperature for 20 minutes , then centrifuged at 3000 rpm for 15 minutes , sera were then removed and added in eppendorf tubes and stored at -30ºC for determination of AGE3 and MDA by using ELISA technique, RBS (manual biochemical kit ).

**Results**

The study showed non-significant difference among the studied group regarding their age (P>0.05), and Diabetic nephropathy group showed high BMI as compared with Diabetic non-Nephropathy and the control groups (P<0.05), Table 1
Table 1: Comparison of age and BMI among the studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Studied groups</th>
<th>No.</th>
<th>Mean±SD</th>
<th>ANOVA</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>Diabetic nephropathy</td>
<td>31</td>
<td>51.22±5.45</td>
<td>3.14</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>Diabetic non-Nephropathy</td>
<td>31</td>
<td>51.13±6.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>28</td>
<td>51.51±5.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>Diabetic nephropathy</td>
<td>31</td>
<td>29.56±4.22</td>
<td>14.56</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Diabetic non-Nephropathy</td>
<td>31</td>
<td>28.13±4.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>28</td>
<td>24.21±3.76</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P<0.05: Significant and ≤0.05 mean significant

The study showed the mean of AGEs was elevated significantly in diabetic nephropathy (DN) group (625.3±177.4 ng/L) compared with diabetic non-nephropathy (397.26±53.34 ng/L) and the control group (230.4±57.5 ng/L) (P<0.001), Table 2.

Table 2: Levels of AGEs in Diabetic patients (with and without nephropathy) and the control group

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>AGEs (ng/L)</th>
<th>ANOVA</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.  Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>31  625.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic non-Nephropathy</td>
<td>31  397.26</td>
<td>90.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control group</td>
<td>28  230.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The study showed the mean of MDA was elevated significantly in DN group (126.5±10.5 nmol/ml) compared with diabetic non-nephropathy (102.1±17.2 nmol/ml) and the control group (67.5±22.3 nmol/ml) (P<0.001), Table 3.
Table 3: Levels of MDA in Diabetic patients (with and without nephropathy) and the control group

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>MDA (nmol/ml)</th>
<th>ANOVA</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>31</td>
<td>126.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Diabetic non-Nephropathy</td>
<td>31</td>
<td>102.1</td>
<td>17.2</td>
</tr>
<tr>
<td>Control group</td>
<td>28</td>
<td>67.5</td>
<td>22.3</td>
</tr>
</tbody>
</table>

The study showed a significant positive correlation between AGEs and MDA in diabetic nephropathy (r: 0.78, P<0.01), which is mean that AGEs levels is elevated proportionally with the elevation of MDA in diabetic nephropathy.

The study showed a significant difference between patients with and without nephropathy regarding duration of diabetes (P<0.001), and the highest mean was recorded in DN patients (10.17±2.21 year) as compared with patients without nephropathy (5.21±2.11 year), Table 4.

Table 4: Comparison between patients with and without nephropathy regarding duration of diabetes

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>Duration of DM (year)</th>
<th>T. Test</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>31</td>
<td>10.17</td>
<td>2.21</td>
</tr>
<tr>
<td>Diabetic non-Nephropathy</td>
<td>31</td>
<td>5.21</td>
<td>2.11</td>
</tr>
</tbody>
</table>

The study showed the mean of RBS was elevated significantly in DN group (261.87±45.64 mg/dl) compared with diabetic non-nephropathy (235.2±75.1 mg/dl) and the control group (101.29±10.6 nmol/ml) (P<0.001), Table 5.

Table 5: Levels of RBS in diabetic patients (with and without nephropathy) and the control group

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>RBS (mg/dl)</th>
<th>ANOVA</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>31</td>
<td>261.87</td>
<td>45.64</td>
</tr>
<tr>
<td>Diabetic non-Nephropathy</td>
<td>31</td>
<td>235.2</td>
<td>75.1</td>
</tr>
<tr>
<td>Control group</td>
<td>28</td>
<td>101.29</td>
<td>10.6</td>
</tr>
</tbody>
</table>
The study revealed that, the highest mean of HbA1c was recorded in DN group (13.68±0.72%) compared with diabetic non-nephropathy (847±2.13%) and the control group (4.91±0.41%) (P<0.001), Table 6.

Table 6: Levels of HbA1c in diabetic patients (with and without nephropathy) and the control group

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>HbA1c (%)</th>
<th>ANOVA</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>31</td>
<td>13.68</td>
<td>0.72</td>
</tr>
<tr>
<td>Diabetic non-Nephropathy</td>
<td>31</td>
<td>8.47</td>
<td>2.13</td>
</tr>
<tr>
<td>Control group</td>
<td>28</td>
<td>4.91</td>
<td>0.41</td>
</tr>
</tbody>
</table>

The study revealed that, the highest mean of UACR was recorded in DN group (258.6±28.56 mg/gm) compared with diabetic non-nephropathy (35.6±11.95 mg/gm) and the control group (20.39±1.72 mg/gm) (P<0.001), Table 7.

Discussion

The study showed the mean of AGEs was elevated significantly in diabetic nephropathy (DN) group followed by diabetic non-nephropathy and the control group. Recent studies have suggested that advanced glycation end-products (AGEs) may be a key factor in the development of metabolic memory in diabetic complications, because AGEs are produced and accumulated irreversibly in the body, depending on the degree of blood sugar regulation and duration (5-7). Another study done by Nenna et al (8) found that, AGEs was elevated significantly in DN patients comparing with the control group and suggested that, AGEs may contribute to the development of diabetic complications. Pasupulati et al (9) found that, patients with nephropathy showed significantly increased AGEs activity compared to DM patients as well as controls and reported that a highly significant correlation exists between an accumulation of AGEs on collagen and the severity of diabetic complications. Several other studies reported an elevation in AGEs levels in DN patients and suggested that AGEs may play an important etiological role in the development of diabetic complications (10,11). The study showed the mean of MDA was elevated significantly in DN group. In agreement with our finding, Mistry et al (5) found that, patients with nephropathy showed significantly increased MDA activity compared to DM patients as well as controls. Moreover, one study showed that there was a significant increase in MDA concentrations in patients with DN compared with both normal subjects and patients with T2DM (10,11). Additionally, several recent studies observed that, Oxidative stress markers like MDA is suggested to be an essential factor in the development and progression of DM as well as in the development of diabetic complications like DN (12-14). Moreover, Sifuentes-Franco et al (15) found that, investigated the status of markers of oxidative stress in DM and reported a significant elevated level of oxidative stress markers in DN group. Singh et al (16) revealed that, levels of MDA was elevated significantly in DN individuals as compared to controls. Different other studies were also found and increase in production of MDA level in the diabetic nephropathy and suggested that, feature of oxidative stress is one of intrinsic factors of diabetic complications (17-19).
without nephropathy regarding duration of diabetes (P<0.001). These findings were close to that reported Shahwan et al (90) showed that a significant difference was observed in the mean duration of diabetes (9.5 ± 1.7 vs. 5.9 ±1.5 years, (P<0.05). In agreement with our findings, Mistry et al (5) showed that, blood sugar glycosylated haemoglobin (HbA1c) in DN patients and in NDN were significantly (P< 0.05) higher than control subjects. Several studies showed that poor glycemic control as measured by HbA1c and blood sugar were strong predictor of nephropathy16-18. Other studies clearly demonstrated the benefits of improving glycemic control and decreasing HbA1c concentration in decreasing the complication rate of T2D (9,14,19). It has been hypothesized that the mechanism by which high glycaemic variability favours diabetic complications may be an increase in oxidative stress, as oxidative stress has been reported to correlate with glycemic variability as reflected by continuous glucose measurements among patients with Type 2 diabetes (20). The study revealed that, the highest mean of UACR was recorded in DN group (258.6±28.56 mg/gm) followed by diabetic non-nephropathy (35.6±11.95 mg/gm) and the control group (20.39±1.72 mg/gm) (P<0.001). Other studies which reported that elevated UACR is one of the major markers of progressive renal damage as complication of diabetes mellitus (21,22). An other study showed that, an increase in urea level is seen when there is damage to the kidney. Increase in blood urea level in the presence of high blood sugar level in diabetic patient indicates damage to the kidney (20). Study conducted by Rohitash et al (23) had found that increase urea and serum creatinine in diabetic rats indicates progressive renal damage. In agreement with our finding, Makita et al (24) found a significant association between serum levels of AGE peptides with duration of DM glycemic control in diabetic patients by analyzing the results from all the diabetic patients. Increased AGE levels have been associated with elevation in renal function tests and referred to renal damage as diabetic complications nephropathy (25). The study showed a significant positive correlation of MDA with blood sugar and HbA1c in diabetic nephropathy patients. Krishnamurthy et al (26) concluded that the inflammatory markers and oxidative stress are raised with elevation in levels of blood sugar and HbA1c in patients who have diabetic nephropathy because of hyperglycemia induced oxidative stress. This indicates that MDA influenced glycemic control in T2DM. In T2DM, the glycaemic control improves, the oxidative stress will partially decrease. The result as well showed that MDA when compared to FBS and HbA1c correlated positively, thus, as fasting blood sugar increases, MDA and HbA1c increase as well. There is growing evidence that more synthesis of highly reactive free radicals, due to hyperglycaemia causes oxidative stress, which further exacerbates the development and progression of type 2 diabetes and its complications (27,28).

Conclusions

This study showed elevated of AGEs, MDA and Poor glycemic control were associated with diabetic nephropathy.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the Erbil Health Directorate and carried out in accordance with approved guidelines”.

References


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Effect of Sperm Morphology on Normal Pregnancy

Samraa Maki Hamed¹, Yusra Salih Khudair²

¹M.B.Ch.B. HD.G.O Stud. Diyala Health Directorate, Iraq. ²Assistant Prof Dr. Obstetric and Gynecology, College of Medicine, Tikrit University, Iraq

Abstract

Background: Sperm morphology has been demonstrated to be a better predictor of fertilization potential than other semen analysis parameter.

Aim: The aim of this study is to determine the influence of the values of sperm morphology in men with normal sperm concentrations, according to the World Health Organization criteria, on the normal pregnancy.

Patients and Methods: A Prospective study conducted in Salah Al-Din general hospital from January to May 2021. The study included 60 pairs of infertile couples and recorded male partner sperm morphology. Based on the sperm morphology of the semen sample collected for the initial evaluation, patients were divided into three groups: Group A (n:20): patients with sperm morphology values between 0% and 4%. Group B (n:20): between 5% and 14%. Group C (n:20): patients with sperm morphology greater than 14%. A study was excluded when mean female age was less than 25 or greater than 40 years to avoid confounding due to extremes in maternal age.

Results: The study revealed that 35% of infertile men (group A) were smoker and compared with 20% of group B and 15% of group C, although the result was non-significant. The study showed that the mean of duration of infertility in group A was 45 year. The highest rate of infertile patients in the study (35%) was with duration of infertility for ≥5 year while only 10% was suffered from infertility less than one year, the study showed no significant differences among the studied groups regarding semen volume, sperm concentration, pH, rate of motility and WBCs cells. The study demonstrated that only 5% (1 of 20) of women who their husbands with abnormal sperm morphology (Group A) succeed to be pregnant normally during the period of the study compared with 30% of the group B and 65% of group C.

Keywords: Sperm morphology; Infertility; Pregnancy rate; Seminal fluid

Introduction

For years, sperm morphology has been a debated indicator of male fertility and success with assisted reproductive technologies (ARTs). While subfertile men have a lower percentage of normal forms when compared with men with proven fertility, the question of “Does form impact function?” remains. While an assessment of sperm morphology is a component of the standard semen analysis, the clinical utility of this is debated⁴. Despite routine application, semen analysis does not provide a direct measure of functional capacity of sperm⁵. No single semen parameter definitely predicts the potential of an individual couple to achieve success with assisted reproduction, however the percentage of sperm with normal morphology is positively correlated with In vitro fertilization (IVF) and pregnancy rates⁶. The 2010 WHO manual defines a morphologically “normal” sperm as having a head (with acrosome), midpiece, and tail. Specifically, a “normal” head has an oval shape with smooth contours⁷. The acrosome
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is clearly visible, well-defined, exhibits a homogenous light-blue staining, and covers 30–60% of the anterior portion of the sperm head. A “normal” midpiece lacks cytoplasmic residues and is axially attached to the head, without forming a definite angle with respect to the head, ≤ 1 μm in width and approximately 1.5 times the head length. The tail should also lack cytoplasmic residues, be apically inserted to the post-acrosomal end of the midpiece, have a length of approximately 45–50 μm long, and be lacking any sharp bends (1). Sperm should be analyzed after being stained via a modified Papanicolaou method. The analyzer should assess at least 200 spermatozoa per sample (6). However, the overall appearance of a sperm preparation does not always reflect the quality of the sperm cells injected into the oocyte. The number of cycles in which individual sperm morphology has been described remains limited (6). In addition, individual sperm morphology has not been evaluated when epididymal or testicular sperm cells were injected or when morphologically abnormal spermatozoa were used for microinjection (in most cases because no other sperm cells were available) (7). The aim of this study is to determine the influence of the values of sperm morphology in men with normal sperm concentrations, according to the World Health Organization criteria, on the normal pregnancy.

Patients and Methods

A Prospective study conducted in Salah Al-Din general hospital from January to May 2021. The study included 60 pairs of infertile couples and recorded male partner sperm morphology. Based on the sperm morphology of the semen sample collected for the initial evaluation, patients were divided into three groups:

1- Group A (n:20): patients with sperm morphology values between 0% and 4%.

2- Group B(n:20): between 5% and 14%.

3- Group C(n:20): patients with sperm morphology greater than 14%.

Group C was considered the control group, and groups A and B were compared to group C. All couples had been trying unsuccessfully to conceive for at least 1 year before being enrolled in the trial.

A study was excluded when mean female age was less than 25 or greater than 40 years to avoid confounding due to extremes in maternal age, and when mean TMC was less than 10 million since prior studies have demonstrated no difference in IUI pregnancy rates after semen samples exceed 9 million TMC. We excluded all studies that reported clinical pregnancies not verified by ultrasound, chemical pregnancies (a positive test for β-human chorionic gonadotropin that did not correlate with clinical or ultrasound findings) or live births that did not provide a count of pregnancies that underwent demise prior to delivery.

Before enrollment, each couple underwent assessment for infertility, by Careful history and physical examination, age of female partner, duration and type of infertility, details of menstrual history, previous pregnancies and miscarriages, illnesses, smoking, drugs, surgery, infertility investigations and/or treatments including previous IUI and/or IVF. It is also important to ask about sexual history including coital frequency and any history of contraception. Written informed consent was obtained from each patient.

The semen sample of the husband was collected by masturbation after 3 - 5 days of sexual abstinence into a clean, dry, wide mouthed container in a private room near the laboratory. The container was labeled with the name of patient and his wife’s’ name, age, time of sample collection and abstinence period. The specimens were kept in an incubator at 37 C° till complete liquefaction. Semen samples were analyzed depending on the guidelines of WHO (2010). Lower reference limits for semen characteristics according to WHO- 2010 criteria.
Results

The present study showed non-significant difference between the studied groups regarding their, Table 1.

Table 1: Distribution of infertile patients in the study according to age

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>21-25</td>
<td>3</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>26-30</td>
<td>7</td>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>31-35</td>
<td>5</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>36-40</td>
<td>3</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>&gt;40</td>
<td>2</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>30.5±3.3</td>
<td></td>
<td>31.6±4.1</td>
</tr>
</tbody>
</table>

P> 0.05

The study revealed that 35% of infertile men (group A) were smoker and compared with 20% of group B and 15% of group C, although the result was non-significant, Figure 1.

Figure 1: Relation of smoking with male infertility
The study showed that the mean of duration of infertility in group A was 4.5 year. The highest rate of infertile patients in the study (35%) was with duration of infertility for ≥5 year while only 10% was suffered from infertility less than one year, Table 2.

Table 2: Duration of infertility of studied patients

<table>
<thead>
<tr>
<th>Duration of infertility</th>
<th>No.</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>1-2</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>3-4</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>≥5</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean duration: 4.5 years

In Table below the main seminal parameters, the study showed no significant differences among the studied groups regarding semen volume, sperm concentration, pH, rate of motility and WBCs cells (P>0.05).

Table 3: Semen parameters in the studied groups

<table>
<thead>
<tr>
<th>Seminal Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume ml</td>
<td>3.1±1.9</td>
<td>3.3±1.2</td>
<td>3.2±1.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>pH</td>
<td>7.31±0.4</td>
<td>7.28±0.3</td>
<td>7.64±0.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Sperm concentration (10⁶ / ml)</td>
<td>24.5±3.6</td>
<td>25.3±4.5</td>
<td>25.8±4.6</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Total sperm No (10⁶/ ejaculate)</td>
<td>52.5±6.8</td>
<td>55.4±8.1</td>
<td>54.6±7.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Total sperm motility (PR+NP%)</td>
<td>47.6±6.3</td>
<td>48.5±5.4</td>
<td>49.1±5.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Progressive motility (PR%)</td>
<td>35.6±3.6</td>
<td>36.4±3.4</td>
<td>35.8±4.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>WBCs ×10⁹/ml</td>
<td>2.41±1.7</td>
<td>2.12±1.12</td>
<td>1.91±1.2</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
The study demonstrated that only 5% (1 of 20) of women who their husbands with abnormal sperm morphology (Group A) succeed to be pregnant normally during the period of the study compared with 30% of the group B and 65% of group C, Table 4.

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Succeed</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Failed</td>
<td>19</td>
<td>95</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>100</td>
<td>20</td>
</tr>
</tbody>
</table>

P<0.01

**Discussion**

The study revealed that 35% of infertile men (group A) were smoker and compared with 20% of group B and 15% of group C, although the result was non-significant, Figure 1. In a cross-sectional on a rural Chinese population, Yang et al. (1) analyzed the relation of male smoking with couple’s infertility. Their study included data from 7,025 couples and found that after adjusting for the confounding factors, the couples were more likely to suffer from infertility if the husband smoked before the first pregnancy. In-depth analysis showed that the risk started after a longer duration of 5-10 years of smoking. Among studies on large number of smokers, Meri et al. (2) analyzed 396 smokers and 546 non-smokers and found that smokers had poor sperm motility and a higher proportion of abnormal sperm and leukocytes. In similar finding, Sarhat et al. (3) in Thi-Qar Province found that, the rate of infertile patients was (33.85%) with duration of infertility for ≥5 year and 9.23% was suffered from infertility less than year with mean duration 4.9 year. The study demonstrated that only 5% (1 of 20) of women who their husbands with abnormal sperm morphology (Group A) succeed to be pregnant normally during the period of the study compared with 30% of the group B and 65% of group C. The importance of sperm morphology has been a topic of debate since the 1970s, and the controversy has increased since the World Health Organization (WHO) adopted the Kruger strict morphology classification system in 1999 (4). When reviewing semen analysis methodology, morphology is the parameter that has undergone the most scrutiny and change over the years. Starting with the McLeod classification and proceeding to the WHO second through fifth editions, the definition of normal sperm morphology has changed significantly (5,6).

Accordingly, the normal reference ranges have transformed from >60% to >50% to >30% to >14% normal forms, and now, according to the WHO fifth edition, >4% normal forms is considered to be within the 95th percentile reference range (7,8). The debate over the significance and utility of morphology is so split that in many forums there is no middle ground or gray area. There are some who believe sperm morphology is the single most important seminal parameter in
determining pregnancy rates(9-11).

Then there are others who believe that sperm morphology is so unreliable that it should be stricken as a component of the semen analysis altogether and should not be considered when counseling couples(12-14). The definition of morphology has changed so many times over the past several decades that it has become nearly impossible to meaningfully compare data from the literature across time(15,16). To complicate matters even more, the interobserver variability in determining normal morphology and the non-standardization of techniques in many labs has resulted in literature that is very heterogeneous and difficult to critique(17-19). Nonetheless, several studies stand out as illuminating this contentious topic(20-24).

In 2006, Nallella et al. reported that a majority of proven fertile men had sperm morphology values in the abnormal range (25). After performing receiver operator characteristic curve analysis, the authors proposed a cutoff value of >11% for Tygerberg/strict morphology. Although their study did not address pregnancy rates in assisted reproduction, it did offer insight into the limitations of morphology and the significant overlap between fertile and infertile males. Karabinus et al. assessed morphology in the context of IUI outcomes, and they found no statistical difference in IUI pregnancy rates among patients stratified by morphology results(22). More specifically, they showed similar outcomes when sperm morphology was <5%, 5%–9%, 10%–19%, 20%–29%, or even R30%. These findings are in contrast to a study by Nikbakht et al. that reviewed 445 women undergoing 820 IUI cycles with controlled ovarian hyperstimulation (25). These authors reported an overall IUI clinical pregnancy rate of 9.9%, and they that found sperm morphology was significantly predictive of IUI success. The most profound differences were seen when comparing subgroups of sperm morphology <5% versus 5%–10% versus >10% (2.1% vs. 10.1% vs. 12.6% pregnancy rates, respectively). Sun et al. have also contributed to the morphology discourse (26). They evaluated IUI outcomes in patients with normal sperm concentration and motility, and they observed no difference in IUI success rates based on morphology in women younger than 35 years. They further reported, however, that IVF/intracytoplasmic sperm injection should be offered as a first-line therapy if normal morphology is <5% and female age is >35 years.

Conclusion

Morphology of sperms is a unique semen parameter, as it is not a direct measure of a specific aspect of sperm activity. Morphology might, however, act as a surrogate marker for other facets of sperm function. The key for investigators will be to determine those aspects of sperm function for which morphology serves as a surrogate.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the College of Medicine and carried out in accordance with approved guidelines”.

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23. Nallella KP, Sharma RK, Aziz N, Agarwal A. Significance of sperm characteristics in the evaluation of male infertility. Fertil Steril


Serum β HCG as a Predictor and Potent Marker for Pregnancy Induced Hypertension in Salah Aldeen General Hospital

Marwah Abdulrahman Ftaikhan¹, Musryia Rashad Hassein²
¹M.B.Ch.B. HD.G.O Stud. Al-Anbar Health Directorate, Iraq, ²Assistant Prof Dr. Obstetric and Gynecology, College of Medicine, Tikrit University, Iraq

Abstract

Aim: This study was conducted to predict gestational hypertension by using serum beta HCG and thereby to follow up the risk patients and to reduce both maternal and perinatal morbidity and mortality.

Patients and Methods: A prospective study was carried out in Tikrit city from the beginning of October 2020 to May 2021 and included 150 women, 25 were lost to follow-up. The rest 125 patients followed up in their second trimester (13-20) weeks, attending Salah Aldeen General Hospital. Routine antenatal investigations were done. All patients underwent thorough clinical and routine obstetrical examination. Venous blood sample was collected for determination of serum β HCG level.

Results: The study showed no significant difference between women with pregnancy induced hypertension (PIH) and Normal mothers regarding their age at the time of booking (P>0.05). The mean age of PIH women was 32.2±5.9 year and normal women was 32.2±6.2 year. Although 26.67% of PIH women were below 20 year of age compared with 12.73% of Normal women. The relationship between β−hCG (absolute) levels and PIH and the association between mean β−hCG level of PIH patients and Normotensives was statistically significant (p< 0.001) and as the level of β−HCG increases the PIH also increases. The study showed that, 66.67% of PIH women have severe elevation in hypertension and majority of women with severe PIH (90%) have Beta hCG level above 80000 mIU/ml and only 10% have level ≤80000 mIU/ml while 60% of women with mild PIH have level ≤80000 mIU/ml. The mean increase of SBP of PIH mothers was 40.1 mm/Hg.

Keywords: pregnancy, Serum β HCG, hypertension, Salah Aldeen General Hospital

Introduction

Hypertensive disorders of pregnancy complicate 5 - 10% of pregnancies worldwide and constitute one of the greatest causes of maternal morbidity and mortality and perinatal morbidity and mortality (1). In developed countries 16% of maternal deaths are attributed to hypertensive disorders. Hypertensive disorders are a frequently encountered complication of pregnancy and remain a major cause of maternal and perinatal morbidity and mortality (2). The Confidential Enquiry into Stillbirths and Deaths in Infancy report cites one in six stillbirths as occurring in pregnancies complicated by maternal hypertension. Hypertensive disorders vary from mild gestational hypertension to severe preeclampsia and have a number of possible etiologies (3). Several tests have been proposed but none has been accepted widely due to their low predictive value (4). The abnormal placentation has been considered as one of the initial events in the disease process hypothesized that during mid-trimester, immunological changes occur in the trophoblasts, resulting in a secretory response, which is seen as a rise in the beta Human chorionic gonadotropin (HCG) levels (5). Hypertensive disorders are also responsible for perinatal mortality and morbidity. Pre-eclampsia is a risk factor for stillbirth, Intrauterine growth restriction (IUGR), Low birth weight (LBW), Preterm delivery, Respiratory distress syndrome, and admission in the neonatal intensive care unit. Hypertensive disorders account for 8-10%
of all preterm births. A variety of biochemical and biophysical markers have been proposed for predicting the development of preeclampsia in pregnancy(6). Pregnancy Induced Hypertension, Gestational Hypertension or Transient Hypertension of pregnancy are terms used to describe new hypertension which appears after mid-term (20 weeks) and resolves within 10 days postpartum without other symptoms of pre-eclampsia in a previously normotensive woman(7). In pregnancy, the placenta forms especially large quantities of Human Chorionic Gonadotropin which is essential to a normal pregnancy and more so in Pregnancy Induced Hypertension. Elevated serum β-hCG in the second trimester has repeatedly been shown to be significantly associated with later PIH. The patients with abnormal β-hCG levels and in very few reports, the free β-subunit (free β-hCG) were reported as possible predictors of pre-eclampsia, pregnancy-induced hypertension, spontaneous miscarriage, low birth weight, preterm delivery and intrauterine growth retardation (IUGR) (8). This study was conducted to predict gestational hypertension by using serum beta HCG and thereby to follow up the risk patients and to reduce both maternal and perinatal morbidity and mortality.

Patients and Methods

A prospective study was carried out in Tikrit city from the beginning of October 2020 to May 2021. The study conducted to determine the role of β HCG in pregnant women, the included 150 women, 25 were lost to follow-up. The rest 125 patients followed up in their second trimester (13-20) weeks, attending Salah Aldeen General Hospital. Routine antenatal investigations were done.

Inclusion criteria

Pregnant women with:

- Singleton
- Gestational age 13-20 weeks as determined by last menstrual period or ultrasound scan.

Exclusion criteria

- Chronic hypertension.
- Molar Pregnancy.
- Diabetes mellitus.
- Anomalous foetus.
- Multiple pregnancies.

All patients were informed about the study and informed written consent was taken before they were enrolled in the study. At the time of enrollment, demographic details were noted, detailed obstetric and medical history was taken. Gestational age was calculated from reliable menstrual history dates and/or 1st trimester ultrasonographical measurement of fetal crown rump length. All patients underwent thorough clinical and routine obstetrical examination. Baseline blood pressure (average of 3 readings) using a sphygmomanometer was recorded using the auscultatory method. Routine antenatal tests were performed as required and indicated.

A routine antenatal investigation was done. 5 ml of venous blood sample was collected and tests were carried out. Estimation of serum β HCG level was done by immunofluorescence immunoassay as manufacture instructions. The cases were followed up in antenatal clinic and were examined 4 weekly till 28 weeks, fortnightly up to 34 weeks and thereafter weekly till delivery. At every visit. Serum β HCG as a predictor and potent marker for pregnancy induced hypertension. Blood pressure was recorded and urine was examined for albumin. PIH included gestational hypertension and preeclampsia. Gestational hypertension was defined as blood pressure 140/90 mmHg on two occasions at least 6 hours apart after 20 weeks of gestation. Preeclampsia was defined as gestational hypertension and proteinuria of at least 1
Severity of PIH

The severity of PIH is assessed by the extent of symptoms. Both blood pressure and proteinuria are dependable indicators of severity. (1)

v Mild PIH: Blood pressure is ≥140/90 mmHg but <160/110 mmHg after 20 weeks gestation, and proteinuria is ≥300 mg/24 hours without exceeding 2.0 g/24 hours or 3+ dipstick

v Severe PIH: Blood pressure is ≥160/110 mmHg, and proteinuria exceeds 2.0 g/24 hours or 3+ dipstick.

Data collection form

A structured format was prepared for collecting the demographic details, personal, obstetric and medical history, data related to all ANC visits, outcomes of serum β-hCG assessment, perinatal events and outcomes.

Results

In this study, 150 women completed the study, 25 were lost to follow-up. The rest 125 patients followed, 15 women developed PIH constituting 12% of the study population. Thus, the incidence of PIH was 12% in our study (Figure 1). When further subdivided, the incidence of preeclampsia was 20% of the PIH (n = 3). Of these 3 preeclamptic patients, 2 women had early-onset preeclampsia (onset before 34 weeks), whereas the rest 1 had late-onset disease. Most of the patients (83.33%) had late-onset PIH (after 34 weeks), whereas the incidence of early-onset PIH (before 34 weeks) was only 16.67% (Table 2).

Figure 1: Incidence of PIH
Table 1: Distribution of pregnancy-induced hypertension into pregnancy-induced hypertension and preeclampsia

<table>
<thead>
<tr>
<th>PIH women</th>
<th>PIH</th>
<th>early-onset (before 34 weeks)</th>
<th>Late Onset (&gt;34 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>3</td>
<td>20%</td>
<td>2</td>
</tr>
<tr>
<td>Continue PIH</td>
<td>12</td>
<td>80%</td>
<td>2</td>
</tr>
</tbody>
</table>

The study showed no significant difference between women with pregnancy induced hypertension (PIH) and Normal mothers regarding their age at the time of booking (P>0.05). The mean age of PIH women was 32.2±5.9 year and normal women was 32.2±6.2 year. Although 26.67% of PIH women were below 20 year of age compared with 12.73% of Normal women, Table 2.

Table 2: Comparison between pregnant women with induced hypertension and normal pregnant women regarding age

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Pregnancy Induced Hypertension</th>
<th>Normal women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>15-20</td>
<td>4</td>
<td>26.67</td>
</tr>
<tr>
<td>21-30</td>
<td>3</td>
<td>20.00</td>
</tr>
<tr>
<td>31-40</td>
<td>4</td>
<td>26.67</td>
</tr>
<tr>
<td>&gt;40</td>
<td>4</td>
<td>26.67</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean ± SD

P>0.05 (non-significant)

Table 3 shows the relationship between β−hCG (absolute) levels and PIH and the association between mean β−hCG level of PIH patients and Normotensives was statistically significant (p<0.001) and as the level of β−HCG increases the PIH also increases.
Table 3: Comparison between PIH and normal pregnant women regarding the level of serum hCG level

<table>
<thead>
<tr>
<th>Beta hCG level (mIU/ml)</th>
<th>PIH</th>
<th>Normal women</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td></td>
</tr>
<tr>
<td>&lt;30,000</td>
<td>0 0.0</td>
<td>12 9.60</td>
<td></td>
</tr>
<tr>
<td>30,000-40,000</td>
<td>0 0.0</td>
<td>40 32.00</td>
<td></td>
</tr>
<tr>
<td>40,001-50,000</td>
<td>1 6.7</td>
<td>41 32.80</td>
<td></td>
</tr>
<tr>
<td>50,001-60,000</td>
<td>2 13.3</td>
<td>23 18.40</td>
<td></td>
</tr>
<tr>
<td>60,001-70,000</td>
<td>1 6.7</td>
<td>5 4.00</td>
<td></td>
</tr>
<tr>
<td>70,001-80,000</td>
<td>0 0.0</td>
<td>4 3.20</td>
<td></td>
</tr>
<tr>
<td>80,001-90,000</td>
<td>4 26.7</td>
<td>0 0.00</td>
<td></td>
</tr>
<tr>
<td>90,001-1,00,000</td>
<td>3 20.0</td>
<td>0 0.00</td>
<td></td>
</tr>
<tr>
<td>&gt;1,00,000</td>
<td>4 26.7</td>
<td>0 0.00</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15 100.0</td>
<td>125 100</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD 96325.3±39757.5 41343.6±24737.5 <0.001

The study showed that, 10 of 15 (66.67%) of PIH women have severe elevation in hypertension (Figure 2). The study also showed that, majority of women with severe PIH (90%) have Beta hCG level above 80000 mIU/ml and only 10% have level ≤80000 mIU/ml while 60% of women with mild PIH have level ≤80000 mIU/ml , Table 4.

Figure 2: Rate of Severity of PIH

Table 4: Relation of beta hCG level with severity of PIH

<table>
<thead>
<tr>
<th>Beta hCG level (mIU/ml)</th>
<th>PIH women (n:15)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild</td>
<td>Severe</td>
</tr>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>≤80000</td>
<td>3 60.0</td>
<td>1 10.0</td>
</tr>
<tr>
<td>&gt;80000</td>
<td>2 40.0</td>
<td>9 90.0</td>
</tr>
<tr>
<td>Total</td>
<td>5 100</td>
<td>10 100</td>
</tr>
</tbody>
</table>

P<0.05
The mean increase of SBP of PIH mothers was 40.1 mm/Hg. The mean increase of SBP of Normal mothers was 9.3 mm/Hg. The difference between them was statistically very highly significant (P<0.001). Similarly, the DBP mean increase of PIH mothers was 24.3 mm/Hg. The mean increase of DBP of Normal mothers was 3.2 mm/Hg. The difference between them was statistically highly significant (P<0.001).

Figure 2: Comparison of increased SBP and DBP between PIH and normal mothers.

Table 5 shows the mode of delivery of the studied patients where cesarean was in majority of the cases (80%) whereas in normotensive patients cesarean was in 48.18% and the association was found to be statistically significant between hypertensive and normotensive patients (p< 0.05).

Table 5: Mode of Delivery in the studied groups

<table>
<thead>
<tr>
<th>Mode of delivery</th>
<th>PIH</th>
<th>Normal women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Vaginal</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>LSCS</td>
<td>12</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>100</td>
</tr>
</tbody>
</table>

P. value: 0.039
Discussion

Hypertension and proteinuria are important complications of pregnancy. Abnormal placentation is one of the important pathologies for the development of GHT. Because of abnormal placentation, there may be an increased synthesis of beta HCG (1). There may be deregulation of lipoprotein lipase in GHT prone women, that causes elevated plasma lipid and lipoprotein levels, may induce endothelial dysfunction is the prominent pathology, usually occurs in early trimester (8-18 weeks) but signs and symptoms occur in a late trimester (1). In this study serum beta HCG estimated in the early second trimester, women with elevated levels, categorized under the high-risk group. So it is easy to identify the high-risk women and kept under regular follow up. It helps in preventing the development of complication in GHT. Since the year 1950 HCG is reported to be elevated in toxemic pregnancy (2,3). Like in present study, Mazhari and Varun (9), Soudararajan et al (10) Muthulakshmi et al (11), Kulkarni et al (12) and Rajesh et al (35) also use hCG production level as a measurement tool for the prediction of pregnancy induced hypertension. In the present study the prevalence of PIH was observed to be in 12% which was comparable to the studies performed by Mazhari and Varun (9) who found that the prevalence of PIH was 13.33% and Pawar et al (14) (11.53) which were in agreement with our finding Table 2 shows the relationship between β−hCG (absolute) levels and PIH and the association between mean β−hCG level of PIH patients and Normotensives was statistically significant (p< 0.001) and as the level of β−HCG increases the PIH also increases. Elevated serum β-hCG in second trimester has repeatedly been shown to be significantly associated with PIH (41). It has been reported that women with markedly elevated maternal serum β-hCG levels has significantly increased risks of having spontaneous miscarriage, preterm delivery and IUGR (15). The estimation of serum β-hCG levels may be helpful in both the early detection of PIH and to initiate necessary treatment to avoid associated complications (16). This will perhaps bring down the maternal and foetal morbidity and mortality (17,18) reported from their study that all those who developed PIH had higher levels of serum β-hCG confirmed 100% correlation between high serum β-hCG levels and development of PIH which in turn significantly increased risks of having poor/adverse pregnancy outcomes (Spontaneous miscarriage, Intra Uterine Growth Retardation (IUGR) and Preterm delivery), where the magnitude of risk correlates with the levels of β-hCG. The present study results indicate that β-hCG determination may have value in the prediction of PIH (19). It is evident from the different studies that, the serum β-hCG levels were significantly higher and significantly correlated with increased risk of PIH in women with increased β-hCG levels in the hypertensive group as compared to those in the normotensive group; which indicates the strong correlation between higher serum β-hCG levels and development of PIH later on during pregnancy (20,21). It seems reasonable to presume from the results of the present experimental study that the increased synthesis and secretion of free β-hCG is associated with immunosuppressive activity of hCG which helps to initiate early treatment so as to minimize and/or avoid adverse effects of PIH (22,23). The study showed that, 10 of 15 (66.67%) of PIH women have severe elevation in hypertension, The study also showed that, majority of women with severe PIH (90%) have Beta hCG level above 80000 mIU/ml and only 10% have level ≤75000 mIU/ml while 60% of women with mile PIH have level ≤80000 mIU/ml. Our study is in accordance to the study performed by Chowdhary et al (24) who reported 60% with severe hypertension and 40.0% with mild hypertension.

Conclusion

The study showed that measuring second trimester beta HCG levels is useful in clinical practice to identify women who will develop PIH in the same pregnancy.

Financial Disclosure: There is no financial disclosure.
Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the Al-Anbar Health directorate and carried out in accordance with approved guidelines”.

References


Determination of Effectiveness of Exclusive Breastfeeding Preparing Program on Knowledge of Primipara Mothers in Kirkuk City Hospitals

Muntaha Hameed Hussein¹, Suhad H. Khairi², Mahasin T. AL-Harbawe³

¹Ph.D, ²Assist. prof. Dr., Department of Maternal and Newborn Nursing - College of Nursing / University of Baghdad.; ³Assist. Prof. Dr., Community Health Nursing / Al-Maarif University College

Abstract

Objective: To determine of effectiveness of exclusive breastfeeding preparing program on knowledge of primipara mothers in kirkuk city hospitals

Methodology: A quasi-experimental design of thirty Primipara women in three hospitals in kirkuk city for the period from March 1st to October 1st 2020. The data collected through stages which include pretest, posttest.

Results: The finding indicate that among thirty study sample the MS and SD were higher in posttest in compare with pretest, and significant differences were found within study sample before and after program at (P=0.05) which indicated that educational program positively effect on the study sample regarding exclusive breastfeeding.

Conclusions: The study concludes that after assessing the knowledge of the study sample, it found that their knowledge was poor and exclusive breastfeeding educational program was effectively improved their knowledge.

Keywords: Effectiveness, Exclusive breastfeeding, Preparing program, Knowledge, Primipara mothers.

Introduction

Breastfeeding is an important origin of food for infant at firstly six months of lifetime, according to the World Health Organization ¹, which confirmed maternal to use it as their primary source of nutrition. Mothers should feed their babies complementary foods in addition to breastfeeding between the ages of 6 months and 2 years.

Exclusive breastfeeding” is interpreted as Only breast milk is given to the newborn; no fluids or hard are given – not even water – except for oral rehydration liquid or vitamin, mineral, or medication droplets / syrups.” It is advised to use it during the first six months of a child’s lifetime, after which supplement feeding should be introduced. on the other hand, Breastfeeding should be continued for at least 2 years age or beyond ²³.

Performance for Natural Growing and progression for neonate and child feed , these practices involve starting breastfeeding during first hour of childbirth, exclusive breastfeeding during first six months of lifetime , adding suitable and sufficient goal of 21.9 million exclusively breastfed infants during the first six months of lifetime in 49 nations by 2015 (United Nations Secretary General, 2013).

As a result of the multiple predictor cluster survey (MICS) in Iraqi (2018), 32.4 % of babies were breastfed during the first hour of delivery,
and (26 percent) of infant were received exclusive breastfeeding within aged (0-5), (85 %) of infant were getting firm nourishment or semi-solid diet in aged (6-8) months while (45 %) of infant aged (6-23) months received the lowest nutritional variety. Five types of described developmental food groups, In the first year of life (12-15 months), 45 % of children continue to breastfeeding whereas in the second year of life (20-23 months), 27 % of infant continue to breastfeeding.

The provision (or lack thereof) of adequate information and support to the mother during both the antenatal and postnatal periods is one of the most important factors that influences the initiation and continuity of breastfeeding. Several studies have found that mothers who receive professional breastfeeding support and advice have more optimistic attitudes about breastfeeding and tend to breastfeed longer 6,7. Breastfeeding initiation rates have been shown to improve with interventions aimed at increasing healthcare providers’ breastfeeding awareness, and additional professional support from healthcare providers continues to enhance the duration of exclusive breastfeeding for mothers 8,9. On the other hand, breastfeeding results are negative when the upholding and advice given by healthcare providers is insufficient and suitable (Montalto et al., 2010). Knowledge of breastfeeding information has a direct and indirect influence on breastfeeding practice. Directly, a more enlightened individual on breastfeeding may practice it but the less enlightened, may doubt its potency and its benefit and therefore may not find it attractive to practice. Society may not accept the practice of breastfeeding due to cultural, religious and economic reasons, coupled with poor health staff attitude 11.

Objective of The Study

To determine of effectiveness of exclusive breastfeeding preparing program on knowledge of primipara mothers in kirkuk city hospitals

Methodology

A quasi-experimental design of thirty Primipara women in three hospitals in kirkuk city for the period from March 1st to October 1st 2020. The study instrument consist of three parts, it include demographic characteristics, reproductive history ,and mothers Knowledge. The data collected through three stages ; pretest stage to assess mothers knowledge , second stage , the educational program design to provide primipara woman with exclusive breastfeeding knowledge, it consisted from 27 items .The program intervention covered through lectures, videos, films, role play and pictures .The third stage was posttest , this done after two weeks from intervention the educational program. Pre and posttest fill questionnaire form by themselves .A descriptive and inferential statistic used to analyzed the data .

Results

Figure 1. Wives’ Age (Years)
The mean age of participants is $24.33 \pm 4.54$; Most of them age 18-24-years ($n = 18; 60.0\%$), followed by those who age 25-31-years ($n = 10; 33.3\%$), and those who age 32-40-years ($n = 2; 6.7\%)$.

![Figure 2. Wives’ Educational level](image)

Concerning wife’s level of education, less than a third are primary school graduates ($n = 9; 30.0\%$), followed by those who each of preparatory school graduates, institute graduates, and college graduates and above ($n = 5; 16.7\%$) for each of them, those who intermediate school graduates ($n = 3; 10.0\%$), those who are unable to read and write ($n = 2; 6.7\%$), and one who reads and writes ($n = 1; 3.3\%)$.

![Figure 3. Husbands’ Educational level](image)
Regarding husband’s level of education, a third are college graduates and above (n = 10; 33.3%), followed by those who are primary school graduates (n = 6; 20.0%), those who are preparatory school graduates (n = 5; 16.7%), those who are institute graduates (n = 4; 13.3%), those who are intermediate school graduates (n = 3; 10.0%), and those who read and write (n = 2; 6.7%).

With respect to wife’s occupation, most are housewives (n = 21; 70.0%) compared to those who are employees (n = 9; 30.0%). For husbands, more than a half are freelancers (n = 16; 53.3%), followed by those who are employees (n = 7; 23.3%), those who are students (n = 5; 16.7%), and those who are out of work (n = 2; 6.7%). Concerning family’s monthly income from family point of view, most reported that they have barely sufficient income (n = 19; 63.3%), followed by those who have a sufficient income (n = 7; 23.3%), and those who have an insufficient income (n = 4; 13.3%). Regarding the residence, the vast majority live in urban areas (n = 29; 96.7%) compared to those one who live in rural area (n = 1; 3.3%).
3.3%). Lastly, With respect to “Family type, less than a half reported that they have been living in nuclear families (n = 14; 46.7%), followed by those who have been living in shared families (n = 11; 36.6%), and those who have been living in extended families (n = 5; 16.7%).

**Table (2) Study Sample Reproductive Variables (N = 30)**

<table>
<thead>
<tr>
<th>Items</th>
<th>Variables</th>
<th>F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Previous abortion</td>
<td>Yes</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>21</td>
<td>70</td>
</tr>
<tr>
<td>2. Any disorders during pregnancy</td>
<td>Yes</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>24</td>
<td>80</td>
</tr>
<tr>
<td>3. Breast care during pregnancy</td>
<td>Yes</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>4. Intend to breastfeeding</td>
<td>Yes</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>5. Delivery type</td>
<td>Normal Vaginal Delivery</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Cesarean Section</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>6. Delivery place</td>
<td>Public Hospital</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Concerning abortion, most study sample reported that they did not experience abortion (n = 21; 70.0%) compared to those who have experienced it (n = 9; 30.0%). Regarding pregnancy-related problems, the majority reported that they do not experience such problems (n = 24; 80.0%) compared to those who experienced them (n = 6; 20.0%). With respect to breast care, all study sample reported that they are caring for their breast (n = 30; 100.0%). All study sample have intend to breastfeed their babies (n = 30; 100.0%). Study sample gave birth through normal vaginal delivery (n = 15; 50.0%) compared to those who delivery cesarean section (n = 15; 50.0%). All study sample gave birth in the public hospitals (n = 30; 100.0%).
Table 3. Overall Difference in Study Sample Knowledge between the Pretest and Posttest Times

<table>
<thead>
<tr>
<th>Paired Samples Test</th>
<th>Paired Differences</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std. Error Mean</td>
<td>Lower</td>
<td>Upper</td>
</tr>
</tbody>
</table>

There is a statistically significant difference in study sample knowledge between the pretest and posttest times at (p = 0.05).

Discussion

Socio-Demographical Characteristics Variables

Among thirty study sample who completed the questionnaire, the main age of the study sample ranged from (18-24) years was (SD 24.33 ±4.54 years), the majority (30%) of the study sample are primary school graduate and (33.3%) of their husband graduate from college and above

Occupationally (70%) of the study sample are house wife, while more than half (53.3%) of their husband are freelancer, (63.3%) of the study sample consider their socio-economic status barely sufficient from their point of view, (96.7%) of them lived in urban area and (46.7%) lived in nuclear families.

The study’s findings concluded that highly percentage of the study sample were young, low socio-educational status, without work, and low economic status as shown in table (1), and figure(1,2,3).

Studies found that age, educational level, no occupation leads to minimize breastfeeding (Rocci & Fernandes, 2015)

Many studies show that socio demographic factors characteristics such as age, education level, occupation, family income and types of family were coincide with this study results (Usha Rani & Bhattacharjee, (2018); Hasan, (2016); Najem & Al-Deen, (2011); Shommo & Al-Shubrumi, (2014)).

Habibil et al., (2018) concluded that a significant relationship between exclusive breastfeeding and mother education, socio-economic status and length for age, and maternal employment. Breastfeeding was closely associated to all socioeconomic variables, maternal educational level, maternal unemployment benefit, and income (Flacking et al., 2007).

Reproductive Characteristics

The reproductive criteria of study sample included (70 %) of study sample with no abortion, the majority (80%) of them do not experience pregnancy-related problems. All (100%) of study sample get care for their breast during pregnancy and have an intention to breastfeed their babies respectively. Half (50%) of
them their delivery type was normal vaginal delivery compared (50%) of Cesarean section respectively. All (100%) of women gave birth in the public hospitals.

Breastfeeding intentions of maternal before conception have an effect on practice of baby feeding (Donath & Amir, 2003). Breastfeeding for 6 months is more likely when mothers have high intention and self-efficacy (Wilhelm et al, 2008). During the antenatal period, all women should be encouraged to plan for breastfeeding their children.

Women’s breastfeeding self-efficacy and intent to breastfeeding were the most significant indicators of breastfeed beginning, continuance, and duration in the first six months following delivery (Brockway et al., 2017).

The type of birth (vaginal vs. caesarean) have no effect on breastfeeding patterns, according to DiGirolamo et al. (2008). While Patel et al., (2003) discovered that the mode of delivery had no influence on breastfeeding.

Mothers who gave delivery naturally had a more favorable attitude toward breastfeeding and had less stress breastfeeding practices than mothers who underwent a caesarean delivery (Imhonde et al., 2012; Carlander et al., 2009).

Bottle-feeding and reduced milk supply are associated to Caesarean deliveries (Zhang et al, 2004).

Concerning of the delivery place, the current study found that all of the mothers gave delivery at hospital. The finding was confirmed by Ravi et al., (2015), who mentions the majority of women’s gave delivery in a hospital.

This finding is confirmed by Pushpa & Chowti (2012), who state that there is no significant association among the site of delivery and women’s information on breastfeeding.

Differences in Study Sample Knowledge between Pretest and Posttest

Thirty mothers who breastfeeding for the first time received sessions about exclusive breastfeeding. Twenty seven questions covered the entire process of exclusive breastfeeding. After statistical assessing, the knowledge of the study sample through pre and posttest.

Before the program only (26.7%), (6.7%) and (20%) of the study sample know the correct definition and the duration and advantages for baby of exclusive breastfeeding respectively.

This results indicate that many study sample have general information about breastfeeding but few of them knew accurate information and how breastfeeding done (Hanafi & El-Ammari, 2014).

The finding indicate that Although most of study sample are primary school graduated the results shows that at pretest 10(3,5,6,8,9,13,18,19,21,22) out of 27 items assessment degree record high which mean that study sample had back ground about breastfeeding information regard less of educational level.

The majority of primigravidas in India (78%) did not know that EBF should be continued for six months, according to a survey (Dhandapany etal., 2008). The majority of primigravidas were unaware that EBF reduces the risk of neonatal jaundice (Kumer et al., 2015). In respect to table (3) the overall analysis revealed that there is a positive significant improvement in the study sample knowledge at p: value (0.05). Kumar et al., (2015) several research reinforce the previous findings, stating that insufficient information, a negative attitude, or inappropriate breastfeeding technique lead to negative results for both mother and newborn. Insufficient breastfeeding is one of main reasons of baby deaths and morbidity, because the mother’s lack of awareness of its importance and benefits. During antenatal education in the waiting room, however, education can play a large role in changing mothers’ attitudes and knowledge about breastfeeding. Iraq and neighboring countries have a common culture that is similar to that for countries with Islamic
religious roots which has a positive attitude toward breastfeeding. Finally, the study participants have a positive response, and the sessions have a good impact on their knowledge.

Conclusions

Based on data analysis and critical interpretation of the finding. The study concluded that after assessing the knowledge of the study sample, it found that their knowledge was poor and exclusive breastfeeding educational program was effectively improved their knowledge.

Recommendations:

1. Developing and implementing of educational programs support and counseling routinely during pregnancy (antenatal and postnatal period through primary health care centers and hospitals.

2. Nurse need to motivate and monitoring the promotion of exclusive breastfeeding for six months by home visit or telephone call and provide mothers with pamphlets and guideline to increase knowledge and practices regarding breastfeeding.

3. Engage family members and husbands effectively in the educational programs, family have a big role in encouraging pregnant women to breastfed their babies after birth.

4. Further studies about changing the conception of insufficient breast milk for mothers.

5. Enhance new mothers understanding rather than education to change their misconception and attitude and behavior through mass media.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the College of Nursing and carried out in accordance with approved guidelines”.

References


5. Multiple Indicator Cluster Survey (MICS6) IRAQ. 2018


Detection the Level of Anxiety and Depression among Diabetic Foot Patients at Al-Najaf Al-Ashraf Teaching Hospitals

Ali Dawood Salman¹, Serwan Jafar Bakey²

¹M.Sc., Department of Psychiatric and Mental Health Nursing, ²Lecturer, Dr., Ph.D. in Adult Nursing, Faculty of Nursing, University of Baghdad, Iraq

Abstract

Objective: The study was done to assess the level of anxiety and depression among diabetic foot patients at Al-Najaf Al-Ashraf Teaching Hospitals. Also to find out relationships between anxiety and depression of diabetic foot patients and their demographic characteristics. Methodology: a descriptive correlational design, the study established for a period from 10th October, 2020 to 1st June, 2021. The study was conducted on a probability (accidental) sample of 120 patients who have a diabetic foot which was selected from Al-Sadder teaching hospital and Al-Furat teaching hospital in Al-Najaf Al-Ashraf Governorate. The researcher is using the Hospital Anxiety and Depression Scale to assess the level of anxiety and depression. Results: The result of the study showed (37.5%) of the study sample had moderate anxiety. Furthermore, the study shows (36.6%) of the study participant have moderate depression. Contingency correlation analysis showed that anxiety and depression is a significant correlation with the number of hospital admission, duration of current admission, and previous amputation. In addition, anxiety was significantly correlated with gender, educational level, duration of the diabetic foot. Besides, depression was significantly correlated with age, economic status, having other chronic diseases.

Keywords: Anxiety, Depression, Diabetic foot

Introduction

Diabetes Mellitus (DM) is one of the most important chronic diseases worldwide, with regards to the impact on health and the fifth leading cause of death worldwide ¹, DM is a chronic genetic disorder that has been recognized as one of the main causes of life with disabilities ².

Diabetes mellitus is a multi-system disorder characterized by impaired glycemic control and is associated with a range of medical complications. Adverse events may occur due to medication dosing and complications of the disorder may slow progress, these factors make individuals with diabetes predisposed to have psychological disturbance such as depression and anxiety ³.

Diabetes mellitus has become a worldwide problem as its prevalence rises. In 1980, 108 million people were diagnosed with diabetes, rising to 422 million in 2017. By 2035, it is expected that 592 million people will have the disease (Sari et al., 2018). All types of diabetes mellitus can lead to complications in many parts of the body and increase the risk of dying prematurely, diabetes was the direct cause of 1.5 million deaths globally (World Health Organization[WHO], 2020). Iraq is living with a DM epidemic, with a prevalence of about 20 percent, this prevalence has risen fourfold in the last four decades, and it is predicted to continue increasing in the future. Long-term DM complications are more frequent in Iraqi patients than in other parts of the world, approaching 80 percent for certain microvascular complications [retinopathy and neuropathy], and 25 to 48 percent for macrovascular complications [cardiovascular diseases and stroke, respectively] .
Diabetic Foot (DF) is a severe diabetic complication characterized by deep tissue lesions in the lower limbs, as well as neurological problems and peripheral vascular disease. The diabetic foot has become more common as the global prevalence of diabetes mellitus has increased 4.

Diabetic Foot Ulceration (DFU) is a serious and potentially limb-threatening complication of diabetes that can lead to pain, tissue necrosis and amputation, and may significantly affect an individual’s well-being and mobility 15. Around 25 percent of people with diabetes will be suffering from DFU during their lifetime. In addition, every 20 seconds a lower limb is amputated due to diabetic complications. In fact, every year 5 percent of all the patients with diabetes develop foot ulcers, and 1 percent require amputation 5.

Foot complications can be the most common cause for hospitalization in people with diabetes, and the duration of stay may be up to 59 percent longer than in people with diabetes hospitalized for a non-foot-related reason. Diabetes complications are associated with amputation rates and life-threatening problems. A foot ulcer affects about 15 percent of diabetic patients, and 14-24 percent of those with foot ulcers need amputation 6.

Diabetic patients suffer from a variety of health problems, including mental and psychological challenges that have an effect on their overall health (Yavati et al., 2011). On the other hand, diabetic foot patients are more likely to experience depression and anxiety than diabetics without foot problems, and depression and anxiety levels increased with hospitalization 6.

Diabetes mellitus and depression are two of the most serious public health problems in the United Kingdom (UK) and around the world 11. Anxiety and diabetes-specific distress, such as not accepting diabetes, worries about complications, concerns about food, feelings of guilt or shame, and distressing social interactions, are often common causes of disability, occupational absenteeism, and lost productivity 7.

Methodology

Design of the Study:

A Descriptive correlation design was conduct on diabetic foot patient in Al-Najaf Al-Ashraf teaching hospitals. The study was carried out to assess the level of anxiety and depression among diabetic foot patient and to find out the relationship between the anxiety and depression and their socio-demographic characteristic.

The Setting of the Study:

The study is conducted in Al-Najaf city/ Al-Najaf Al-Ashraf Health Directorate/ Al-Sadder teaching hospital and Al-Forat teaching hospital.

Sample and Sampling of the Study:

Anon-probability (accidental) sample including 120 diabetic foot patients which were selected from Al-Najaf Al-Ashraf teaching hospitals.

Instrument of the Study:

The research instrument which was consisting of two parts, part 1 list of common items for assessing demographic characteristics of patient with diabetic foot and information about their illness and part 2 Hospital Anxiety and Depression Scale (HADs) to assess the anxiety and depression among diabetic foot patients.

Part I: Socio-Demographic Characteristic

This section is dedicated to the collection of socio-demographic data including age, gender, residence, marital status, economic status, education level, occupational status, and information about their disease including duration of diabetes mellitus, duration of diabetic foot, if they have other chronic diseases, smoking history, alcohol history, number of admissions, duration of current hospitalization if they have a previous amputation and complications of diabetes mellitus.
Part II: Hospital Anxiety and Depression Scale

This scale assesses psychological morbidity (depression and anxiety) the scale consists of 14-item: seven items for anxiety (e.g. “I feel tense or wound up”; “Worrying thoughts go through my mind”) and seven items for depression (e.g. “I enjoy the things I used to enjoy”; “I have lost interest in my appearance”) \(^8\). The score for each scale ranges from 0 to 21 and each item has a choice of four response statements (scored 0–3). A Total score which divided into four levels: Scores represent: normal 0-7, Mild 8-10, Moderate 11-14, severe 15-21 for anxiety and depression.\(^{33}\)

Validity and Reliability of Scale:

Validity of Scale:

The instrument was introduced to a panel of (12) experts with more than ten years of experience in their field to make the instruments more valid using content and face validity methods.. The changes have been made in accordance with the recommendations of the experts.

Reliability of the Scale:

The scale was previously used and according to Terkawi et al (2017), the reliability had shown that for Time 1 and Time 2, Cronbach’s alfa for the HADS anxiety subscale were 0.83 (95 percent confidence interval [CI]: 0.79–0.88) and 0.87 (95 percent confidence interval [CI]: 0.83–0.91), respectively. For Time 1 and Time 2, Cronbach’s s for the HADS depression subscale were 0.77 (95 percent CI: 0.7–0.83) and 0.8 (95 percent CI: 0.75–0.86), respectively. Internal consistency was found to be adequate for both HADS subscales at both time points among patients.

Method of Data Collection:

After determining the validity and reliability, data was collected using an ‘Arabic version’ self-report questionnaire as a means of data collection, as well as an semi-structured interview with patients who do not read or write, unless they refused to participate in the research. The questionnaire takes about 15 to 20 minutes to complete. Where the data was collected from 10th January 2021 to 10th May 2021.

Method of Statistical Analysis

The statistical package for social sciences (SPSS) version 24.0 application of statistical analysis framework was used to analyze the results. The resulting research was analyzed and evaluated using the subsequence statistical data analysis technique.

Results

Table (1) Distribution of the sample according to demographic characteristics

<table>
<thead>
<tr>
<th>Section</th>
<th>Divisions</th>
<th>Frequency N= 120</th>
<th>Percent Total :100.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-39 years</td>
<td>7</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>40-49 years</td>
<td>36</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>50-59 years</td>
<td>45</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td>60 years and more</td>
<td>32</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td>Mean = 53.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Std. Deviation = 9.652</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (1) Distribution of the sample according to demographic characteristics

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male</th>
<th>66</th>
<th>55.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>54</td>
<td>45.0</td>
</tr>
<tr>
<td>Residence</td>
<td>Urban</td>
<td>74</td>
<td>61.7</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>46</td>
<td>38.3</td>
</tr>
<tr>
<td>Marital Status</td>
<td>Married</td>
<td>91</td>
<td>75.8</td>
</tr>
<tr>
<td></td>
<td>Widowed</td>
<td>23</td>
<td>19.2</td>
</tr>
<tr>
<td></td>
<td>Divorced</td>
<td>6</td>
<td>5.0</td>
</tr>
<tr>
<td>Economic status</td>
<td>Satisfied</td>
<td>10</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>Satisfied to same extent</td>
<td>57</td>
<td>47.5</td>
</tr>
<tr>
<td></td>
<td>Unsatisfied</td>
<td>53</td>
<td>44.2</td>
</tr>
<tr>
<td>Occupational status</td>
<td>Employee</td>
<td>20</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>Retired</td>
<td>12</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Unable to work</td>
<td>25</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td>Unemployed</td>
<td>8</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Free job</td>
<td>8</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Housewife</td>
<td>47</td>
<td>39.2</td>
</tr>
<tr>
<td>Educational level</td>
<td>Don’t read and write</td>
<td>45</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td>Read and write</td>
<td>17</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>Primary school</td>
<td>23</td>
<td>19.2</td>
</tr>
<tr>
<td></td>
<td>Secondary school</td>
<td>5</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>Preparatory school</td>
<td>4</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Diploma</td>
<td>9</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Bachelor</td>
<td>17</td>
<td>14.2</td>
</tr>
<tr>
<td>Do you have another chronic disease</td>
<td>Yes</td>
<td>55</td>
<td>45.8</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>65</td>
<td>54.2</td>
</tr>
<tr>
<td>Type of chronic disease</td>
<td>Hypertension</td>
<td>27</td>
<td>49.1</td>
</tr>
<tr>
<td></td>
<td>CVA</td>
<td>24</td>
<td>43.6</td>
</tr>
<tr>
<td></td>
<td>Asthma</td>
<td>4</td>
<td>7.3</td>
</tr>
</tbody>
</table>
Table (1): Distribution of the sample according to demographic characteristics

<table>
<thead>
<tr>
<th>Duration of DM</th>
<th>1-5 years</th>
<th>6-10 years</th>
<th>11-15 years</th>
<th>16-20 years</th>
<th>21 years and more</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>31</td>
<td>37</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>Duration of diabetic foot</td>
<td>1-10 Days</td>
<td>11-20 Days</td>
<td>21-30 Days</td>
<td>31 Days and more</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>28</td>
<td>23</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Do you smoker</td>
<td>Yes</td>
<td>No</td>
<td>Quit smoke</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>55</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you drinking Alcohol</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>118</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of admission</td>
<td>1-2 times</td>
<td>3-4 times</td>
<td>5 times and more</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>32</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>duration of current hospitalization</td>
<td>1-2 days</td>
<td>3-4 days</td>
<td>5 days and more</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>39</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you have previous amputation</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complication of DM</td>
<td>CVA</td>
<td>Retinopathy</td>
<td>Neuropathy</td>
<td>Nephropathy</td>
<td>Other complication</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>40</td>
<td>10</td>
<td>24</td>
<td>32</td>
</tr>
</tbody>
</table>

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2 days</td>
</tr>
<tr>
<td>3-4 days</td>
</tr>
<tr>
<td>5 days and more</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>CVA</td>
</tr>
<tr>
<td>Retinopathy</td>
</tr>
<tr>
<td>Neuropathy</td>
</tr>
<tr>
<td>Nephropathy</td>
</tr>
<tr>
<td>Other complication</td>
</tr>
</tbody>
</table>
### Table (2) Assessment of anxiety status among diabetic foot patients:

<table>
<thead>
<tr>
<th>Section</th>
<th>Divisions</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety</td>
<td>Normal</td>
<td>19</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>32</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>45</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>24</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Mean 11.29**  
**St Deviation 3.42**

### Table (3) Assessment of depression status among diabetic foot patients:

<table>
<thead>
<tr>
<th>Section</th>
<th>Divisions</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression</td>
<td>Normal</td>
<td>20</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>29</td>
<td>24.2</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>44</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>27</td>
<td>22.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Mean 11.93**  
**St Deviation 3.68**

### Table (4) Correlation between anxiety and depression with demographic characteristics among diabetic foot patients:

<table>
<thead>
<tr>
<th>Section</th>
<th>Anxiety</th>
<th>Depression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>p-value</td>
</tr>
<tr>
<td>Age</td>
<td>.257</td>
<td>.483</td>
</tr>
<tr>
<td>Gender</td>
<td>.432</td>
<td>.000</td>
</tr>
<tr>
<td>Residence</td>
<td>.223</td>
<td>.098</td>
</tr>
<tr>
<td>Marital Status</td>
<td>.213</td>
<td>.458</td>
</tr>
<tr>
<td>Economic status</td>
<td>.291</td>
<td>.086</td>
</tr>
<tr>
<td>Occupational status</td>
<td>.376</td>
<td>.180</td>
</tr>
</tbody>
</table>
Cont... Table (4) Correlation between anxiety and depression with demographic characteristics among diabetic foot patients:

<table>
<thead>
<tr>
<th></th>
<th>.446</th>
<th>.040</th>
<th>.344</th>
<th>.584</th>
</tr>
</thead>
<tbody>
<tr>
<td>Educational level</td>
<td>.144</td>
<td>.471</td>
<td>.265</td>
<td>.028</td>
</tr>
<tr>
<td>Do you have another chronic disease</td>
<td>.160</td>
<td>.957</td>
<td>.312</td>
<td>.167</td>
</tr>
<tr>
<td>Type of chronic disease</td>
<td>.323</td>
<td>.304</td>
<td>.277</td>
<td>.621</td>
</tr>
<tr>
<td>Duration of DM</td>
<td>.432</td>
<td>.001</td>
<td>.293</td>
<td>.260</td>
</tr>
<tr>
<td>Duration of diabetic foot</td>
<td>.166</td>
<td>.335</td>
<td>.163</td>
<td>.772</td>
</tr>
<tr>
<td>Do you smoker</td>
<td>.434</td>
<td>.000</td>
<td>.413</td>
<td>.000</td>
</tr>
<tr>
<td>Do you drinking Alcohol</td>
<td>.434</td>
<td>.000</td>
<td>.404</td>
<td>.001</td>
</tr>
<tr>
<td>Number of admission</td>
<td>.495</td>
<td>.000</td>
<td>.426</td>
<td>.000</td>
</tr>
<tr>
<td>Complication of DM</td>
<td>.301</td>
<td>.452</td>
<td>.332</td>
<td>.250</td>
</tr>
</tbody>
</table>

Part I: Discussion of Socio-demographic Data of the Study Sample:

Table(1) shows that nearly thirds of the age of the study sample were between (50-59) years at an age mean of 53.43 ± 9.65 years, this result coincides with the result of previous studies, Chen et al. (2020) asserted that the mean age of DFU patients was 59.4±10.1 years. Polikandrioti et al. (2020) reported that 32.8% of their study participant had ages from (51-60) years. Ahn et al. (2018) reported that the median age of the study sample was 58 years. Saber & Daoud (2018) reported that the mean age of the patients was 53.7 ± 12.08 years. Mohammed et al. (2016) stated that most of the study sample was that at age mean 54.8 ± 11.05 years. Sehlo et al (2016) noted that 56.55 ± 3.32 mean of the age in their study. Neeru et al.(2015) found that the mean age of the patients having diabetic foot ulcers was 52.36 ± 7.8 years. 

Regarding gender, the results of the current study showed that males were more than females with diabetic foot, according to the findings of this study, which are consistent with earlier findings that show that the males were more than females.

Regarding residence, the study reported that less than two-thirds of the study sample living in the urban area, the result of this study congruent with other studies, Saber & Daoud, (2018) stated that more than two-thirds of diabetic foot patients who participate in their study dwelled in urban areas. The result also agree with determining that there are just 21.3% DF patients living in the rural area and the other lives in the urban areas. Agree with asserted that more than half of the study participants was lives in urban areas. While noted that more than three-quarters of their study sample resided in urban areas.

Concerning the marital status, the study finding shows that three-quarters of DF patient participant in the study were married, this result is agree with previous studies reported that more than three-quarters
of DF patients were married 29.

As for the presence of other chronic diseases, the patients who didn’t have another chronic disease are more than half of the study sample, the result of this study congruent with Mohammed et al. (2016) documented that the patients with other comorbid conditions were more than one quarter while the patients without other chronic diseases they were less than three quarters in their study sample, furthermore, the study by 24 noted that more than three-quarters of DF patients did not have other chronic diseases.

As for those who have chronic diseases, the current study showed that most of them had hypertension, this result agree with previous studies showed highly percentage of DF patients have hypertension 4,6,23,25,27,32.

Regarding the duration of diabetic foot, the study shows that the majority of the study sample have diabetic foot more than one month, this result agrees with Messenger et al. (2018) stated that less than three quarters have diabetic foot more than 1 month, Also documented 34 the mean of DF duration was 1.6 months. While noted 24 the median of DF duration was 11 weeks that also agree with our study.

With regard to previous amputations, the result found that half of DF patients had a previous amputation, and this result does not completely agree with previous studies that showed that the percentage of patients who did not have a previous amputation was more than those who had it 16, 18, 25, 31.

Table (2) shows that more than one-third of patients with diabetic foot participants in the current study have moderate anxiety and a quarter of them have mild anxiety. The study by Chen et al. (2020) asserted that less than two-thirds of the study sample were had mild anxiety while nearly one-quarter of them suffering from moderate anxiety. Likewise, reported 26 that DF patients who had low anxiety were more than two-third while just (16.9%) had moderate anxiety. Besides that, indicated by 24 that the mean score of anxiety was 11.46 ± 5.14 (moderate anxiety) this result agrees with the current study that indicated the mean score of anxiety was 11.29 ± 3.42.

Table (3) indicates that more than one-third of the patients in the current study have moderate depression and about a quarter of them have mild depression. the study by Chen et al. (2020) stated that more than half of the study sample had mild depression while less than one-third of them had moderate depression. In addition, 26 found that more than two-thirds of the DF patients in their study experienced mild depression. According to Pearson et al. (2014), 28.3 percent of DF patients suffer from moderate to severe depression. Moreover, 24 that the mean score of depression was 9.39 ± 5.90 moderate depression.

Concerning the result related to the correlation between anxiety and socio-demographic data in table (4) the existing study exposes that there is no significant correlation between anxiety and demographic data of the study sample related to (age, residence, marital status, economic status, occupational status, if they have another chronic disease, type of other chronic diseases, duration of DM, smoking history, alcohol history, and complication of DM.

Regarding the result related to the correlation between depression and socio-demographic data in table (4) the existing study exposes that there is no significant correlation between depression and demographic data of the study sample related to (gender, residence, marital status, educational level, type of other chronic diseases, duration of DM, duration of DF, smoking history, alcohol history, and complication of DM.

Conclusion

The researcher concluded that the level of anxiety and depression among diabetic foot patients was moderate and was associated with the number of times patients were admitted to hospitals and the length of stay of patients in the hospital, and was also significant associated with patients who had a
previous amputation.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** “All experimental protocols were approved under the Department of Psychiatric and Mental Health Nursing and carried out in accordance with approved guidelines”.

**References**


19. Mikhael, E, Hassali, M, Hussain, S. Need of Self-Management Education for Iraqi Diabetic


Assessment the Complications of Caesarean Section among Women’s attending AL - Diwaniyah Maternity and Pediatric Hospital/ Iraq

Ghufran Fadhil Abo-Khuwait¹, Najmah Mahmood Meran²

¹Academic Nurse, Ministry of Health/ AL Diwaniyah Directorate, Iraq, ²Assist. Prof, Collage of Medicine/ University of Baghdad, Iraq

Abstract

Aims: This study aimed to assess complications of caesarean section among pregnant women attending AL Diwaniyah Maternity and Pediatric Hospital; and find out relationship between complications of caesarean section and socio-demographic data of women.

Methods: A descriptive cross-sectional study design is conducted for the period of December 26th 2020 to June 1st 2021. The validity of the questionnaire is determine through a panel of experts and reliability was achieved through a pilot study. By a purposive sample is selected among pregnant women who undergo caesarean section, data was collected through the use of questionnaire and interview techniques; and analyzed through the descriptive and inferential statistic.

Results: Findings indicate participants age, the mean age was 29±7.723, the age 20-29 years old (n=217; 43.4%), a not read and write (n=149; 29.8%), more than half of studied participants were housewife (n=339; 67.8%), most of study participants were overweight (n=248; 49.6%). Findings showed that the factors associated with caesarean section were post-partum hemorrhage, prolonged operation wound infection and postpartum infection among studied sample. Regarding fetal complication, Low Apgar score (n=348; 69.6%), respiratory distress syndrome (n=352; 70.4%), and perinatal asphyxia (n=468; 93.6%), among studied sample. The education and BMI have been significant relationship with their associated factors of caesarean section at p-value <0.05.

Key-wards: Assessment, Caesarean Section , Complications.

Introduction

The importance of caesarean sections in preventing difficulties during childbirth and lowering maternal and fetal mortality rates was widely recognized [1]. However, in the recent decade, the rise in caesarean section births has been a major source of concern for public health officials around the world. In most countries, the caesarean section rate (CSR) has above the World Health Organization’s recommended range of 10–15 percent (WHO) [2]. Within the first 10 days after a cesarean delivery, infection is the most common consequence. Infection rates without prophylactic antibiotics are around 85%, whereas infection rates with prophylactic antibiotics were only around 5% [3]. All patients undergoing cesarean delivery should get prophylactic antibiotics; a single dose of a first-generation cephalosporin or ampicillin is sufficient [3]. Although CS was a safe procedure, it puts mothers and their newborns at risk of short- and long-term health concerns when it is performed without medical necessity. The majority of CS problems, on the other hand, stem from the underlying source of the condition Obesity, huge infant size, extended labor, multiple pregnancy, and early birth are all factors that increase the risk of problems in some women [4]. The additional risk connected with the procedure itself must be considered in the absence of a clear medical
justification. Maternal care, both short- and long-term [5]. Therefore, this study aimed to assess complications of caesarean section among women’s attending AL Diwaniyah Maternity and Pediatric Hospital; and find out relationship between complications of caesarean section women’s and socio-demographic data.

**Methodology**

A descriptive cross-sectional study design is conducted for the period of December 26th 2020 to June 1st 2021. A questionnaire deal with complication of caesarean section related to women’s which composed of (11) items; and complications of caesarean section related to fetus which composed of (6) items. The validity of the questionnaire is determine through a panel of experts and reliability is achieved through a pilot study. By a purposive sample is selected among those who are undergo caesarean section, data was collected through the use questionnaire and interview techniques; and analyzed through the descriptive and inferential statistic.

**Results**

**Table 1: Distribution of the study sample by their demographic characteristics**

<table>
<thead>
<tr>
<th>Demographic Variables</th>
<th>Rating</th>
<th>No=500</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Mean± S.d= 29±7.723)</td>
<td>&lt;20 years old</td>
<td>56</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>20-29 years old</td>
<td>217</td>
<td>43.4</td>
</tr>
<tr>
<td></td>
<td>30-39 years old</td>
<td>158</td>
<td>31.6</td>
</tr>
<tr>
<td></td>
<td>40 and older</td>
<td>69</td>
<td>13.8</td>
</tr>
<tr>
<td>Residents</td>
<td>Urban</td>
<td>271</td>
<td>54.2</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>229</td>
<td>45.8</td>
</tr>
<tr>
<td>Education</td>
<td>Not read and write</td>
<td>149</td>
<td>29.8</td>
</tr>
<tr>
<td></td>
<td>Read and write</td>
<td>59</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>Primary school</td>
<td>44</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>Secondary school</td>
<td>32</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>Middle school</td>
<td>57</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>Institute</td>
<td>72</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>College</td>
<td>87</td>
<td>17.4</td>
</tr>
<tr>
<td>Occupation</td>
<td>House wife</td>
<td>339</td>
<td>67.8</td>
</tr>
<tr>
<td></td>
<td>Employ</td>
<td>102</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>Retired</td>
<td>2</td>
<td>.4</td>
</tr>
<tr>
<td></td>
<td>Student</td>
<td>17</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Private sector</td>
<td>40</td>
<td>8.0</td>
</tr>
<tr>
<td>BMI(kg/m2)</td>
<td>Thin (&lt;18.5)</td>
<td>21</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>Normal (18.5-24.9)</td>
<td>213</td>
<td>42.6</td>
</tr>
<tr>
<td></td>
<td>Overweight (25-29.9)</td>
<td>248</td>
<td>49.6</td>
</tr>
<tr>
<td></td>
<td>Obesity (≥30)</td>
<td>18</td>
<td>3.6</td>
</tr>
</tbody>
</table>
This table represents the descriptive statistics of socio-demographic information of the women in terms of frequencies and percentage. Out of (500) women participated in this study.

Table 2: Maternal complications of Caesarean Section.

<table>
<thead>
<tr>
<th>List</th>
<th>Women complication</th>
<th>Rating</th>
<th>No=500</th>
<th>%</th>
<th>Mean</th>
<th>S.d.</th>
<th>Ass.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pulmonary embolism, which may cause death</td>
<td>No</td>
<td>496</td>
<td>99.2</td>
<td>1.01</td>
<td>0.089</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>4</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Anesthetic complication</td>
<td>No</td>
<td>486</td>
<td>97.2</td>
<td>1.03</td>
<td>0.165</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>14</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sever bleeding during the operation, leading to an emergency hysterectomy</td>
<td>No</td>
<td>486</td>
<td>97.2</td>
<td>1.03</td>
<td>0.165</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>14</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Injury to the intestine</td>
<td>No</td>
<td>496</td>
<td>99.2</td>
<td>1.01</td>
<td>0.089</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>4</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Injury to the urinary bladder, ureters</td>
<td>No</td>
<td>494</td>
<td>98.8</td>
<td>1.01</td>
<td>0.109</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>6</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Deep vein thrombosis (DVT)</td>
<td>No</td>
<td>493</td>
<td>98.6</td>
<td>1.01</td>
<td>0.118</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>7</td>
<td>1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Post-partum hemorrhage</td>
<td>No</td>
<td>64</td>
<td>12.8</td>
<td>1.87</td>
<td>0.334</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>436</td>
<td>87.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Surgical site infection</td>
<td>No</td>
<td>12</td>
<td>2.4</td>
<td>1.98</td>
<td>0.153</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>488</td>
<td>97.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Restrict the number of children</td>
<td>No</td>
<td>467</td>
<td>93.4</td>
<td>1.07</td>
<td>0.249</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>33</td>
<td>6.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Endometritis</td>
<td>No</td>
<td>106</td>
<td>21.2</td>
<td>1.79</td>
<td>0.409</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>394</td>
<td>78.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Mastitis</td>
<td>No</td>
<td>494</td>
<td>98.8</td>
<td>1.01</td>
<td>0.109</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>6</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

"Mean (1.5), yes indication (mean = <1.5), no indication (mean=≥ 1.5), S.d= Standar deviation"

This table presents the mean analysis for assessing the complication of caesarean section among women. Findings showed that the complication with caesarean section were post-partum hemorrhage, prolonged operation wound infection and postpartum infection among studied sample.
### Table 3: Fetal complication of Caesarean Section

<table>
<thead>
<tr>
<th>List</th>
<th>Fetal complications</th>
<th>Rating</th>
<th>No=500</th>
<th>%</th>
<th>Mean</th>
<th>S.d.</th>
<th>Ass.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low Apgar score</td>
<td>No</td>
<td>152</td>
<td>30.4</td>
<td>1.70</td>
<td>0.460</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>348</td>
<td>69.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Respiratory distress syndrome (RDS)</td>
<td>No</td>
<td>148</td>
<td>29.6</td>
<td>1.70</td>
<td>0.457</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>352</td>
<td>70.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Perinatal asphyxia</td>
<td>No</td>
<td>32</td>
<td>6.4</td>
<td>1.94</td>
<td>0.245</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>468</td>
<td>93.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Neonatal sepsis</td>
<td>No</td>
<td>477</td>
<td>95.4</td>
<td>1.05</td>
<td>0.210</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>23</td>
<td>4.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Early neonatal deaths</td>
<td>No</td>
<td>473</td>
<td>94.6</td>
<td>1.05</td>
<td>0.226</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>27</td>
<td>5.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Stillbirth</td>
<td>No</td>
<td>479</td>
<td>95.8</td>
<td>1.04</td>
<td>0.201</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>21</td>
<td>4.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

“Mean (1.5), No indication (mean = <1.5), Yes indication (mean=≥ 1.5), S.d= Stander deviation”

This table presents the mean analysis for assessing the complication of caesarean section among fetus. Findings showed that the most common fetal complication with caesarean section are low Apgar score, respiratory distress syndrome and perinatal asphyxia among studied sample.

### Table 4: Relationship between complication of Caesarean Section and Women their Demographic Characteristics

<table>
<thead>
<tr>
<th>Demographic Data</th>
<th>Chi-Square Value</th>
<th>D.f</th>
<th>P-Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/years</td>
<td>4.346</td>
<td>3</td>
<td>0.226</td>
<td>NS</td>
</tr>
<tr>
<td>Residents</td>
<td>1.697</td>
<td>1</td>
<td>0.193</td>
<td>NS</td>
</tr>
<tr>
<td>Education</td>
<td>20.811</td>
<td>6</td>
<td>0.002</td>
<td>S</td>
</tr>
<tr>
<td>Occupation</td>
<td>0.954</td>
<td>4</td>
<td>0.917</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>45.802</td>
<td>3</td>
<td>0.000</td>
<td>HS</td>
</tr>
</tbody>
</table>
Findings shows that there were no-significant relationship between women demographic characteristics and their complication of caesarean section at p-value >0.05 except, the education and BMI have significant relationship with their complications of caesarean section at p-value <0.05.

**Discussion**

Table 1: Concerning participants age, the mean age is 29±7.723, the age 20-29 years old (n=217; 43.4%), followed by those who are age 30-39 years and old (n=158; 31.6%), followed by those who are age 40 years and older (n=69; 13.8%) and those who age <20 years old (n=56; 11.2%). This results come because those age groups were the age of production. In terms of residents, more than half of studied sample are urban residents (n=271; 54.2%) compared for those who are rural residents (n=229; 45.8%) due to the hospital covered by the study were located in urban areas so, the women residents in those areas. As well as, urban residents were four times more likely to give birth through cesarean section than those who came from rural resident [6].

With respect to education level, studied sample express a not read and write (n=149; 29.8%), followed by those who were collage graduated (n=87; 17.4%), followed by those who are institute graduated (n=72; 14.4%), and followed by those who are primary and secondary graduate (n=44; 8.8% and n= 32; 6.4%) respectively. Occupation related findings, more than half of study participants are housewife (n=339; 67.8%), followed by those who were employed (n=102; 20.4%), and followed by those who are students and retired. In regards with body mass index, most of study participants were overweight (n=248; 49.6%), followed by those who were normal weight (n=213; 42.6%), followed by those who are thin (n=21; 4.2%), and followed by those who were obese (n=18; 3.6%).

The above findings come with findings of study conducted in Kurdistan region, Iraq. Results indicated that the (55.5%) were within age 20-29 years at mean age=28, (51%) were basic education, (61.7%) urban residences with normal to overweight [7].

In recent years, caesarean section rates continue to evoke worldwide concern because of their steady increase, lack of consensus on the appropriate caesarean section rate and the associated short- and long-term risks. Our findings showed according to mean that the surgical site infections were the most common complications with cesarean section followed by post-partum hemorrhage and endometritis among studied sample. The perinatal asphyxia were most common complications of cesarean section among fetus followed by respiratory distress syndrome and low Apgar score.

This findings come consisting with findings of study conducted in Public Hospitals in Northern Ethiopia. Their findings showed that the post-partum infection and post-partum hemorrhage were the most complications occurs after delivery [8].

According to the American College of Obstetricians and Gynecologists report, cesarean birth significantly increased a woman’s risk of pregnancy-related fatality (35.9/100,000) compared to a woman who delivered vaginally (9.2/100,000) deaths. The rates of operation wound infection, post-partum hemorrhage and babies respiratory distress syndrome were also common after cesarean section [9, 10].

**Conclusion**

Surgical site infection and post-partum hemorrhage were the most common complications of cesarean section among women. Perinatal asphyxia, respiratory distress syndrome and low Apgar score were most common fetal complication of cesarean section.

**Recommendation**

Health providers should follow WHO recommendations for the cesarean section. A manual booklet of side effects of cesarean section and how to manage it should be write in simple words and use
attractive pictures given to the women and family.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the AL Diwaniyah Directorate and carried out in accordance with approved guidelines”.

References
Prevalence of Drugs Use among Secondary-School Students in Al-Diwaniya City

Noor Adil Mayeh¹, Kareem Rishaq Sachit²

¹M.Sc., Department of Psychiatric and Mental Health Nursing, College of Nursing, University of Baghdad, Iraq. ²Assist. Prof. Department of Psychiatric and Mental Health Nursing, College of Nursing, University of Baghdad, Iraq

Abstract

A descriptive study design using a convenience random sample of (N=423) students. This sample is distributed throughout AL-Diwaniya Teaching Directorate. Data were collected through the use of an online questionnaire; and analyzed through the descriptive and inferential statistical approach. This study aims to identify the prevalence of Drug abuse among secondary school students. Results indicate that drugs use is low prevalent among secondary school students. Conclusion and Recommendations: Drug use prevalence is associated with students’ gender, age, family member, residency, house ownership, monthly income, parents’ education, and mothers’ occupation. The Ministry of Health should prioritize the Drug use in public health by educating the parents and adolescents the health hazards of substance use through health programs and social media.

Keywords: Drug use, Nursing, Secondary School Students.

Introduction

Substance misuse is linked to a variety of social and health issues, as has been shown in recent years. Substance misuse is the leading cause of death and disability in the world. UNODC estimates that about 5.2% of the world population has used illicit drugs (¹). Using of illicit drugs and the misuse of therapeutic drugs has elevated over the last two decades and has spread to every corner of the globe; nearly 7 billion people abuse narcotics worldwide, with 76 million suffering from their effects; the youth in this country are the most affected (⁷). Studies on substance abuse among young people conducted in different parts of the world show that there is a growing trend in drug use among this population. According to a study conducted in Nigeria on social demographic factors correlated with psycho active substance abuse by motorcycle operators, those who used drugs the most were those who were younger in age (⁴). In secondary schools, the use of psychoactive substances and other medications tends to be widespread and varies across African countries. Senior students in Ghana, for example, have been found to misuse different forms of substances at a lower rate than in other parts of the African continent and West Africa as a whole (⁸). Kenya is also grappling with the widespread use of narcotics and other addictive substances in secondary schools. Alcohol, tobacco, bhang, and miraa were the most frequently abused substances in Kenyan schools, according to a survey. These results revealed that substance abuse was widespread in secondary schools, with 57.9% of students admitting to having used drugs ⁶. In Eastern countries, substance abuse has skyrocketed to the point that it has become a major social issue. According to figures from 2003, the average age of initiation of substance addiction has dropped to 14-16 years. According to statistics, addiction and issues linked to substance abuse was the cause of 34% of divorces ⁵. Substance abuse most often starts in early adolescence as a result of social economic issues and cultural norms prevalent...
at this period. Long-term studies of drug misuse have discovered that the use of tobacco, alcohol, marijuana, and other drugs occurs in this order \(^3\).

**Methodology**

A quantitative descriptive study used assessment approach with questionnaire items is conducted to explore prevalence of drug use among secondary-school students in AL-Diwaniya City for the periods of 27.2.2021 to 8.4.2021.

Students were informed that their participation was voluntary in the study. The purpose and the benefits of the study was explained by the researcher. After they agreed to participate in the study, anonymous questionnaire was published online for the participants to participate in present study.

A convenient sample of \((N=423)\) students is selected throughout the use of non-probability sampling approach. The study sample is distributed throughout AL-Diwaniya Teaching Directorate.

The students test their prevalence of drug use with the constructed questionnaire. This questionnaire was created as a screening method to identify those who are likely to have drug use or not. A constructed questionnaire was used as a method for collecting data.

After completing the required approvals, data was collected by the use of a present questionnaire with the self-report techniques with students online. The researcher introduced himself to the participants and explained the purpose of the study in order to get oral agreement. The questionnaire fill out an answer by the participants (students). The researcher gathered the questionnaire after participant’s self-administration online on individual bases. Approximately each self-report took (15 to 20) minutes. Data collection was performed for the period of 27.2.2021 to 8.4.2021.

**Results of the study**

Table 1: Prevalence of Drugs Use among Secondary School Students

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>F</th>
<th>%</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>383</td>
<td>90.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>24</td>
<td>5.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>16</td>
<td>3.8</td>
<td>2.91</td>
<td>8.157</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>423</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

f: Frequency, %: Percentage, M: Mean for total score, SD: Standard Deviation for total score, None= 0 – 14, Low= 15 – 28, Moderate= 29 – 42, High= 43 – 56
This table indicates that drugs use are prevalent low among 5.7% of students, and prevalent moderately among 3.8% only.

Table 2. Assessment of Drugs Use Prevalence among Secondary School Students (N=423)

<table>
<thead>
<tr>
<th>List</th>
<th>Items</th>
<th>Responses</th>
<th>f</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Do you take some medications for non-medical purposes?</td>
<td>Never</td>
<td>372</td>
<td>87.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rarely</td>
<td>23</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sometimes</td>
<td>26</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Often</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most of time</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Have you used more than one drug at a one time?</td>
<td>Never</td>
<td>360</td>
<td>85.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rarely</td>
<td>47</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sometimes</td>
<td>13</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Often</td>
<td>3</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most of time</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Do you consume drugs more than once a week?</td>
<td>Never</td>
<td>361</td>
<td>85.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rarely</td>
<td>24</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sometimes</td>
<td>37</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Often</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most of time</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Have you misused medication in the past?</td>
<td>Never</td>
<td>372</td>
<td>87.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rarely</td>
<td>22</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sometimes</td>
<td>27</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Often</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most of time</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Have you tried to stop using a certain drug and found it difficult?</td>
<td>Never</td>
<td>372</td>
<td>87.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rarely</td>
<td>13</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sometimes</td>
<td>23</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Often</td>
<td>14</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most of time</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>Do you feel guilty when using drugs?</td>
<td>Never</td>
<td>372</td>
<td>87.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rarely</td>
<td>22</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sometimes</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Often</td>
<td>26</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most of time</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>Are your relationships affected by drug abuse?</td>
<td>Never</td>
<td>372</td>
<td>87.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rarely</td>
<td>12</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sometimes</td>
<td>25</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Often</td>
<td>14</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most of time</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Do you find it easier to be in the company of friends who use drugs than people who don’t?</td>
<td>Never</td>
<td>372</td>
<td>87.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rarely</td>
<td>23</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sometimes</td>
<td>26</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Often</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most of time</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Cont... Table 2. Assessment of Drugs Use Prevalence among Secondary School Students (N=423)

<table>
<thead>
<tr>
<th></th>
<th>Has anyone ever indicated that your abuse is a big problem?</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>372</td>
</tr>
<tr>
<td></td>
<td>Rarely</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Sometimes</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Often</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Most of time</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Does drug use make you violent?</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>372</td>
</tr>
<tr>
<td></td>
<td>Rarely</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Sometimes</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Often</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Most of time</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Does drug abuse negatively affect your ability to work?</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>372</td>
</tr>
<tr>
<td></td>
<td>Rarely</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Sometimes</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Often</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Most of time</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Have you ever been arrested for drug use, possession or driving?</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>384</td>
</tr>
<tr>
<td></td>
<td>Rarely</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Sometimes</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Often</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Most of time</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Have you ever had physical symptoms of withdrawal when you stopped using drugs?</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>372</td>
</tr>
<tr>
<td></td>
<td>Rarely</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Sometimes</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Often</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Most of time</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Have you ever lost consciousness while using drugs?</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>383</td>
</tr>
<tr>
<td></td>
<td>Rarely</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Sometimes</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Often</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Most of time</td>
<td>2</td>
</tr>
</tbody>
</table>

**M.S: Mean of Score, Assess: Assessment**

This table presents the assessment of drugs use prevalence; the table shows that 6.1% of students are sometimes taking some medication for non-medical purpose; 11.1% of them are rarely used more than one drug at a one time; 8.7% are sometimes consume drugs more than once a week; 6.4% are sometimes misuse drugs they stopped using drugs; 5.9% are sometimes feel guilty when using drugs; 5.9% are sometimes have affected relationship because of drug use; 6.1% are sometimes feel easier to be in the company of friends who use drugs than people who don’t; 5.9% are sometimes indicated by others that abuse is a big problem; 5.7% are rarely violent due to drug use; 6.6% are sometimes have affected ability in works due to drug use; only 5.4% are responding they rarely arrested for drug use, possession or driving; 5.7% are sometimes have physical symptoms of withdrawal when they stopped using drugs; and 5.4% are rarely lost consciousness while using drug.

**Discussion of the Study Result**

The table 1 indicated that drugs use are prevalent low among 5.7% of students, and prevalent moderately among 3.8% only. The table 2 showed
that 6.1% of students are sometimes taking some medication for non-medical purpose; 11.1% of them are rarely used more than one drug at a one time; 8.7% are sometimes consume drugs more than once a week; 6.4% are sometimes misused medication in the past; 5.4% are sometimes tried to stop using a certain drugs and found difficulty to do; 6.1% are often feel guilty when using drugs; 5.9% are sometimes have affected relationship because of drug use; 6.1% are sometimes find it easier to be in the company of friends who use drugs than people who don’t; 5.9% are sometimes indicated by others that abuse is a big problem; 5.7% are rarely violent due to drug use; 5.6% are sometimes have affected ability in works due to drug use; only 5.4% are responding they rarely arrested for drug use, possession or driving; 5.7% are sometimes have physical symptoms of Withdrawal when they stopped using drugs; and 5.4% are rarely lost consciousness while using drug. The low prevalence of drug use may be explained by the religious and tribal factor in the governorate, as the use of narcotic drugs is considered socially unacceptable behavior. In addition, the role of the Ministry of Interior that limits the spread of such phenomena is not lost because they are crimes punishable by law. Although most studies indicate an increase in drug use prevalence, one study supports the current results that found 9.8% of adolescents are using illegal drugs (2).

Conclusion

Drug use is low prevalent among 5.7% and moderately prevalent among 3.8% of secondary students. Drug use prevalence is associated with students’ gender, age, family member, residency, house ownership, monthly income, parents’ education, and mothers’ occupation. Cultural factor, socioeconomic factor, and level of education are key moderators in prevalence of substance use among secondary schools.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the College of Nursing and carried out in accordance with approved guidelines”.

References


The Effect of Keeler’s Strategy on Cognitive Achievement and Learning Offensive Skills in Volleyball

Alia Hussein Obaid1, Ahmed Hussein Jiyad2, Alaa Kazem Armout3

1Assistant Prof. Dr. University of Kufa/Faculty of Education for Girls / Department of Physical Education and Sports Sciences / IRAQ, 2Lecturer. Dr. Ministry of Education / Najaf Governorate / Institute of Arts / IRAQ, 3Assistant Prof. Dr. University of Qadisiyah/Faculty of Physical Education and Sports Sciences / IRAQ

Abstract

Excellence in the sports field has become one of the essential metrics on which the progress of nations and peoples is viewed. In order to draw up valid scientific policies represented in the use of modern strategies and methods to prepare the appropriate educational and training curricula in order to reach the learners to the highest levels, individual learning strategies must be used, which is self-learning, as they take into account the individual differences between the learners and reduce the time and effort required for this process. Keller’s strategy is for individual or personal learning. One of the strategies that achieve this goal.

The researchers believe that learning with the Keeler strategy is a personal process, as learning cannot be given or indoctrinated but must result from the educated person’s experience and ability to retrieve his memory to perform better than what he witnessed. The field of physical education and education. The researchers identified the research community with students of the second stage in the College of Physical Education and Sports Sciences - the University of Kufa for the academic year 2017-2018, numbering 30 students, divided into two groups, each group consisting of (12) students, one of whom was chosen randomly to represent the experimental group to which the educational program will be applied. Under consideration is what will be applied to the other, which is the control group, the prescribed curriculum, and according to the method used, and the seven female students were chosen to represent the sample that will be conducted on the exploratory experiment.

Key words: cognitive achievement Keeler’s strategy, skills, volleyball

Introduction

Excellence in the sports field has become one of the essential metrics on which the progress of nations and peoples is viewed 1. And in order to draw up valid scientific policies represented in the use of modern strategies and methods to prepare appropriate educational and training curricula in order to reach the learners to the highest levels, individual learning strategies must be used, represented by self-learning, as they take into account the individual differences between learners and reduce the time and effort required for this process 2. Keeler’s strategy is for individual or personal learning. One of the strategies that achieve this goal.

Here, the researchers believe that learning with the Keeler strategy is a personal process, as learning cannot be given or indoctrinated but must result from the educated person’s experience and ability to retrieve his memory perform better than what he saw. In the field of physical education and education.

Research Problem

By informing researchers of many studies and research and through their experience that they have
been working in the educational field for quite some time, the teacher and the teacher alike must pay attention to modern education and move learners from education based on indoctrination often to positive education and the transfer of knowledge to The learner is an active medium that goes in line with recent developments and makes the learner more effective in the educational process by creating situations in which he is more upbeat and active.

Therefore, the researchers decided to use a new strategy in education using the Keeler strategy and identify its impact on cognitive achievement and learning offensive volleyball skills for students.

**Research objectives**

- Designing an educational curriculum according to Keeler’s strategy in cognitive achievement and learning offensive skills in volleyball for students.
- Recognizing the effect of Keeler’s strategy on cognitive achievement and learning offensive skills in volleyball for students.

**practical part**

Research Methodology:

Research Methodology: The researchers used the experimental method for its suitability and research requirements and the appropriate means to prove its hypothesis.

**Research Community and Sample**

The researchers identified the research community with students of the second stage in the College of Physical Education and Sports Sciences - the University of Kufa for the academic year 2017-2018, numbering 30 students, divided into two groups, each group consisting of (12) students, one of whom was chosen randomly to represent the experimental group to which the educational program will be applied Under consideration is what will be applied to the other, which is the control group, the prescribed curriculum, and according to the method used, and the seven students were chosen to represent the sample that will be conducted on the pilot experiment.

**Steps of conducting a search**

achievement test:

To identify the effect of Keeler’s strategy on cognitive achievement and in order to achieve the goal of the research, the researchers used the modified cognitive test by “Thaier Rashid (and others)” (), which measures its 31 paragraphs on the cognitive aspects of the offensive skills understudy with three alternatives for each paragraph that the laboratory chooses The correct answer is among them.

**The pilot experiment:**

To ensure obtaining reliable results, an exploratory experiment was conducted on (6) female students who did not enter the main experiment and from the same research community at exactly eight-thirty in the morning on Saturday 17/11/2017 in order to identify the most critical obstacles and errors that the researcher may face when Implementation of the main experiment as well as knowing the time taken to implement the skill tests and the extent of the research sample’s interaction in its Implementation Grades according to the skill performance appraisal forms.

**Scientific basis for the tests:**

In order to identify the scientific bases for the tests used, and after the researcher was informed of many sources, it became clear that they are codified on the Iraqi environment and were used in several studies as well as using them on similar samples. Constancy, objectivity).

**Equivalence of the two research groups:**

“In order for the researcher to return the difference to the experimental factor, the experimental and control groups must be completely equal in all their conditions, except for the experimental variable that
affects the experimental group”, the researchers divided the sample into two groups at random, and in order to achieve this, the researcher performed the equivalence process between the two research groups for the tests understudy, a t-test was used for independent samples, and the results showed that there were no significant differences between the two groups, which confirms the equivalence between them as shown in Table (1)

Table (1). It shows the equivalence between the experimental and control groups in cognitive achievement tests, serving skills, crushing hitting and blocking in volleyball, and the calculated and tabular (T) values and their statistical significance

<table>
<thead>
<tr>
<th>the test</th>
<th>Measurement</th>
<th>Experimental groups</th>
<th>control groups</th>
<th>Value (T)</th>
<th>denotation statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Std. deviation</td>
<td>Mean</td>
<td>Std. deviation</td>
</tr>
<tr>
<td>Cognitive achievement</td>
<td>Degree</td>
<td>15.416</td>
<td>7.076</td>
<td>15.916</td>
<td>7.656</td>
</tr>
<tr>
<td>Transmission</td>
<td>Degree</td>
<td>1.916</td>
<td>0.900</td>
<td>2</td>
<td>0.953</td>
</tr>
<tr>
<td>Straight smash</td>
<td>Degree</td>
<td>1.416</td>
<td>0.999</td>
<td>1.333</td>
<td>0.984</td>
</tr>
<tr>
<td>Straight Accelerator</td>
<td>Degree</td>
<td>1.583</td>
<td>1.083</td>
<td>1.916</td>
<td>0.900</td>
</tr>
</tbody>
</table>

**Pre-test:**

The control and experimental groups were pre-tested to determine the students’ skill level at 8:30 a.m. on Sunday and Monday, 22 and 23/11/2017, and the same assessors gave their scores to each student.

**Curriculum**

After preparing the requirements to implement the research experiment, work on the program (the experiment) began on the corresponding Tuesday, at a rate of two academic units per week for eight weeks, at a rate of (16) sixteen academic units.

Below is an explanation of how to apply the tutorial:

The researchers distributed the educational material into independent academic units and placed it in the time frame as follows:

The educational material was divided into 16 educational units distributed over eight weeks, with two educational units per week, with a time of 90 minutes for each educational unit.

According to Keeler’s strategy, the researchers applied for the educational program to the first group (experimental). In contrast, the curriculum was applied to the second group (control) for students of the second stage in the Faculty of Physical Education, in which the following steps were followed:

1- The academic unit time for the experimental and control groups is (90) minutes.

2- Inform the students of the date of the academic unit and prepare before its start (at the break).

3- Read the instructions and explain how to use the Keeler strategy with an explanation of how to deal with the program.
4- The beginning of the academic unit is in the classroom instead of the external arena in the college, starting with the educational section, during which the lecture is displayed on a 51-inch plasma screen (LCD) via a computer (laptop), starting with displaying the written and readable text and then displaying the images. Explanations that show what has been read in the text, noting that this is followed by a presentation of a video clip that also expresses the content of the text. At the end of this part, the evaluation questions are presented to the students, consisting of three Annex 2 questions, and the students must answer them with supervision by the observers, provided that they are informed of the extent to which the answer is correct or not. This is called feedback, which is a characteristic of reinforcement, which means that notifying the student of the correctness of his answer reinforces him and increases the probability of repeating the correct response later.

5- After completing this part of the lesson and with the help of the assistant staff, we move to the outside arena to apply the rest of the educational unit parts.

6- The second part of the educational unit takes place in the external arena and according to the sequence of the educational unit as in the method followed, except for the educational section that was given in the classroom for the experimental group.

7- In this part, the student applies the skill to teach himself and according to what he understood, absorbed and learned from the educational part under the supervision of the assistant staff, through skill training according to three levels, and each student must start from the level he deems appropriate for his abilities and capabilities, and then move to the higher level. Then the higher only after he has mastered the performance of that skill, provided that this is done under the direct supervision of the responsible professor and the staff who helps him in carrying out the lecture, as follows:

8- In performing the exercises for the skill of serving from the top, three circles were drawn, the first with a diameter of 7 m, the second with a diameter of 5 m, and the third with a diameter of 3 m. That this be done under the supervision of the observers present during the performance of the exercises.

9- As for the skill of crushing, three squares have been created in three levels, the first of which is 3 m long, the second 2.5 m, and the third 2 m. A space with full supervision of the observer present while performing the exercises.

10- As for the skill of the wall, three sites are created, each site contains a certain height of the network, the first height is 1.70 cm, the second is 1.90 cm, and the third is 2 m. Which he chose under the supervision of one of the observers present in the exercise ground. However, it was emphasized that the sample members need to constantly attend the educational units, not to be absent, and to cooperate with the assistant staff.

11- In the last section of the lecture, i.e. the concluding section, its duration is (3 minutes), which includes general relaxation and calming exercises, as well as a small game for the purpose of recreation and suspense, after which the tools and balls are collected and orderly withdrawal to the classroom.

Post-Tests

The post tests were conducted for the period from 25-1-2018 -27-1-2018 and included skill tests and cognitive achievement for the experimental and control groups.

Statistical means:

The researcher used the statistical bag (SPSS) to process the data.

Results

Presenting and analyzing the results of the pre and post tests of the experimental group in cognitive achievement, serving skills, hitting the smasher and
individual blocking in volleyball:

**Table (2). It shows the arithmetic means, standard deviations, the calculated and tabular (T) values and their statistical significance for the tests under study, before and after the experimental group.**

<table>
<thead>
<tr>
<th>the test</th>
<th>Measurement</th>
<th>Experimental groups</th>
<th>control groups</th>
<th>value (t) calculated</th>
<th>indication statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Std. deviation</td>
<td>Mean</td>
<td>Std. deviation</td>
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<tr>
<td>Cognitive achievement</td>
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<td>15.416</td>
<td>7.076</td>
<td>60.166</td>
<td>5.859</td>
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<td>Transmission</td>
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<td>0.900</td>
<td>7.083</td>
<td>0.792</td>
</tr>
<tr>
<td>Straight smash</td>
<td>degree</td>
<td>1.416</td>
<td>0.999</td>
<td>6.333</td>
<td>0.651</td>
</tr>
<tr>
<td>Straight Accelerator</td>
<td>degree</td>
<td>1.583</td>
<td>1.083</td>
<td>6.333</td>
<td>0.651</td>
</tr>
</tbody>
</table>

Presenting and analyzing the results of the pre and post tests of the control group in cognitive achievement, serving skills, hitting the smasher and individual blocking in volleyball:

**Table (3). It shows the arithmetic means, standard deviations, the calculated and tabular (T) values and their statistical significance for the tests under study, before and after the control group.**

<table>
<thead>
<tr>
<th>the test</th>
<th>Measurement</th>
<th>Experimental groups</th>
<th>control groups</th>
<th>value (t) calculated</th>
<th>indication statistic</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td>Mean</td>
<td>Std. deviation</td>
<td>Mean</td>
<td>Std. deviation</td>
</tr>
<tr>
<td>Cognitive achievement</td>
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</tr>
<tr>
<td>Transmission</td>
<td>degree</td>
<td>2</td>
<td>0.953</td>
<td>5.166</td>
<td>0.717</td>
</tr>
<tr>
<td>Straight smash</td>
<td>degree</td>
<td>1.333</td>
<td>0.984</td>
<td>5</td>
<td>0.603</td>
</tr>
<tr>
<td>Straight Accelerator</td>
<td>degree</td>
<td>1.916</td>
<td>0.900</td>
<td>4.916</td>
<td>0.668</td>
</tr>
</tbody>
</table>

(*) Tabular value (T) under the degree of freedom (11) and the probability of error (0.05) = (2.20).

Presenting and analyzing the results of the post-tests of the two experimental and control groups in cognitive achievement, serving skills, hitting the smasher and the blocking wall:
Table (4). the arithmetic means, standard deviations, the calculated and tabular (T) values and their statistical significance.

For the post-tests of the experimental and control groups

<table>
<thead>
<tr>
<th>the test</th>
<th>Measurement</th>
<th>control groups</th>
<th>value (t) calculated</th>
<th>indication statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measurement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean  Std. deviation</td>
<td>Mean  Std. deviation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitive achievement</td>
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<tr>
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</tr>
<tr>
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<td>degree</td>
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<td>5 0.603</td>
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<tr>
<td>Straight Accelerator</td>
<td>degree</td>
<td>6.333 0.651</td>
<td>4.916 0.668</td>
<td>5.042</td>
</tr>
</tbody>
</table>

(*) Below the significance level (0.05) and the degree of freedom (22)

Discussing and analyzing the results of the post-tests of the experimental and control groups in cognitive achievement, transmission skills, smash hits, and individual blocking:

It is evident from the results of Table (4), after processing the results of the post-tests for the experimental and control groups, that the calculated T-value in the cognitive achievement test amounted to (8.272), as for the skill tests in question (sending, crushing and individual blocking) for the control and experimental groups and after Statistically processed, the value of (T) calculated in the transmission skill test reached (5.990), in the crushing beating skill test, it amounted to (5.011), and in the individual blocking wall skill test, it reached a value of (5.042), and it is clear from the above that the calculated (T) values for the variables under research are it is greater than the tabular value of (2.20) at a degree of freedom (22) and a probability of error (0.05), and the researcher attributes that the superiority of the experimental group is due to the use of the Keeler strategy, including the principles it contains, including self-learning, since this strategy is the best credible for this type of learning. Which has outperformed many well-known teaching strategies as it provides sufficient opportunity for the learner through freedom in the time he takes to learn and gives the student the opportunity to start learning from the level he considers appropriate for his mental abilities and physical capabilities.

Through the foregoing presentation, analysis and discussion of the results of the tribal, follow-up and posterior tests for the experimental and control groups, and the superiority of the experimental group over the control group by using Keeler’s strategy in cognitive achievement and teaching offensive skills in volleyball through the clear impact of using Keeler’s strategy on cognitive achievement and teaching offensive skills in volleyball. Through the superiority of the experimental group that learned according to this strategy, whether in the cognitive achievement of the educational material included in the educational program, or in teaching offensive skills in volleyball, which are the skills of (serving from the top, crushing beating, and individual blocking).
The researcher would also like to confirm that the good application of this strategy with all its components and the emphasis on the cognitive aspect contributed to the achievement of the research objectives.

Based on the foregoing, we find that the skill performance has a close and direct relationship with the cognitive aspect of the individual, as the educational process for any skillful performance in volleyball must be at the beginning of the early stages of learning to focus on the cognitive aspect of these skills in order to help in gaining performance faster and better than Learn it.

Thus, the objectives of the research have been achieved in the impact of Keeler’s strategy on cognitive achievement and learning offensive skills in volleyball, and to identify the priority of the two research groups, which was represented by the superiority of the experimental group over the control group.

Conclusions

In light of the results of the tests, their analysis and discussion, the researcher reached the following conclusions:

1. There are significant differences between the results of the pre and post tests for the experimental and control groups in all research variables in favor of the two groups.

2. The advantage of learning using Keeler’s strategy over the adopted strategy.

3. The use of Keeler’s strategy has a clear and significant effect in increasing the cognitive achievement, the learning process and the speed of learning the skills of serving, smashing and blocking in volleyball.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the Faculty of Education for Girls and carried out in accordance with approved guidelines”.

References

Effectiveness of an Educational Program on Nurses- Midwives Knowledge about Preterm Labor in Maternity Hospitals at Baghdad City

Bushra Thiab Obead1, Iqbal Majeed Abbas2
1Ph.D. Student, Academic Nursing Specialist, Baghdad Health Directorate Al-Risafa Sector, Ministry of Health Iraq, 2Ph.D. Emeritus Professor and Academic Supervisor, Baghdad College of Medical Sciences / Nursing Department

Abstract

Objectives: To determine the effectiveness of the educational program on nurses-midwives knowledge concerning preterm labor and to identify the relationship between nurses- midwives knowledge and study variables.

Methodology: A quasi-experimental design conducted on non-probability (purposive) sample of forty five nurses- midwives selected during period 17th October 2020 to 1st March 2021. The study is conducted at the four hospitals of Ministry of Health (Baghdad health directorate in Al-Karhk and Al-Risafa) sector. in obstetric wards and delivery room and neonatal Unit. The questionnaire form is consisted of two parts which included socio-demographic characteristics of the nurses-midwives and knowledge about preterm labor and birth. Reliability of the questionnaire was determined through internal consistency of correlation coefficient (R=0.807) and validity of content was determined through reviewing it by ten experts in different specialists was determined through pilot study. Descriptive and inferential statistics were used to analyze the date.

Result: The result of study revealed that their (33.3%) of study sample their age was (26-30) years old with mean and standard deviation is (32.68 ± 8.31) years, and less of half of the study sample was graduated from midwifery secondary school, half of study sample were married, 46.7% of them had less than / equal to (5) years of experience in nursing and midwifery field. And (80%) of the them did not practices midwifery as a profession inherited, (78%) of them were working in Government Hospital only , (70%) of (Nurses- Midwives) were not practicing midwifery at midwife home , (91%) of them having knowledge about preterm labor from Practical experience and (30%) of them suffered from preterm labor during their fertile age.

Keywords: Education, Effectiveness, Preterm labor, Nurses-Midwives, Knowledge

Introduction

Preterm birth (PTB) is still a widespread public health problem responsible for high perinatal mortality and long-term morbidity worldwide despite improved perinatal care programs, it still remains a major leading cause of perinatal mortality, mainly in developing countries (1). In developing countries, the main causes of preterm births include infectious diseases and poor availability and accessibility of health care resources. In high-income countries, the increase in the number of preterm births is linked to conception among older women and increased number of multiple pregnancies as a result of usage of fertility drugs. In some developed countries, medically unnecessary
inductions and cesarean section deliveries before full term also increase preterm birth rates. In rich and poor countries, many preterm births remain unexplained (2).

Preterm labor and birth are associated with traumatic life sites (eg, close family death, domestic violence, Anxiety about losing food, home or partner) either indirectly by associated risk behaviors. Many of these risk factors present together, such as chlamydia endocervicitis and third trimester bleeding. Rarely, cervical insufficiency results in spontaneous PTB. Preterm labor occurs for a variety of reasons. Most preterm births happen spontaneously, but some are due to early induction of labor or caesarean section, whether for medical or non-medical reasons. Common causes of preterm labor include multiple pregnancies, infections and chronic conditions such as diabetes and high blood pressure; however, often no cause is identified. There could also be a genetic influence. Better understanding of the causes and mechanisms will advance the development of solutions to prevent preterm birth (3).

Preterm labor is defined as the presence of uterine contractions of sufficient frequency and intensity to effect progressive effacement and dilation of the cervix prior to term gestation (between 20-37 wk). It has been the most concerning complication among pregnant women and affects 10% of all pregnancies. Annually 1 million neonatal deaths occur due to preterm birth. Unfortunately, there are no statistics in the Ministry of Health on preterm labor, but only on caesarean sections, normal vaginal delivers and abortion. The four major factors leading to preterm labor are: decidua hemorrhage, intrauterine infection, excessive uterine stretch, and maternal or fetal stress. Nurses-midwives are often the main fact of contact during the primary assessment and direct bedside care to women experiencing signs and symptoms of PTL; therefore, knowledge of identification, care, and treatment is essential. Nurses as frontline clinical experts are in an ideal position to address the complete needs of pregnant women and their support systems (4).

Nurses-midwives Knowledge about medications used to inhibit PTL enhances the nurse’s ability to recognize efficacy of tocolysis therapy and potential harmful side effects. Nurses-midwives should focus education on individualized pregnant women needs, home care and supplier follow-up instructions, and signs and symptoms that require seeking immediate medical attention such as ruptured membranes, bleeding, increasing frequency and intensity of contractions as well as decreased fetal movement (5).

Nurses-midwives knowledge and skills, timely identification of signs, symptoms, complications, cooperative and communication are essential for nurses-midwives caring for women in PTL and who give birth preterm.(6)

The most common interventions suggested to prevent preterm birth, such as bed rest, tocolytics, antibiotics and cervical cerclage have been proven to have little or no benefit. The goal of treatment is only to delay delivery in order to allow for the transfer of the pregnant woman to the most appropriate hospital and for administration of corticosteroids. Premature preterm labor newborn are at greater risk for cerebral palsy, delays in development, hearing problems and sight problems (7).

Methodology

A quasi-experimental design was conducted from 17th October 2020 to 1st March 2021 to determine the effectiveness of the educational program conducted on nurses-midwives in the labor room about general knowledge concerning preterm labor, in obstetric wards and delivery room and neonatal unit in five maternity hospitals at Baghdad city: Fatima Al – Zahra, Al -Elwia maternity, iben Al-Balady and Al-Karkh maternity Teaching hospital. Questionnaire format and education program were constructed to determine the effectiveness of educated program. None probability (purposive) sample was used to select the sample of 45 nurses and midwives.
A pilot study was carried out for the period from (1st of September to 9th of October /2020). The validity of the questionnaire was estimated through a panel of experts related to the field of the study, and its reliability was estimated through split half approach which was statistically accepted. The study instrument consisted of two parts which include:

- demographic data such as age, Level of education, Social Status, Years of experience in nursing and midwifery field, Sources of knowledge about preterm labor, Reproductive statues for the midwife, and knowledge of nurses - midwives towards preterm labor which consisted of four main domains include:
  - (26) questions related nurses-midwives knowledge regarding Preterm Labor,
  - (13) questions related nurses-midwives knowledge about premature birth.

Scale of the questionnaire is (multiple choice) the correct answer code was (2) and the wrong answer code was (1). The implementation of the educational program was done within nine educational sessions during one week at delivery room In addition each nurses/midwives had one (30-45) minute session with the researcher how give information about preterm labor and answered questions on preterm labor, pretest and giving lectures.

The researcher distributed the questionnaire for nurses after taking their agreeable to participate in this study the interview was conducted with volunteer nurses It took about 20 to 40 minutes for interviewing and after that the questionnaire was collected. Statistical analyses were conducted by using statistical package for social science (SPSS) version (20) Data analysis was employed through the application of descriptive and inferential statistical approaches which were performed through the computation of the following: frequencies, percentage, standard deviation, t-test and Chi-squar.

### Results and Discussion

**The present study as shown in table (1)** that the highest percentage (33.3%) of study sample was (26-30) years old with mean and standard deviation (x̅ ± SD) is (32.68 ± 8.31) years, and less of half of the study sample was graduated from midwifery secondary school, half of study sample were married, 46.7% of them had less than / equal to (5) year of Experience in Nursing and midwifery field. And (80%) of them did not practices midwifery as a profession inherited , (78%) of them were work in Government Hospital only , (70%) of (Nurses- Midwives) were not practicing midwifery at midwife home , (91%) of them having knowledge about preterm labor from Practical experience and (30%) of them Suffered from preterm labor during their fertile age. These results agree with other study finding indicate that that the highest percentage (were in the age group ranging between 20 years to less than 30 years) and disagree with study was conducted by Hasan who reported that the highest percentage at age group ranged between (26-30) years with mean and standard deviation was (33.84 ± 9.40). The present study shows the majority (86.6%) of them had secondary nursing school. The finding agrees with study stating that the highest percentage (57.5 %) of the nurses are graduated from secondary school.

**Table (2)** show the highest percentage (35.6%) their job description were skilled midwife this result is consistent with study stating that the highest percentage (77.3%) of the sample were skilled midwives. Regarding years of experience the majority of study sample had less than 5 years in all field of nursing and midwifery such as (nursing field emergency department, delivery room, neonatal intensive care unit (NICU)).

this result was in agreement with study by Qusay who reported that more than forty of the participants (78%) had less than five years of nursing experience in premature units (NICU). Ramasamy et.al found that the highest percentages (76.7%) of the participant had (1-5) years of experience in nursing field. The present study was disagreement with other study who reported that more than half of nurse/midwives (53.3%) had 1– 9 years’ experience in the labor room. The highest percentage 41(91.1%) of
the (Nurses and Midwives) having knowledge about preterm labor from Practical experience as shown in figure (1): The present study was disagreement with Nury who reported that the majority of them (73.3%) had participated in training courses regarding care during labor and delivery. 15(33.3%) of them having knowledge about preterm labor from academic study 10 (22.2%) of them having the same percentage knowledge about preterm labor from attending training courses and browse the Internet respectively and 8(17.8 %) of them having knowledge about preterm labor from medical journals and scientific books and magazines. Also Nuriy reported that the majority of sample (73.3%) had participated in training courses regarding care during labor and delivery (13).

The present study shows there are (low MS & RS) in most items in pretest period, this current study agree with Niazi who stated that the majority of (60.3%) midwives were poor knowledge in Baghdad City hospitals due to several reasons (14). Except item No: (1): Definition of preterm labor, Item No: (17): Secondary of postpartum hemorrhage and item No: (21): When are cervical ceceral suture removed: Had (moderate MS & RS) in pretest (before implementation of the program) for study sample this indicates that the nurses- midwives had good information about preterm birth. As shown in table (3).

While the current study revealed there are (high and moderate mean score &relative sufficiency ) in some of items in post1 test (after implementation of the program) for study sample. The finding of present study is similar with study was conducted at Sudan by Hassan & Nasr who reported that the impact of an educational program on nurses’ knowledge and nursing management of preterm labor patients receiving tocolytics. they reported all nurses archived better scoring in both knowledge and skills after implementing the program (15)

The current finding is in agreement with hassan et al to assess nurses midwives practices about pain management during labor before and after implementation of educational program, they found there are (high MS&RS) after implementation of education program (16).

The current study revealed there are show (Low MS &RS) in post 1 test in item No (5): Rupture of the amniotic fluid, item No : (6): Pregnant women with a BMI above 35 , item No : (8): cause and predisposing factors for preterm labor, item No : (14): Test is performed for the pregnant mother who suffer from preterm labor after the implementation of the educational training program this is may be due to over loaded work in delivery room in addition to the lack in the number of staff to conducting the job in the delivery room.

The present study shows that (high and moderate MS &RS) in most items related to knowledge of nurses and midwives regarding preterm labor in post 2 test period, Except item No: (2) Signs and symptoms of preterm labor, item No (5): Rupture of the amniotic fluid, item No (6): Pregnant women with a BMI above 35, item No (7): There are causes and risk factor for preterm labor, item No (9): Methods of diagnosing preterm labor, item No (13): Complications of premature birth to the newborn on Longtime, item No (18): The most common indicator of (stimulation) to preterm labor, item No (22): Termination of pregnancy when the pre rupture of membranes occurs , item No (23): Information about vaginal progesterone supplements, item No (25): Side effects of, item No (26): modern treatments for rupture of the amino membrane at 32week. Had (Low MS &RS) after implementation of the program in Post-2- test period. related to general knowledge of nurses and midwives about preterm labor. The weak of results regarding Tocolytics drugs that have occurred through the research indicate that the midwife or nurse is not interested in understand and search for knowledge related to pharmaceutical field when given to the pregnant woman during labor and adopted only to the attending doctor and this therefore leads to undesirable results. So the current
result show the (low MS & R.S) in nurses – midwives knowledge about medications of preterm labor in pre and post 2 test. The present study was disagreement with study conducted in Egypt by Hassan & Nasr who revealed that there were an improvement of the nurse’s knowledge in all items (definition, indication, contraindication and side effect) regarding the knowledge of tocolytics drugs (8), as show in table (3).

Table (4) show that there was (low MS&RS) in all items of nurses and midwives knowledge in pretest for study sample. The current study agrees with other study conducted in Punjab city and reported that the majority of nurses had less than “good” in the pretest. While in the post test had good and excellent level of knowledge after teaching program regarding care of low birth weight baby (17).

Also item No (5): (Low body temperature of premature birth ) had (moderate MS &RS) in pretest (before implementation of education program) for study sample, Similar findings were reported by a study in Sudan which showed that the nurses knowledge improved to be good by majority of the nurses when testing their knowledge in preventing hypothermia premature babies’ (18). While there are (high and moderate MS&RS) in some items of nurses-midwives knowledge in (post 1 and post 2 test). This result agreement with Young el at who reported that the training intervention highly improved of nurses –midwives in knowledge after implementation of education program (19).

Table (5): show there are significant differences between (pretest and post 1 knowledge test). The results of current study are similar with other studies stating to assess the effectiveness of information booklet on management of preterm babies this result revealed the overall mean posttest knowledge score was higher than the mean pre-test knowledge score (20).

While there are no significant differences between (post 1 and post 2 knowledge) after the implementation of education program for nurse-midwives regarding pre-term labor.

The present study disagreed with other studies stating that there are significant correlations between pretest and posttest periods after the implementation of education program for nurses- midwives’ knowledge regarding fetal investigations (10).

Table (6) shows that there are no statistical significant differences between overall knowledge of nurses-midwives about Pre-term labor (pre and post term) and studied variables such as: ( Level of education , marital status, years of service in nursing, years of experience in emergency, years of service in delivery room, years of service in NICU, midwifery profession inherited , specialized training courses in preterm birth and preterm labor.

Also Ali who reported that there were no statistical significant difference between nurse-midwives knowledge and some socio demographic characteristics such as: age ,educational level,marital status, number of years of total service, number of years of service (experience) in the delivery room, number of training courses related to pregnancy and childbirth (21).

There is statistical significant differences between overall knowledge of nurses-midwives and Age groups in (pretest). This findings were similar with Hassan who reported that a significant direct (positive) correlation had been found between age of participants and their overall knowledge score (8).

This findings were disagree with Farrell who found a total of (31) nurse and (36) midwives were included in the study and control respectively. There were no differences regarding the Age and other Socio-Demographic Variables (22).

There is a statistical significant association between pre and post variables and overall knowledge at (p.value: 0.005) and there is no statistical significant association between socio-demographic variables and overall knowledge, except age groups there was a
statistical significant with overall knowledge in pretest as shown in table (6).

**Table (1): Distribution of the study sample according to socio demographic characteristics (n=45)**

<table>
<thead>
<tr>
<th>No</th>
<th>Socio Demographic Characteristic</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>21-25</td>
<td>8</td>
<td>17.8</td>
</tr>
<tr>
<td>2</td>
<td>26-30</td>
<td>15</td>
<td>33.3</td>
</tr>
<tr>
<td>3</td>
<td>31-35</td>
<td>9</td>
<td>20.0</td>
</tr>
<tr>
<td>4</td>
<td>36-40</td>
<td>3</td>
<td>6.70</td>
</tr>
<tr>
<td>5</td>
<td>41-45</td>
<td>6</td>
<td>13.3</td>
</tr>
<tr>
<td>6</td>
<td>46 &amp; Above</td>
<td>4</td>
<td>8.90</td>
</tr>
</tbody>
</table>

\[ \bar{x} \pm SD \quad 32.68 \pm 8.31 \]

<table>
<thead>
<tr>
<th>2</th>
<th>Level of education</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Graduate Nursing School</td>
<td>2</td>
<td>4.40</td>
</tr>
<tr>
<td>2</td>
<td>Graduate secondary nursing school</td>
<td>7</td>
<td>15.6</td>
</tr>
<tr>
<td>3</td>
<td>Graduate secondary of Midwifery school</td>
<td>20</td>
<td>44.4</td>
</tr>
<tr>
<td>4</td>
<td>Graduate Institute of Higher Health Professions</td>
<td>4</td>
<td>8.90</td>
</tr>
<tr>
<td>5</td>
<td>Graduate Institute of Midwifery</td>
<td>9</td>
<td>20.0</td>
</tr>
<tr>
<td>6</td>
<td>Graduate college of nursing</td>
<td>3</td>
<td>6.70</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3</th>
<th>Social Status</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Single</td>
<td>14</td>
<td>31.1</td>
</tr>
<tr>
<td>2</td>
<td>Married</td>
<td>23</td>
<td>51.1</td>
</tr>
<tr>
<td>3</td>
<td>Divorced</td>
<td>5</td>
<td>11.1</td>
</tr>
<tr>
<td>4</td>
<td>Widow</td>
<td>3</td>
<td>6.70</td>
</tr>
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</table>
Table (2) Distribution of study sample according to job description and years of experience (n=45).

<table>
<thead>
<tr>
<th>No</th>
<th>Variables</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Job description</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>University Nurse Trainer</td>
<td>2</td>
<td>4.40</td>
</tr>
<tr>
<td></td>
<td>Older University Nurse</td>
<td>5</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Head of University Nurse</td>
<td>1</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td>Technical nurse</td>
<td>1</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td>Skilled midwife</td>
<td>16</td>
<td>35.6</td>
</tr>
<tr>
<td></td>
<td>Oldest Skilled midwife</td>
<td>10</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>Head of Skilled midwife</td>
<td>10</td>
<td>22.2</td>
</tr>
<tr>
<td>2</td>
<td>Years of experience in nursing field</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤ 5 year</td>
<td>21</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td>5 – 9 years</td>
<td>12</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td>10 – 14 years</td>
<td>8</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td>15 year and above</td>
<td>4</td>
<td>8.9</td>
</tr>
<tr>
<td>3</td>
<td>Years of Experience in Emergency department</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤ 5 year</td>
<td>40</td>
<td>88.9</td>
</tr>
<tr>
<td></td>
<td>5 – 9 years</td>
<td>3</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>10 – 14 years</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>≤ 15 years</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>4</td>
<td>Years of Experience in Delivery room</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤ 5 year</td>
<td>24</td>
<td>53.3</td>
</tr>
<tr>
<td></td>
<td>5 – 9 years</td>
<td>15</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>10 – 14 years</td>
<td>5</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>≤ 15 years</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>5</td>
<td>Years of Experience in Neonatal intensive care unit (NICU)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤ 5 year</td>
<td>39</td>
<td>86.7</td>
</tr>
<tr>
<td></td>
<td>5 – 9 years</td>
<td>6</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>10 – 14 years</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Result out of this figure reveals that the highest percentage 41(91.1%) of the (Nurses and Midwives) having knowledge about preterm labor from Practical experience, 8(17.8%) of them having knowledge about preterm labor from medical journals and scientific books and magazines.

Table (3): Differences between pre and post variables for the overall knowledge of nurses-midwives about preterm labor for study sample (n=45).

<table>
<thead>
<tr>
<th>Paired Samples Test</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Knowledge</td>
<td>Mean</td>
<td>Std. Deviation</td>
</tr>
<tr>
<td>Pre knowledge</td>
<td>11.80</td>
<td>3.24</td>
</tr>
<tr>
<td>Post 1 knowledge</td>
<td>13.80</td>
<td>13.79</td>
</tr>
<tr>
<td>Post 1 knowledge -</td>
<td>2.00</td>
<td>12.95</td>
</tr>
<tr>
<td>Post 2 knowledge</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( t\) = t-test, \( P.V \) = Probability value, \( Sig \) = Significant at \( P \leq 0.05 \).
Table (4): Differences between socio-demographic variables and overall knowledge of nurses-midwives about pre-term labor for study sample (n=45) Chi-square.

<table>
<thead>
<tr>
<th>No</th>
<th>socio-demographic variables</th>
<th>pre</th>
<th>Post</th>
<th>df</th>
<th>( \chi^2 ) crit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \chi^2 )</td>
<td>obs</td>
<td>p.value</td>
<td>sig</td>
</tr>
<tr>
<td>1</td>
<td>Age Groups</td>
<td>9.600</td>
<td>.0087</td>
<td>S</td>
<td>7.326</td>
</tr>
<tr>
<td>2</td>
<td>Level of education</td>
<td>8.346</td>
<td>0.138</td>
<td>NS</td>
<td>2.616</td>
</tr>
<tr>
<td>3</td>
<td>Marital status</td>
<td>0.457</td>
<td>0.928</td>
<td>NS</td>
<td>2.002</td>
</tr>
<tr>
<td>4</td>
<td>Years of service in nursing</td>
<td>3.804</td>
<td>0.283</td>
<td>NS</td>
<td>2.812</td>
</tr>
<tr>
<td>5</td>
<td>Years of experience in emergency</td>
<td>1.050</td>
<td>0.789</td>
<td>NS</td>
<td>6.344</td>
</tr>
<tr>
<td>6</td>
<td>Years of service in delivery room</td>
<td>1.980</td>
<td>0.583</td>
<td>NS</td>
<td>4.186</td>
</tr>
<tr>
<td>7</td>
<td>Years of service in NICU</td>
<td>0.865</td>
<td>0.352</td>
<td>NS</td>
<td>0.570</td>
</tr>
<tr>
<td>8</td>
<td>Is the midwifery profession inherited</td>
<td>0.085</td>
<td>0.771</td>
<td>NS</td>
<td>0.386</td>
</tr>
<tr>
<td>9</td>
<td>Specialized training courses in preterm birth and preterm labor</td>
<td>1.029</td>
<td>0.310</td>
<td>NS</td>
<td>0.599</td>
</tr>
</tbody>
</table>

**Conclusion**

The study was concluded that there was a statistically significant difference in nurses-midwives overall knowledge related to preterm labor between the pretest and posttest for study sample. While there are no statistically significant association between post 1 knowledge and post 2 knowledge. After the implementation of education program for nursery-midwives regarding Pre-term labor. There is statistical significant differences between overall knowledge of nurses-midwives and studied variables such as: Age Groups in (pretest). While there was no any association between knowledge of nurses – midwives and studies variables.

**Recommendation:**

Based on the study results, the study recommended that: Continuous and update of education program for nurses –midwives development and improvement of their knowledge for dealing with pregnant women who suffering from preterm labor.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Ministry of Health and all experiments were carried out in accordance with approved guidelines.

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Effectiveness of an Educational Program on Nurses- Midwives Performance about Preterm Labor in Maternity Hospitals at Baghdad City

Bushra Thiab Obead¹, Iqbal Majeed Abbas²

¹Ph.D., Student, Academic Nursing Specialist, Baghdad Health Directorate Al-Risafa Sector, Ministry of Health, Iraq, ²Ph.D. Emeritus Professor and Academic supervisor, Baghdad College of Medical Sciences / Nursing Department, Iraq

Abstract

Objectives: determined the effectiveness of an educational program on nurses - midwives performance related to preterm labor and to Identify the relationship between the performance of nurses- midwives and study variables.

Methodology: A quasi-experimental design (one group pre & post ) was conducted on non-probability (purposive) sample of forty five nurses- midwives selected during period from (17th October 2020 to 1st March 2021).The study is conducted at the four maternity hospitals (Baghdad health directorate in Al-Karhk and Al-Risafa) sector. The study sample was selected from obstetric wards and delivery room and neonatal unit .The questionnaire form is consisted of socio-demographic characteristics and Performance of nurses -midwives towards preterm labor through (checklist).

Result: The result of study revealed that the (33.3%) of study sample was (26-30) years old with mean and standard deviation (x̅ ± SD) is (32.68 ± 8.31) years, and less of half of the study sample was graduated from midwifery secondary school, half of study sample were married, 46.7% of them had less than / equal to (5) year of Experience in Nursing and midwifery field. And (80%) of the them did not performances midwifery as a profession inherited , (78%) of them were working in Government Hospital only , (70%) of (Nurses- Midwives) were not practicing midwifery at midwife home , (91%) of them having knowledge about preterm labor from Practical experience and (30%) of them Suffered from preterm labor during their fertile age.

The result of study revealed there are (low GMS &RS) in most items in pretest period regarding Performance of Nurses – Midwives. While there are (high and moderate GMS &R.S) in all items in post 1 test and post 2 observation (after implementation of the program) for study sample.

Keywords: Education, Effectiveness, Nurses-Midwives, Program, Preterm labor and Performance.

Introduction

Preterm birth (PTB) is still a widespread public health problem responsible for high perinatal mortality and long-term morbidity worldwide despite improved perinatal care programs, it still remains a major leading cause of perinatal mortality, mainly in developing countries (¹).

Preterm birth is one of the most troubling problems facing obstetrics today. In spite of all of the available refined research and therapeutic technology, the preterm birth rate has remained the same for the last 40 years. One birth in 10 occurs prematurely. Preterm labor demonstrates itself in a variety of ways. It is essential to differentiate true preterm labor from preterm contractions or other conditions that present with similar symptoms. True preterm labor requires
rapid clinical intervention in the obstetric triage setting. The prevention of preterm birth may be the most important problem facing maternity care providers.

In developing countries, the main causes of preterm births include infectious diseases and poor availability and accessibility of health care resources. In high-income countries, increase in the number of preterm births is linked to conception among older women and increased number of multiple pregnancies as a result of usage of fertility drugs. In some developed countries, medically unnecessary inductions and cesarean section deliveries before full term also increase preterm birth rates. In rich and poor countries, many preterm births remain unexplained.

To prevent preterm labor and delivery, a number of strategies have been developed. When choosing a strategy to prevent preterm birth, however, physicians must remember that preterm delivery arises from three separate conditions: iatrogenic preterm labor, premature rupture of membranes, and idiopathic preterm labor.

Preterm labor and birth are associated with traumatic life sites (e.g. close family death, domestic violence, Anxiety about losing food, home or partner) either indirectly by associated risk behaviors. Many of these risk factors present together, such as chlamydia endocervicitis and third trimester bleeding and cervical insufficiency.

Nurses and midwives are key players in empowering individuals and families and in promoting health safety and changes in health behavior throughout the life-course. The vital role of nurses or midwives in antenatal care is screening of pregnant women who are at risk for premature labor.

The role of nurses or midwives in childbirth is to ensure a safe environment for the mother and the birth of newborn and provide physical and emotional support. Nurses begin evaluating the mother through obstetrics history and determine the frequency, duration, intensity of uterine contraction, cervical dilatation and effacement and evaluating fetus during the labor, various techniques are used to assess maternal status. These techniques provide an ongoing source of data to determine the woman’s response and progress in labor which included: Assess maternal vital signs temperature, blood pressure, pulse, respiration, and pain, Prepare pregnant women for possible ultrasonography, amniocentesis, administer tocolytic agent or steroid therapy as prescribed. And assess for side effects of tocolytic therapy such as decreased maternal Blood pressure, Dyspnea, Chest pain, FHS >180beats/min FHS >180beats/min.

Objectives: To determine the effectiveness of an educational program on nurses - midwives performance related to preterm labor and to identify the relationship between the performance of nurses-midwives and study variables.

Methodology

A quasi-experimental design (one group pre & post) was conducted from (17th October 2020 to 1st March 2021), on nurses - midwives in five maternity hospitals at Baghdad city: Fatima Al-Zahra, Al-Elwia maternity, Iben Al-Balady, Dhari Al-Fayyad and Al-Karkh maternity Teaching hospital. None probability (purposive) sample was used to select the sample of (45) nurses and midwives. A pilot study was carried out for the period from (1st of September 2020 to 9th of October 2020). The validity of the questionnaire was estimated through a panel of ten experts related to the field of the study, and its reliability was estimated through Split half approach which was statistically accepted. The study instrument was consisted of two part: socio-demographic characteristics of the nurses-midwives and performance of nurses -midwives towards preterm labor through (checklist). used by the researcher for three observations, with sufficient time (30 to 45 minutes). An instrument comprised of (18) items It was constructed through the use of three levels of likert scale for the implementation of Performance items. The rating score of checklist was (three) for always, (two) for some times and (one) for...
never with cut-off point (2). Performance tests were used for pre, post1, and post 2 observation after one month.

The checklist consists of (131) items (nine main domains), each one of these included several items: Infection control and prevention includes (5) items, pregnant woman during stages of labor) includes (10) items, Preparing pregnant woman for birth) includes (9) items, to first stage of labor) includes (12) items, second stage of labor includes (10) items, second stage of labor includes (10) items, premature newborn includes (23) items, third and fourth stage of early labor includes (19) items, instructions for mothers and their relatives includes (29), nursing documentation includes (14) items.

By observation and recorded checklist immediately after implementation of an educational program regarding preterm labor. Nurses- Midwives were exposed to the post2 test questionnaire about (Performance) was conducting from the end of program during the first follow-up after 4 weeks of the post1test to identify their Performance regarding preterm labor. Data analysis was employed through the application of descriptive and inferential statistical approaches.

**Results and Discussion**

The present study was reported that the highest percentage (33.3%) of study sample are at age group ranged between (26-30) years old with mean and standard deviation (x̅ ± SD) is (32.68 ± 8.31) as shown in table (1). These findings are consistent with A quasi-experimental study it used three tools: (structured interview questionnaire, pre-post-test knowledge assessment sheet and an observation checklist) who reported that the highest percentage (were in the age group ranging between 20 years to less than 30 years)(7).

Also the present study was disagreement with other study who reported who reported that the highest percentage at age group ranged between (26-30) years with mean and stander deviation was (33.84 ± 9.40). (8)

The highest percentage of the study sample (44.4%) were secondary of midwifery school graduate. While the lowest percentage (4.40 %) and (6.70) were graduated from nursing school and (6.70%) graduate from college of nursing as shown in table (1). This result agreed with other study who reported that majority (86.6%) of them had secondary nursing school (9). Also agreed with other study who reported that the highest percentage (57.5%) of the nurses are graduated from secondary school, also mentioned that the (2.5 %) are from college of nursing. Half of the study samples were married. (10)

**Table (2)**: The result in current study shows that the highest percentage (35.6%) their job description were skilled midwife this result is consistent with other study employed who reported that the highest percentage (77.3%) of the sample were skilled midwives(8).

The majority of study sample had less than 5 years in all felid of nursing and midwifery such as (nursing field Emergency department, Delivery room, Neonatal intensive care unit (NICU)), this result was agreement with other study who reported 11 that more than forty of the participants (78%) had less than five years of nursing experience in premature units (NICU)(11). Ramasamy et al., found that the highest percentages (76.7%) of the participant had (1-5) years of experience in nursing field (12). The present study disagreement with study who reported that more than half of nurse/midwives (53.3%) had 1– 9 years’ experience in the labor room (13).

The Result

Reveals That there are (low GMS &RS) in most items in pretest period regarding all performance of Nurses – Midwives toward preterm labor. Except item No (2.1) Preparation the necessary instrument and equipment for delivery room, item No (2.2): Assessment of the health status of the pregnant
woman, item No (2.3): Assessment of the health status of the fetus, item No (2.5) performance related to first stage of labor, item No (2.6): performance related to second stage of labor, item No (2.8): Nurses-Midwives performance during forth stage of labor, regarding Nurses- midwives performance related to premature newborn item No (3.3): If the new born heart rate lasts below than 60 minutes despite the effective ventilation for 30 seconds, Domains (4): instruction and dominoes (5): Documentation. Had (high and moderate GMS &R.S) in all items in post 1 and post 2 test (after implementation of the program) for study sample. as show in table (3).

There are significant differences between (pre and post 1 observation and there are significant differences between (pre and post 2 observation). There are significant differences between (post 1 and post 2 observation) after the implementation of education program for nurse- midwives regarding pre-term labor. The percent study agreed with study Who revealed that there were a high significant relation between studied nurses’ total performances and personnel characteristics in post intervention (14).

There is no statistical significant differences between socio-demographic variables and overall performance of nurses-midwives about preterm labor.

The results of this study was disagreed with study who reported that there are insignificant relation between studied nurses socio-demographic characteristics and their performances strategies used in the prevention and management of PPH and normal labor (15).

The findings of the present study are almost similar with the results of previous study who found there is no significant association between performance of nurses/midwives and their level of education during labor and delivery (16).

Also findings of the present study disagree with study who reported that Nurses’ specialty had a significant effect on their total score of performances in the field of post-natal care (17).

This study differ with study who approved that there were no relation between nurses performances and general characteristics P value at 0.05 level. (18)

| Table (1): Distribution of the study sample according to socio demographic characteristics, (n=45) |
|---|---|---|
| No | Socio Demographic Characteristic | Frequency | % |
| 1 | Age (years) |  |
|  | 21-25 | 8 | 17.8 |
|  | 26-30 | 15 | 33.3 |
|  | 31-35 | 9 | 20.0 |
|  | 36-40 | 3 | 6.70 |
|  | 41-45 | 6 | 13.3 |
|  | 46 & Above | 4 | 8.90 |
|  | $\bar{x} \pm SD$ | 32.68 ± 8.31 |
| 2 | Level of education |  |
|  | Graduate Nursing School | 2 | 4.40 |
|  | Graduate secondary nursing school | 7 | 15.6 |
|  | Graduate secondary of Midwifery school | 20 | 44.4 |
|  | Graduate Institute of Higher Health Professions | 4 | 8.90 |
Cont... Table (1): Distribution of the study sample according to socio demographic characteristics, (n=45)

<table>
<thead>
<tr>
<th>Social Status</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>14</td>
<td>31.1</td>
</tr>
<tr>
<td>Married</td>
<td>23</td>
<td>51.1</td>
</tr>
<tr>
<td>Divorced</td>
<td>5</td>
<td>11.1</td>
</tr>
<tr>
<td>Widow</td>
<td>3</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Table (2) Distribution of study sample according to job description and years of experience(n=45).

<table>
<thead>
<tr>
<th>No</th>
<th>Variables</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Job description</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>University Nurse Trainer</td>
<td>2</td>
<td>4.40</td>
</tr>
<tr>
<td></td>
<td>Older University Nurse</td>
<td>5</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Head of University Nurse</td>
<td>1</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td>Technical nurse</td>
<td>1</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td>Skilled midwife</td>
<td>16</td>
<td>35.6</td>
</tr>
<tr>
<td></td>
<td>Oldest Skilled midwife</td>
<td>10</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>Head of Skilled midwife</td>
<td>10</td>
<td>22.2</td>
</tr>
<tr>
<td>2</td>
<td>Years of experience in nursing field</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤ 5 year</td>
<td>21</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td>5 – 9 years</td>
<td>12</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td>10 – 14 years</td>
<td>8</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td>15 year and above</td>
<td>4</td>
<td>8.9</td>
</tr>
<tr>
<td>3</td>
<td>Years of Experience in Emergency department</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤ 5 year</td>
<td>40</td>
<td>88.9</td>
</tr>
<tr>
<td></td>
<td>5 – 9 years</td>
<td>3</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>10 – 14 years</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>≤ 15 years</td>
<td>1</td>
<td>2.2</td>
</tr>
</tbody>
</table>
Table (3) Nurses- Midwives performance regarding preterm labor before and after implementation of educational program for study sample (n=45)

<table>
<thead>
<tr>
<th>No</th>
<th>Variables</th>
<th>Pre observation</th>
<th>Post 1 observation</th>
<th>Post 2 observation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GMS</td>
<td>RS</td>
<td>ASS</td>
<td>GM</td>
</tr>
<tr>
<td>1</td>
<td>Infection control and prevention</td>
<td>1.774</td>
<td>59</td>
<td>L</td>
</tr>
<tr>
<td>2</td>
<td>Performance Related to pregnant woman during stages of labor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>Preparation the necessary instrument and equipment for delivery</td>
<td>2.787</td>
<td>92</td>
<td>H</td>
</tr>
<tr>
<td>2.2</td>
<td>Assessment of the health status of the pregnant woman</td>
<td>1.253</td>
<td>41</td>
<td>L</td>
</tr>
<tr>
<td>2.3</td>
<td>Assessment of the health status of the fetus</td>
<td>1.183</td>
<td>39</td>
<td>L</td>
</tr>
<tr>
<td>2.4</td>
<td>Preparing pregnant mother for birth</td>
<td>2.416</td>
<td>80</td>
<td>M</td>
</tr>
<tr>
<td>2.5</td>
<td>performance related to first stage of labor</td>
<td>1.550</td>
<td>51</td>
<td>L</td>
</tr>
<tr>
<td>2.6</td>
<td>performance related to second stage of labor</td>
<td>1.833</td>
<td>61</td>
<td>L</td>
</tr>
<tr>
<td>2.7</td>
<td>performance related to third stage of early labor</td>
<td>2.328</td>
<td>77</td>
<td>M</td>
</tr>
<tr>
<td>2.8</td>
<td>Nurses- Midwives performance during forth stage of labor</td>
<td>1.678</td>
<td>55</td>
<td>L</td>
</tr>
<tr>
<td>3</td>
<td>Nurses- midwives performance related to premature newborn</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>Preparing the necessary equipment for neonatal resuscitation</td>
<td>2.497</td>
<td>83</td>
<td>M</td>
</tr>
<tr>
<td>3.2</td>
<td>Immediately Care for premature newborn after birth</td>
<td>2.320</td>
<td>77</td>
<td>M</td>
</tr>
<tr>
<td>3.3</td>
<td>If the new born heart rate lasts below than 60 minutes despite the effective ventilation for 30 seconds</td>
<td>1.755</td>
<td>58</td>
<td>L</td>
</tr>
<tr>
<td>3.4</td>
<td>After 30 seconds the ventilation and compression is stopped and the heart rate, breathing and color are re-assessed:</td>
<td>2.265</td>
<td>75</td>
<td>M</td>
</tr>
<tr>
<td>4</td>
<td>Instruction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>for Self-care after discharge</td>
<td>1.771</td>
<td>59</td>
<td>L</td>
</tr>
<tr>
<td>4.2</td>
<td>planning to next pregnancies</td>
<td>1.631</td>
<td>54</td>
<td>L</td>
</tr>
<tr>
<td>4.3</td>
<td>danger signs after post-partum period</td>
<td>1.518</td>
<td>50</td>
<td>L</td>
</tr>
<tr>
<td>4.4</td>
<td>danger signs to the newborn</td>
<td>1.465</td>
<td>48</td>
<td>L</td>
</tr>
<tr>
<td>5</td>
<td>Nursing Documentation</td>
<td>2.340</td>
<td>78</td>
<td>L</td>
</tr>
</tbody>
</table>

Ass = Assessment  F = Frequency, Percentage, GMS = Grand Mean Score, R.S = Relative Sufficiency (L = Low less than 75) , M = Moderate = 75 - 87.5 , H = High = 87.6 – 100).
Conclusion

The study was concluded there was (low GMS & RS) in most items of Nurses - Midwives performance regarding preterm labor before implementation of educational program, while there are (high & moderate GMS & RS) in most items after implementation of educational program.

The study was concluded there was a statistically significant difference in nurses - midwives overall performance related to preterm labor between the pretest and post 1 test for study sample, also there are significant differences between (post 1 and post 2 performance) after the implementation of education program for nurse- midwives regarding pre-term labor and there are no statistical significant differences between socio-demographic variables and overall performance of nurses-midwives.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Ministry of Health, Iraq and all experiments were carried out in accordance with approved guidelines.

Recommendation:

Every hospital should update the guidelines during the intervention during stages of labor and immediate resuscitation of premature baby Guidelines for nurses- midwives to instruction and prevention of preterm labor should be used as routine hospital care.

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Effectiveness of Instructional Program Regarding Health Protective Behaviors for Women with Gestational Diabetes on their Self Efficacy in Primary Health Care Centers at Baghdad City

Sagdh Hameed Kareem¹, Iqbal Majeed Abbas²
¹Ph.D., Student, Academic nursing Specialist, MScN, Ministry of Health, Iraq, ²Ph.D. Emeritus Professor and Academic Supervisor, Baghdad college of Medical Sciences/ Nursing Department

Abstract

Objective: The present study to identify the relationship between these pregnant women’s self efficacy study variables, and determine the effectiveness of Gestational Diabetes health protective behaviors-oriented Instructional program on their self-efficacy.

Methods: A descriptive analytic study (two-groups Pretest-Posttest) used to determine the Effectiveness of Instructional Program Regarding Health Protective Behaviors for Women with Gestational Diabetes on Their Self Efficacy on Pregnancy Outcome in Primary Health Care Centers at Baghdad City.

Keywords: Women, Health Protective Behaviors, Gestational Diabetes, Primary Health Care Centers, Baghdad.

Introduction

Diabetes has become a global pandemic because of sedentary self efficacy, urbanization, and increasing incidence of obesity. Prevalence of diabetes is on the rise in developing countries such as India and China. As the incidence of diabetes is rising in epidemic proportion, more women of childbearing age are at increased risk of diabetes during pregnancy. In fact, a high prevalence of gestational diabetes mellitus (GDM) of the order of 18% has been reported from India. agreed with Women with GDM are at high risk for developing diabetes later in life. Thus, GDM provides a unique opportunity to study the early pathogenesis of diabetes and to develop interventions to prevent the disease.

Abnormal metabolic environment due to hyperglycemia has a profound impact on maternal and fetal outcome. Indians belong to higher risk for developing diabetes due to their ethnicity. The present study was conducted to determine the maternal and fetal outcomes of pregnancies complicated with diabetes mellitus vis-a-vis non diabetic pregnancies, in a the city of Baghdad Iraq

Methodology

A descriptive analytic study conducted from 17th October 2020 to 30th January 2021, purposive sample consisted of 50 pregnant women with gestation diabetic were selected from (8) primary health centers, four from Al karkh department, and another four from Al Rusafa in Baghdad City.

The questionnaire from was designed and data collection consists of three main parts include: socio- demographic characteristics, reproductive characteristics & This part is comprised of 49 items in five domains of Self Efficacy of Pregnant Women with Gestational Diabetes,

Diabetes during pregnancy is associated with higher maternal and fetal morbidity. Therefore, early screening, detection, close monitoring, and
Intervention is essential to reduce maternal and fetal short- and long-term adverse effects, especially in high-risk groups. Pregnancy provides an opportunity to the clinician to control the disease process and inculcate self efficacy practices in these pregnant woman.

**Dissection**

The present study reports that the highest percentage (29%) of study sample is at age group ranging between (40 – 44) years old, with mean and SD = 32.38±7.22 years); this result disagree with agreed with (2) it was reported that the half of the study age ranged between 35 and 40 years; participants were and also reported that lowest percentage (14 %) of their study sample is within ideal age between (45-49) years for pregnant.

The highest percentage (24%) of the study sample was graduated from Graduate junior high, while the lowest percentage (9% ) were Neither read nor write. This result was in agreement by a study who reported that a majority of pregnant women (65.5%) indicated that pregnant women should receive on the education agreed with (3) and The highest percentage (30%) of study sample was government of self employed while the lowest percentage (16 %) of study sample were retired. Diabetes mellitus is the leading cause affect their continuous absenteeism from their work and between 10 % to 18% of pregnant believed that it hampers functions of daily life agreed with (4). The highest percentage (40 %) of study sample with enough to some extent Socio-economic status. The finding of the present study is not consistent with a study which revealed that pregnant women who belong to a low socio-economic status had severe symptoms of diabetes mellitus than the pregnant women of high Socio-economic status agreed with 5-7.

The study shows that The finding of present study indicates that majority of the study sample was 5 pregnancy & above and Grand multi Para (had ≥ 5 deliveries ), the lack of health education , in correct and lack of using of family planning methods and vogues of having large families (especially in rural areas) accounted for increased the complication associated with high parity. agreed with (5) indicate also that is one of these causes of frequent births lead to women with gestational diabetes; the result is in consistent with agreed with (7). Nearly Approximately less than half of the sample in present study had ≥ 5 deliveries pregnancies which are in ideal reproductive numbers while the other half may expose too many complications. So, the health protective Behaviors for women with gestational diabetes on their self efficacy and pregnancy outcome is of high obstetrical complications agreed with (8) who has reported that women with parity are greater than five this is almost inducted to complications as many great of number pregnant with diabetes have then less parity The result indicates that the highest percentage (42%) of the study sample have used Mixed feeding; while (28%) of study sample have used Artificial feeding who reported that through increasing breast feeding rate in Australia, the future rates of obesity in Australia will be reduced; a recent publication from the U.K. presents a more comprehensive way of calculating the benefits to be realized in the future from reducing diabetes mellitus, obesity and asthma as a result of breast feeding; the finding agrees with American Academy of Pediatrics, which report that breastfeeding is important because of the nutritional, health, immunological, development, and psychological benefits that breast milk provides for infant and newborn. previous studies have also show that breastfeeding is associated with a lower risk of gestational with diabetes in the mother, agreed with (9). and The result of study sample indicated that the highest percentage (44%) was first pregnancy at age between (21-25 ) years old which means ideal age for become pregnancy. While the lowest percentage (6 %) was the first to have them in the age (31years & above) which means was old prima.

The study resulted ( 42 %) shows that the highest percentage (38%) of study sample has Caesarean births; while the lower percentage (8%) has Normal delivery with forceps agreed with (10) who reported
that schedule the date delivery (either an induction of labor or cesarean delivery), especially if there are risk factors, such as: hypercalcemia levels, nephropathy, worsening retinopathy, hypertension or preeclampsia, or if the Newborn is smaller or larger than normal; if delivery before the due date is planned. agreed with (11) USF Health.

Table (1): Differences between (pretest and Posttest) Variables for the overall domains of self efficacy among pregnant women with gestational diabetics (N=50).

<table>
<thead>
<tr>
<th>Paired Sample Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-Post Test</strong></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td>Lower</td>
</tr>
<tr>
<td>Mean</td>
</tr>
</tbody>
</table>

\( t = t\text{-test}, df= \text{degree of freedom} \quad \text{Sig.} = \text{Significant at } p\text{-value } \leq 0.05 \)

Table (1): show There are Significant Differences between pre observation Health behaviors and post the overall domains test periods While there are No Significant Differences between Post 1 the overall domains and Post 2 the overall domains. after the implementation of education program for self efficacy among pregnant women with gestational diabetic

Table (2) Association between Socio-Demographic Variables and Overall of Self-Efficacy for Women with Gestational Diabetes among study sample (n=50).

<table>
<thead>
<tr>
<th>No</th>
<th>Independent variables: Socio-Demographic Variables</th>
<th>Overall self-efficacy</th>
<th>df</th>
<th>( \chi^2 ) crit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pre</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>( \chi^2 ) obs</td>
<td>p. value</td>
<td>sig</td>
</tr>
<tr>
<td>1.</td>
<td>Age Groups</td>
<td>4.241</td>
<td>0.515</td>
<td>NS</td>
</tr>
<tr>
<td>2.</td>
<td>Level of Education</td>
<td>12.798</td>
<td>0.046</td>
<td>S</td>
</tr>
<tr>
<td>3.</td>
<td>Occupation</td>
<td>2.908</td>
<td>0.406</td>
<td>NS</td>
</tr>
<tr>
<td>4.</td>
<td>Economic and social status</td>
<td>3.125</td>
<td>0.210</td>
<td>NS</td>
</tr>
<tr>
<td>5.</td>
<td>Type family</td>
<td>0.784</td>
<td>0.676</td>
<td>NS</td>
</tr>
<tr>
<td>6.</td>
<td>Gravidity (No. of pregnancies)</td>
<td>0.457</td>
<td>0.499</td>
<td>NS</td>
</tr>
<tr>
<td>7.</td>
<td>Parity (No. of deliveries)</td>
<td>3.640</td>
<td>0.457</td>
<td>NS</td>
</tr>
</tbody>
</table>


Table (2) shows that there are no statistical significant relationship between overall self-efficacy in pretest and reproductive variables. In posttest there are a statistical significant relationship between overall self-efficacy and studied variables (gravidity, parity, family history of diabetes, and type of delivery), while there are no a statistical significant relationship between overall self-efficacy and the rest of reproductive variables.

**Conclusion**

Health behaviors during pregnancy are those that encourage or improve health-related self-efficacy, both maternal and fetal and also decrease risk factors. One of the challenges is to fully understand gestational diabetes and the hormonal changes and altered carbohydrate metabolism that are associated with it during fetal development.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** “All experimental protocols were approved under the Ministry of Health and carried out in accordance with approved guidelines”.

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Effectiveness of Instructional Program Regarding Health Protective Behaviors for Women with Gestational Diabetes on Pregnancy Outcome in Primary Health Care Centers and Maternity Hospital at Baghdad City

Sagdh Hameed Kareem¹, Iqbal Majeed Abbas²
¹Ph.D., Student, Academic Nursing Specialist, MScN, Ministry of Health, Iraq. ²Ph.D. Emeritus Professor and Academic Supervisor, Baghdad college of Medical Sciences/ Nursing Department

Abstract

Background:- Diabetes is a chronic disease in which pregnant with gestational Diabetes require lifelong health Protective Behaviors for Effective on Pregnancy Outcome

Methods: descriptive research design group Pre test used to determine the Effectiveness of Instructional Program Regarding Health Protective Behaviors for Women with Gestational Diabetes on Pregnancy Outcome in Primary Health Care Centers and Maternity Hospital at Baghdad City.

Methodology: descriptive research -l study conducted from 17th October 2020 to 30th January 2021, purposive sample Consisted of 50 pregnant women with gestation diabetic were selected from (4) primary health centers, and tow from Al karkh department, and another Two from Al Rusafa in Baghdad City. And selected from (4) Maternity Hospital Two from Al karkh department, and another Medical City Department.

Results : The present study reports that the highest percentage (29%) of study sample is at age group ranging between (40 – 44) years old, with mean and SD = 32.38±7.22 years) the study sample was graduated from Secondary school graduate, while the lowest percentage (9% ) were Neither read nor write This result was in agreement by a study who reported that a majority of pregnant women (65.5%) indicated that pregnant women should receive on the education and The highest percentage (30%) of study sample was government of self employed while the lowest percentage (16 %) of study sample were .

Keywords: Instructional Program Regarding, Behaviors, Women, Diabetes Pregnancy

Introduction

Diabetes has become a global pandemic because of sedentary health behaviors, urbanization, and increasing incidence of obesity. Prevalence of diabetes is on the rise in developing countries such as India and China. As the incidence of diabetes is rising in epidemic proportion ¹ more women of childbearing age are at increased risk of diabetes during pregnancy. In fact, a high prevalence of gestational diabetes mellitus (GDM) of the order of 18% has been reported from India. agreed with (¹) Women with GDM are at high risk for developing diabetes later in life. Thus, GDM provides a unique opportunity to study the early pathogenesis of diabetes and to develop interventions to prevent the disease. Abnormal metabolic environment due to hyperglycemia has a profound impact on maternal and fetal outcome. Indians belong to higher risk ² for developing diabetes due to their ethnicity agreed with (²) The present study was conducted to determine the effect of the health behaviors of pregnant women with gestational diabetes and the outcome of pregnancy in the city of Baghdad, Iraq.
Objective: To assess the health protective behaviors of pregnant women with gestational diabetes for the study group before implementation of program. & to determine the effectiveness of Gestational Diabetes health protective behaviors-oriented Instructional program on pregnancy outcome.

Decoction

The present study reports that the highest percentage (29%) of study sample is at age group ranging between (40 – 44) years old, with mean and SD = 32.38±7.22 years as shown in table 1. This result disagree with agreed with (3) that the study sample was graduated from Secondary school graduate, while the lowest percentage (9%) were Neither read nor write. This result was in agreement by a study who reported that a majority of pregnant women (65.5%) indicated that pregnant women should receive on the education agreed with 4 and The highest percentage (30%) of study sample was government of self employed while the lowest percentage (16%) of study sample were. agreed with 5 . and the majority of study sample was grand multi gravida and grand multi Para, equal and more than (had ≥ 5 deliveries it was reported that the lack of health education, in correct and lack of using of family planning methods and with having large families) accounted for increased the complication associated with high parity. by6 and nearly Approximately less than half of the sample in present study had ≥ 5 deliveries pregnancies which are in ideal reproductive numbers while the other half may expose too many complications agreed with 8 .

The findings of present study supported evidence is available in the study reported that in cross-sectional analysis of over 200,000 women delivered between 1992-1998 in New South Wales Australia, the incidence of diabetes mellitus complications found to be increased significantly from parity onwards. agreed with 9 and the result indicates that the highest percentage (42%) of the study sample have used Mixed feeding; while (28%) of study sample have used Artificial feeding agreed with 10 and The result of study sample indicated that the highest percentage (44%) was first pregnancy at age between (21-25 ) years old which means ideal age for become pregnancy. While the lowest percentage (6%) was the first to have them in the age (31years & above) which means was old prima gravida . and The study resulted in (42 %) shows that the highest percentage (38%) of study sample has Caesarean births; while the lower percentage (8%) has Normal delivery with forceps agreed with 11 . Table (5 ) shows that is statistical significant relationship between gravidity and type of delivery. In posttest there are a statistical significant relationship between overall health behaviors and studied variables (parity, Personal history of a previous gestational diabetes and type of delivery), while there are no a statistical significant relationship between overall health behaviors and the rest of reproductive variables.

Health behaviors before , from pre-pregnancy to 15–20 weeks’ gestation and older age were associated with an increased risk of gestation diabetes mellitus.

Result

Data were collected through the use of questionnaire by personal interview technique

Table (1) Assessement of Protective Health Behaviors related Pregnant Women with Gestational Diabetes in Pretest and Post test among. (n=50)

<table>
<thead>
<tr>
<th>No.</th>
<th>Domain of Health Behaviors</th>
<th>Study Sample (n=50) Pretest</th>
<th>Study Sample (n=50) Posttest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GMS</td>
<td>GRS</td>
<td>Assessment</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Continued... Table 1: Assessment of Protective Health Behaviors related to Pregnant Women with Gestational Diabetes in Pretest and Posttest among (n=50)

<table>
<thead>
<tr>
<th></th>
<th>Protective Health Behaviors</th>
<th>F</th>
<th>%</th>
<th>Ass.</th>
<th>n</th>
<th>R.S</th>
<th>No Response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Personal hygiene</td>
<td>15.76</td>
<td>59.23</td>
<td>NR</td>
<td>2.47</td>
<td>82.57</td>
<td>M</td>
</tr>
<tr>
<td>2</td>
<td>Dental care for Pregnant Women with Gestational Diabetes</td>
<td>1.78</td>
<td>59.54</td>
<td>NR</td>
<td>2.4</td>
<td>80</td>
<td>M</td>
</tr>
<tr>
<td>3</td>
<td>Feet care for pregnant women with gestational diabetes domain</td>
<td>1.74</td>
<td>58.38</td>
<td>NR</td>
<td>2.74</td>
<td>79.78</td>
<td>M</td>
</tr>
<tr>
<td>4</td>
<td>Nutrition for pregnant women with gestational diabetes domain</td>
<td>1.78</td>
<td>59.5</td>
<td>NR</td>
<td>2.03</td>
<td>77.6</td>
<td>M</td>
</tr>
<tr>
<td>5</td>
<td>Sleep and Rest for women with gestational diabetes domain</td>
<td>1.77</td>
<td>59</td>
<td>NR</td>
<td>9.86</td>
<td>81.98</td>
<td>M</td>
</tr>
<tr>
<td>6</td>
<td>Exercises for women with gestational diabetes domain</td>
<td>1.71</td>
<td>60.28</td>
<td>NR</td>
<td>2.34</td>
<td>77.6</td>
<td>M</td>
</tr>
<tr>
<td>7</td>
<td>Follow Up Medical management for women with gestational diabetes domain</td>
<td>2.03</td>
<td>68.2</td>
<td>L</td>
<td>2.4</td>
<td>79.8</td>
<td>M</td>
</tr>
<tr>
<td>8</td>
<td>Follow-up self-pregnant women with gestational domain</td>
<td>1.85</td>
<td>59.77</td>
<td>NR</td>
<td>2.33</td>
<td>78.99</td>
<td>M</td>
</tr>
</tbody>
</table>

F = Frequency, % = Percentage, GMS = Mean Score, Ass. = Assessment, n = Number of sample, R.S = Relative Sufficiency, No response (NR) < 66.66, low (L) = 66.66 – 77.76, Moderate (M) = 77.77 – 88.88, High (H) = 88.89 – 100

Table (1) shows that the GMS mean scores with No Response Relative Sufficiency (R.S) in all items of Protective Health Behaviors related to Pregnant Women with Gestational Diabetes before implementation, while there are moderate levels of grand mean score and relative sufficiency in all items after implementation program.

Table (2): Differences between (pretest and posttest two) Variables for overall domains health Protective behavior among pregnant women with gestational diabetes (N=50).

<table>
<thead>
<tr>
<th>Pre-Post Test</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>-260.0400</td>
<td>1489.06238</td>
<td>210.58522</td>
<td>-683.22685 – 163.14685</td>
<td>-1.235</td>
<td>49</td>
<td>0.05</td>
</tr>
<tr>
<td>Post</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( t = t\)-test, df = degree of freedom Sig. = Significant at p-value = \( \leq 0.05 \)
Table (2): show That is Significant Differences between pre test observation Health behaviors and post overall domains test periods While there are No Significant Differences between Post 1 overall domains and Post 2 overall domains. after the implementation of education program for health protective Behaviors for women with gestational diabetes on their self efficacy and pregnancy outcome.

Table (3): Association between Reproductive Variables and Overall Health Protective Behaviors for Women with Gestational Diabetes among study sample in post test (n=50).

<table>
<thead>
<tr>
<th>No.</th>
<th>Independent variables: Reproductive Variables</th>
<th>Overall Health Protective Behaviors</th>
<th>df</th>
<th>$\chi^2$ crit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>df</td>
<td>$\chi^2$ obs</td>
<td>p. value</td>
</tr>
<tr>
<td>1</td>
<td>Age Groups</td>
<td>5</td>
<td>5.484</td>
<td>0.360</td>
</tr>
<tr>
<td>2</td>
<td>Level of Education</td>
<td>6</td>
<td>0.492</td>
<td>0.998</td>
</tr>
<tr>
<td>3</td>
<td>Occupation</td>
<td>3</td>
<td>0.062</td>
<td>0.995</td>
</tr>
<tr>
<td>4</td>
<td>Economic and social status</td>
<td>2</td>
<td>0.064</td>
<td>0.996</td>
</tr>
<tr>
<td>5</td>
<td>Type family</td>
<td>2</td>
<td>0.295</td>
<td>0.863</td>
</tr>
<tr>
<td>6</td>
<td>Gravidarum (No. of pregnancies)</td>
<td>1</td>
<td>7.352</td>
<td>0.007</td>
</tr>
<tr>
<td>7</td>
<td>Parity (No. of deliveries)</td>
<td>4</td>
<td>10.199</td>
<td>0.037</td>
</tr>
<tr>
<td>8</td>
<td>Age At First Pregnancy / Years</td>
<td>3</td>
<td>1.389</td>
<td>0.708</td>
</tr>
<tr>
<td>9</td>
<td>Degree of consanguinity between the spouses</td>
<td>1</td>
<td>0.038</td>
<td>0.846</td>
</tr>
<tr>
<td>10</td>
<td>family history of diabetes.</td>
<td>1</td>
<td>0.038</td>
<td>0.846</td>
</tr>
<tr>
<td>11</td>
<td>Personal history of a previous gestational diabetes</td>
<td>1</td>
<td>0.206</td>
<td>0.650</td>
</tr>
<tr>
<td>12</td>
<td>Type of delivery</td>
<td>1</td>
<td>13.018</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table (3) shows that is statistical significant relationship between gravidarum and type of delivery. In posttest there are a statistical significant relationship between overall health behaviors and studied variables (parity, personal history of a previous gestational diabetes and type of delivery), while there are no a statistical significant relationship between overall health behaviors and the rest of reproductive variables.

Conclusion

The result study on based, discussion and critical interpretation of the findings, the researcher concluded the followings : in conclusion gestational diabetic mellitus (GDM) incidence on basis of the health behaviors and self efficacy criteria was common among pregnant women in Iraq.
Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: “All experimental protocols were approved under the Ministry of Health and carried out in accordance with approved guidelines”.

References
Assessment of Metabolic Profile in Relation with Polycystic Ovary Syndrome

Wasan Wajdi¹, Intesar Jabbar Khadum²

¹M.B.Ch.B., F.I.B.M.S, C.A.B.O.G, Consultant Obstetrician and Gynaecologist, Baghdad Medical College, University of Baghdad, Baghdad Teaching Hospital, Iraqi Board for Medical Specialization,²M.B. Ch.B., Baghdad Teaching Hospital, Iraqi Board for Medical Specialization

Abstract

Polycystic ovary syndrome (PCOS) is a common endocrinopathy that, by the most strict definition, affects 5 to 10% of women of reproductive age. There is not complete consensus about the definitive criteria for the diagnosis of PCOS, which necessitates the searching for new biomarkers for definitive diagnosis. This study is a case/control study carried on one hundred (100) women divided into two groups: 50 women with PCOS, and 50 women were with absent features of PCOS represent a control group who were attending the outpatient clinic of Gynecology department at Baghdad Teaching Hospital during the period from March to September, 2018. A fasting blood sample was obtained from all women during the early follicular phase (days 2–4 of the spontaneous cycle). Lipid profile, fasting blood glucose and fasting blood insulin, luteinizing hormone (LH), follicular stimulating hormone (FSH), and testosterone were analyzed by immune chemiluminescence method. There were no significant differences between the two groups regarding total cholesterol, low density lipoprotein -cholesterol, high density lipoprotein -cholesterol and fasting blood glucose. On the other hand, PCOS patients showed higher serum concentration of triglycerides and insulin resistance than controls.

Key words: Polycystic ovary syndrome, insulin, Lipid profile

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine illnesses in women of reproductive age. It is characterized by chronic oligo-or anovulation, hyperandrogenism and polycystic ovaries, and is associated with several long-term health consequences, such as infertility, obesity, hypertension, dyslipidemia, insulin resistance and type II diabetes mellitus [¹]. There are three main definitions for PCOS. The Rotterdam definition is the most widely used which proposes that PCOS can be diagnosed in any woman presenting with at least two of the three hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology (PCOM). By contrast, the 2006 Androgen Excess and PCOS Society (AE–PCOS) Position Statement requires the presence of hyperandrogenism, which must be accompanied by evidence of ovarian dysfunction in the form of ovulatory dysfunction and/or PCOM [²].

Each individual criterion used to define PCOS has clinical consequences by itself. Androgen excess might result in cutaneous manifestations, such as hirsutism, acne and alopecia; ovulatory dysfunction and chronic oligomenorrhea might result in infertility and endometrial hyperplasia and/or carcinoma; and isolated PCOM is associated with a risk of ovarian hyperstimulation syndrome only during ovulation induction [³]. The pathophysiology of PCOS is far from fully understood. However, over the last decades potential underlying mechanisms have been proposed. An increased GnRH pulse frequency resulting in LH hypersecretion has been reported. LH stimulates the ovarian theca cells to produce androgens, such as testosterone. Because of a relative FSH deficiency,
testosterone is incompletely aromatized by the granulosa cells, resulting in hyperandrogenemia. Moreover, although the ovaries are the main source of androgen excess in PCOS, also the adrenal glands contribute to the existing hyperandrogenism\[4\].

In PCOS, hyperandrogenism, hyperinsulinemia and altered intraovarian paracrine signaling can disrupt follicle growth. Moreover, FSH does not seem to increase to the threshold levels which are required to stimulate normal follicular maturation resulting in follicular arrest. The accumulation of small antral follicles result in elevated AMH levels. These increased AMH levels seem to add to the anovulation in PCOS by reducing both primordial follicle growth and follicle sensitivity to FSH\[5\].

Materials and Methods

1: The Study Population: This study is a case/control study carried on one hundred women all are married who were attending to the outpatient clinic of Gynecology department at Baghdad Teaching Hospital during the period from March to September, 2018. The study sample divided into two groups: Group I: fifty women with PCOS and Group II: fifty women were healthy matched for age and BMI with absent features of PCOS represent a control group.

All women were subjected to the following:

1- Verbal consent was obtained from each subjects included after explaining aims and dimensions of the study.

2- Socio-demographic information about age, weight, height, obstetric and gynecological history also obtained

3- General physical, vital signs, abdominal examination have been performed to all women.

4- Transabdominal ultrasound done by senior doctor (Radioloist) by ultrasound machine (Philips HD 11xE) using the curvilinear probe.

2: Biochemical Assays: Serum levels of glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c) were measured by the enzyme-calorimetric methods. Low-density lipoprotein-cholesterol (LDL-c) levels were calculated by using Friedwald et al.\[6\] equation as follows:

\[
\text{LDL-c (mg/dl)} = \text{TC - HDL-c - (TG/5)}.
\]

3: Enzyme-calorimetric method was used for measurement of fasting blood insulin with a commercial ready kit according to the manufacturer’s instructions.

4: Homeostasis Model of Assessment-Insulin Resistance (HOMA-IR) index for the assessment of insulin resistance was measured by calculation using Matthews et al.\[7\] equation as follows:

\[
\text{HOMA-IR} = \frac{[\text{glucose (mg/dl)}* \text{insulin (IU/ml)}]}{405}.
\]

Insulin resistance (IR) was accepted as HOMA-IR \( \geq 2.5 \)

5: Hormone analyzes: Luteinizing hormone (LH), follicular stimulating hormone (FSH), and testosterone were analyzed by immune chemiluminescence method (Roche-Hitachi Modular Analytics E-170, Indianapolis, IN, USA)

6: Statistical Analysis

Statistical package for Social Sciences (SPSS version 20) was used for data analysis, and Microsoft Excel to generate graphs. Continuous variables were expressed as a mean\( \pm \) standard deviation (SD). The student test (t-test) was used to compare means of these variables. The statistical tests were two sided, and a \( P \leq 0.05 \) was considered statistically significant.

Results and Discussion

1: Anthropometric Data and Hormonal Status of the Study Population:

Table (1) shows the anthropometric data and hormonal status of PCOS patients and controls. The
two groups were comparable in age, BMI, W/H ratio as well as FSH concentration with no significant differences. However, PCOS patients showed higher serum concentration of LH (7.12±1.91 IU/L) than controls (4.42±1.44 IU/L) with a highly significant difference (p<0.001). Accordingly the LH/FSH ratio was significantly higher in PCOS patients (1.51±0.08) than controls (1.04±0.4)(p=0.016). Likewise, PCOS patients showed higher serum testosterone than controls (0.84±0.27 ng/mL vs. 0.44±0.18 ng/mL) with a highly significant difference (p<0.001).

Table 1: The anthropometric data and hormonal status of PCOS patients and controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>PCOS patients</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>27.3 ± 7.1</td>
<td>24.51 ± 5.29</td>
<td>0.881</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>26.3 ± 7.0</td>
<td>26.7 ± 6.4</td>
<td>0.932</td>
</tr>
<tr>
<td>W/H ratio</td>
<td>0.76±0.06</td>
<td>0.73±0.03</td>
<td>0.711</td>
</tr>
<tr>
<td>FSH(IU/L)</td>
<td>4.81 ± 1.32</td>
<td>4.29±2.11</td>
<td>0.622</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>7.12±1.91</td>
<td>4.42±1.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>1.51±0.08</td>
<td>1.04±0.4</td>
<td>0.016</td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>0.84±0.27</td>
<td>0.44±0.18</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. BMI, body mass index; W/H: waist/hip; FSH: follicular stimulating hormone, LH: luteinizing hormone.

The hormonal profile of PCOS patients and controls revealed a significant elevation in LH, LH/FSH ratio in patients compared with the controls. Such results were frequently reported by several previous studies (8,9). In fact, LH is an essential player in the pathophysiology of PCOS. Increased levels of LH (with consequence increased LH/FSH) ratio is caused by pulsatile release of GnRH from the hypothalamus. However, some other studies reported no significant differences in serum levels of LH between PCOS patients and controls (10). Likewise, testosterone levels were found to be significantly elevated in PCOS women compared with controls. This results also agree with vast majority of previous studies (11,12).

However, total serum testosterone cannot be a reliable markers for PCOS because about 65% of testosterone % is bound to SHBG. This is particularly important for obese women with IR because they are very likely to have low SHBG levels.

2 : Metabolic Status Data of the Study Population

The metabolic status data of PCOS patients and controls are listed in table (2). There were no significant differences between the two groups regarding total cholesterol, LDL-cholesterol, HDL-cholesterol and fasting blood glucose. On the other hand, PCOS patients showed higher serum concentration of...
triglyceride than controls (89.12±9.81mg/dL vs. 77.41±8.44, p=0.038).

Likewise, PCOS patients had significantly higher concentration of HOMA-IR than controls (2.12±1.04 vs. 1.1±0.92, p=0.041).

Table 2: The metabolic status data of PCOS patients and controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>PCOS patients</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>179.61±21.13</td>
<td>163.39±14.38</td>
<td>0.391</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>89.12±9.81</td>
<td>77.41±8.44</td>
<td>0.038</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>106.4±11.71</td>
<td>101±13.92</td>
<td>0.449</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>48.17±6.88</td>
<td>55.84±7.18</td>
<td>0.297</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>87.52±11.43</td>
<td>84.18±12.82</td>
<td>0.761</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.12±1.04</td>
<td>1.1±0.92</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. LDL: low density lipoprotein, HDL: high density lipoprotein, HOMA-IR: Homeostatic model assessment-Insulin resistance.

The metabolic profile analysis in the present study revealed a significant higher TG and HOMA-IR levels than controls. These results are comparable with many previous studies. In one study including 166 women with PCOS and 277 controls, Chae et al. (38) reported an elevation of TG (≥150 mg/dL) in 26.7%, of PCOS patients versus 1.0% among controls (P < 0.001); prevalence of low HDL-C (<50 mg/dL) 30.0% among patients and 3.0% among controls (P = 0.004). More recently, Lath et al. (13) studied lipid profile in 80 women with PCOS and other 40 healthy women. They found that each of TC, TG and LDL-c were significantly higher in patients with PCOS than controls. Dyslipidemia is common in PCOS compared to weight matched controls, with higher triglycerides and lower high density lipoprotein cholesterol (14). Several mechanisms have been proposed to explain this disorder mainly attributed to IR which present in 50%-70% of PCOS. For example, Resistance to the action of insulin on lipoprotein lipase in peripheral tissues may contribute to elevated TG levels. The significantly higher value of HOMA-IR in PCOS group compared with controls has also been reported frequently (15,16).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Depressions Level among Adolescents Undergoing Chemotherapy Treatment at Pediatric Teaching Hospitals in Baghdad City

Ali K. Ouda¹, Qahtan Q. Mohammed²
¹MSC Student, ²Assistant Prof, University of Baghdad, College of Nursing, Psychiatric and Mental Nursing Department, Baghdad City

Abstract

Objectives: A descriptive correlational study, using an evaluation approach, is conducted to evaluate the level of depression for adolescents undergoing chemotherapy at pediatric hospitals in Baghdad City from December 15th 2020 to June 17th 2021.

Methodology: A self-report questionnaire is developed for the purpose of the study. Content validity and internal consistency reliability are determined through a pilot study. A purposive “non-probability” sample, of (45) adolescents, is selected for the present study. All adolescents have signed a consent form for their agreement to participate in the study with no obligation as part of the ethical considerations.

Results: Results of the study indicate that adolescents, who are middle age and males, develop depressive symptoms greater than others after being treated with chemotherapy. Adolescents’ depression has highly significant relationship with their socio-demographic characteristics of relapse, complications, mothers’ occupation, gender, education, monthly income, duration of treatment, age, fathers’ occupation, fathers’ education, mothers’ education and age at diagnosis.

Conclusion: The study concludes that most of the adolescents have experienced moderate level of depression as a result of their being exposed to the chemotherapy treatment and the study confirms that the issues of looking forward, sleeping, feeling, walking, energy, pain, appetite have major influence on the prognosis of the health problem of depression.

Recommendation: The study recommends that counseling services are important issue to deal with in providing treatment to these adolescents especially those who are males, middle age, and developing complications and relapse. Well-designed and constructed education program can be presented to these adolescents for the purposes of increasing their awareness toward the disease and making them able to prevent complications and relapse associated depression.

Keywords: Depressions Level, Adolescents, Chemotherapy Treatment

Introduction

On a global scale, cancer and depression, when viewed separately, are responsible for immense human suffering. In 2008, cancer affected an estimated 169 million years of healthy life worldwide, and the World Health Organization (WHO) predicts that by 2030, unipolar major depression will be the leading cause of disease burden. When cancer and depression coexist, the symptom burden and mortality of each illness are amplified, as the treatment of one complicates the management of the other¹. These issues are particularly pressing for Adolescents and Young Adults (AYA), who have seen the rates of cancer and depressive disorders ².

The prevalence of cancer in the AYA population continues to grow, despite the fact that the majority
of cancer patients are elderly. These younger patients have specific psychosocial issues, such as depression, that necessitate complex clinical management. Despite these challenges, there is a noticeable lack of research literature on the best psychiatric treatment for depression in AYA cancer patients [3].

Each year, nearly 70,000 adolescents and young adults are diagnosed with cancer in the United States, with tens of thousands more completing cancer care[4]. Cancer is still the leading cause of disease-related death among teenagers in the United States, trailed only by death from injuries, suicide, and homicide. Adolescents with acute lymphocytic leukemia have a 50% 5-year survival rate compared to 80 percent for children with the disease. 9 The annual rate of improvement in 5-year survival for adolescents has been less encouraging across all cancer types, especially for those with breast cancer and leukemias [5].

Physical symptoms including fatigue, anorexia, and sleep disturbance are typical side effects of an underlying malignancy or its treatment. It’s still up for discussion whether and how these somatic symptoms can be included in the diagnosis of a depressive disorder. Furthermore, clinicians and patients’ common assumptions that depression is a “anticipated” reaction to a cancer diagnosis or a life-limiting disease cloud reliable measurement of depression prevalence[6]. Young women with breast cancer are more likely than older women to develop triple-negative basal-cell breast cancer, which is an aggressive type of the disease. Despite the fact that colorectal cancer incidence is declining in the United States, rates of diagnosis (as well as worse outcomes) have increased among the AYA population [7].

Psychiatric syndromes are common in people who have cancer. Adjustment conditions with signs of depression or anxiety have a wide range of incidence, ranging from 16 percent to 42 percent. Minor depression or dysthymia is estimated to affect 20% of cancer patients. 28 Although exact figures are difficult to come by, cancer patients have significantly higher rates of all mood disorders than the general population, with MDD occurring up to three times more often in cancer patients[8].

Methodology

A descriptive correlational study using an evaluation approach is designed to examine the degree of depression among adolescents undergoing chemotherapy at pediatric hospitals in Baghdad City, From December 15th 2020 to June 17th 2021

Setting of study

The study was conducted at Oncology Teaching Center and Central Teaching Hospital of Pediatric in Baghdad city, this hospital and center is the designated agencies for data collection.

Sampling

A purposeful “non-probability” sample of (45) adolescents undergoing chemotherapy was chosen. This sample was chosen from Oncology Teaching Center and Central Teaching Hospital of Pediatric in Baghdad city.

Instrument of study

To evaluate depression level among adolescents undergoing chemotherapy treatment at Pediatric Teaching Hospitals in Baghdad City, a self-administered questionnaire was developed to assess it was constructed through the review of related literatures and previous studies. The questionnaire consisted of two parts:

Part 1: The socio-demographic characteristics of the patient:

Patients’ socio-demographic characteristics consist of (9) items which include age, gender, age at diagnosis, education level, parents’ education level, parents’ occupation, monthly income, duration of chemotherapy, complications and relapse.
Part II: Knowledge of nurses regarding nursing management of thrombolytic therapy for patients with acute myocardial infarction.

This part embraces (21) item which are measured on 3-level type Likert scale of always =3, sometimes =2 and never = 1 and valued as mild = (21-35), moderate = (36-49) and high = (50-63).

Statistical Methods

The analysis of the data was used through descriptive statistics (frequencies, percentages, and the mean of score and standard deviation) and statistical inferential. It includes the following:

1. Cronbach Alpha Correlation Coefficient:
   This test is used for the determination of the internal consistency of the study instrument.

2. T-test:
   This test is employed for the comparative differences between adolescents’ depression relative to their age and gender.

3. Multiple linear Regression:
   This test is applied to determine the relationship between adolescents’ depression and their socio-demographic characteristics.

Results and Discussion

Part I: Discussion of Adolescents’ Socio-demographic Characteristics (Table 1):

Based on these factors, the majority of the adolescents in the present sample who have developed depressive disorders are in their middle years. In terms of gender, the current research discovered that male adolescents account for more than half of the patients. Boys have a higher relative risk of depression during early and late adolescence than children, according to study findings. A randomized clinical trial is being performed on ninety-two (92) cancer-stricken adolescents and young adults (48 PRISM, 44 Usual Care). They are (43 percent) females, and (73 percent) are (12-17) years old, according to the results.

According to the findings, the majority of depressed adolescents are diagnosed between the ages of 12 and 14, and are treated for 1 to 5 months. These results show that these adolescents developed cancer while still in their early teenage years.

Park and colleagues (2015) found that cancer is diagnosed in advanced school years in their assessment descriptive research. Many teenagers and their parents have been reported as having low achievement levels in relation to their education (Table 1). These results show that low-educated teenagers and their parents are more likely to develop depressive disorders than those who are well-educated and have never had cancer. Unfortunately, there is no supporting evidence for these findings in the literature.

According to the report, more than half of adolescents have sufficient family income, with more than a third of their fathers working and the majority of their mothers being housewives. A randomized clinical trial is being performed on ninety-two (92) cancer-stricken adolescents and young adults (48 PRISM, 44 Usual Care). According to the findings, a quarter of them (24%) come from low-income households.

According to the findings, many suicidal adolescents have acquired depression-related complications and have relapsed after treatment.

And compared to children who did not obtain a depression diagnosis during primary school, children who receive a depression diagnosis during primary school have a significantly higher chance of a depression relapse during adolescence.

Part II: Discussion of Adolescents’ Level of Depression (Table 2):

During the data analysis, the researchers discovered that many adolescents developed and endured mild...
depression as a result of their chemotherapy treatment (Table 4-2). This finding suggests that teenagers who are diagnosed with cancer and are undergoing chemotherapy treatment.

Adolescents with cancer are more likely to experience depression due to delays in their developmental trajectory, increased physical symptom burden, and a higher risk of developing aggressive disease, according to the literature. Adolescents with depression have significantly higher rates than those without cancer when compared with older adults\[11\].

**Part II: Discussion of the Comparative Differences between Adolescents’ level of depression Relative to their age and Gender (Table 3,4):**

According to the results of such a comparison, middle-aged and male teenagers grow and experience, as well as becoming more susceptible to depression following chemotherapy treatment, than others (Table 4-4). According to a national survey of US adolescents aged 12–17 (N=101 685), the average incidence of depression between the ages of 12 and 17 is (13.6 percent) among male subjects and (36.1 percent) among females. At the age of (12) years, the sex disparity in incidence is substantial (5.2 percent in female subjects versus 2.0 percent in male subjects, P0.0001), and it is substantially greater at the ages of (13) through (17) years than at the age of (12) years (P-values0.05)\[12\].

Female participants are (2.8) times more likely than males to experience depression at the age of twelve, and between (3.1 and 4.0) times more likely to develop depression at the ages of thirteen and fourteen (13 through 16). At the age of 17, the relative risk of (2.2) is smaller than at the age of (12)\[13\].

**Conclusion:** It has been well-founded throughout the course of the study that male and middle age adolescents experienced depressive disorders more than others. The study ascertains that many depressed adolescents have developed complications associated with depression and experienced relapse far along. Most of the adolescents have experienced moderate level of depression as a result of their being exposed to the chemotherapy treatment.

The study confirms that the issues of looking forward, sleeping, feeling, walking, energy, pain, appetite have major influence on the prognosis of the health problem of depression. Adolescents, who are middle age and males, develop depressive symptoms greater than others after being treated with chemotherapy. Adolescents’ depression is affected by their socio-demographic characteristics of relapse, complications, mothers’ occupation, gender, education, month income, duration of treatment, age, fathers’ occupation, fathers’ education, mothers’ education and age at diagnosis.

**Recommendations:** The study recommended Counseling services are important issue to deal with in providing treatment to these adolescents especially those who are males, middle age, and developing complications and relapse. Well-designed and constructed education program can be presented to these adolescents and their parents for the purposes of increasing their awareness toward the disease and making them able to prevent complications and relapse associated depression. Regular and periodic investigations should be carried out to monitor the adolescents’ health status. And Further research can be conducted on the same topic with wide-range sample size, variety of variables and different setting.
### Table (1): Adolescents’ Socio-demographic Characteristics

<table>
<thead>
<tr>
<th>Socio-demographic variables</th>
<th>Groups</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (Years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-14</td>
<td>22</td>
<td></td>
<td>48.9</td>
</tr>
<tr>
<td>15-18</td>
<td>23</td>
<td></td>
<td>51.1</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td></td>
<td>53.3</td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
<td></td>
<td>46.7</td>
</tr>
<tr>
<td><strong>Age at Diagnosis (Years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-15</td>
<td>41</td>
<td></td>
<td>91.2</td>
</tr>
<tr>
<td>16-20</td>
<td>4</td>
<td></td>
<td>8.8</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t read and write</td>
<td>3</td>
<td></td>
<td>6.7</td>
</tr>
<tr>
<td>Read and write</td>
<td>42</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>Primary school graduate</td>
<td>37</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>Middle school graduate</td>
<td>13</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Secondary school graduate</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Duration of Chemotherapy (Months)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5</td>
<td>23</td>
<td></td>
<td>51.1</td>
</tr>
<tr>
<td>6-10</td>
<td>12</td>
<td></td>
<td>26.7</td>
</tr>
<tr>
<td>11-15</td>
<td>6</td>
<td></td>
<td>13.3</td>
</tr>
<tr>
<td>16-20</td>
<td>2</td>
<td></td>
<td>4.4</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>2</td>
<td></td>
<td>4.4</td>
</tr>
<tr>
<td><strong>Monthly Income</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enough</td>
<td>55</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Almost Enough</td>
<td>26</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Not Enough</td>
<td>17</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td><strong>Complications</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4</td>
<td></td>
<td>4.4</td>
</tr>
<tr>
<td>No</td>
<td>95</td>
<td></td>
<td>95.6</td>
</tr>
<tr>
<td><strong>Relapse</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>49</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>No</td>
<td>51</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>45</td>
<td>100</td>
</tr>
</tbody>
</table>

Freq. = frequency, % = percentages
Table (2): Evaluation of Adolescents’ Level of Depression

<table>
<thead>
<tr>
<th>Depression Level</th>
<th>Group Size</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (21-35)</td>
<td>10 (22.22%)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Moderate (36-49)</td>
<td>35 (77.78%)</td>
<td></td>
</tr>
<tr>
<td>Severe (50-63)</td>
<td>10 (22.22%)</td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Comparative Differences between Adolescents’ Depression with Respect to Their Age

<table>
<thead>
<tr>
<th>Variance</th>
<th>Group Size</th>
<th>Mean</th>
<th>S.D</th>
<th>t-test</th>
<th>Degree of Freedom</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equal Variance Assumed</td>
<td>22</td>
<td>55.364</td>
<td>5.141</td>
<td>1.051</td>
<td>43</td>
<td>0.299</td>
</tr>
<tr>
<td>Equal Variance not Assumed</td>
<td>23</td>
<td>53.739</td>
<td>5.224</td>
<td>1.051</td>
<td>42</td>
<td>0.299</td>
</tr>
</tbody>
</table>

$t = t$-statistics; Sig. = significance, S.D = Standard Deviation

Table (4): Comparative Differences between Adolescents’ Depression with Respect to Their Gender

<table>
<thead>
<tr>
<th>Variance</th>
<th>Group Size</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>t-test</th>
<th>Degree of Freedom</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equal Variance Assumed</td>
<td>22</td>
<td>54.636</td>
<td>4.924</td>
<td>0.129</td>
<td>43</td>
<td>0.898</td>
</tr>
<tr>
<td>Equal Variance not Assumed</td>
<td>23</td>
<td>54.435</td>
<td>5.542</td>
<td>0.129</td>
<td>42</td>
<td>0.898</td>
</tr>
</tbody>
</table>

$t = t$-statistics; Sig. = significance

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Mothers Quality of Life towards their Children with Nephrotic Syndrome at Pediatric Teaching Hospitals in Baghdad City

Asawer Ihsan Ali¹, Khatam M. Al- Mosawi²

¹MSC Student, University of Baghdad, College of Nursing, Department of Pediatric Nursing, Baghdad City, Iraq.
²Assist. Professor, University of Baghdad, College of Nursing, Department of Pediatric Nursing

Abstract

A descriptive correlational study was conducted to assess the quality of life of mothers toward their children with nephrotic syndrome on in children’s teaching hospitals in Baghdad, which received mothers of children with nephrotic syndrome during the period from (the nineteenth of September 2020 to the twenty-second June 2021).

The results of the study showed the age of children ranges from one to five years, and most of the diagnostic age is less than three years. mothers were mostly between the ages of 20-29. More than half of mothers are unemployed. The highest percentage of mothers were found to have a bachelor’s degree. the results of the study showed that mothers of children with nephrotic syndrome suffer from moderate (63.0%) overall level of quality of life in the five domains of, physical, psychological, social, environmental, beliefs and spiritual. All social and demographic characteristics of the mother do not affect the quality of life except for age, monthly income, level of education, and psychological distress.

Recommendations: Health and educational institutions should hold special meetings and celebrations for mother and child. Nephrotic Syndrome is educational and entertaining at the same time. Availability of multidisciplinary team of supportive care as well as follow-up out-patient clinic that include pediatric nurses, renal physicians, social workers, dietitian, psychotherapists and physiotherapist to assist children and their mother in maintaining near normal lifestyle at highest possible level of emotional feeling.

Keywords: Mothers Quality, children and nephrotic syndrome.

Introduction

Nephrotic syndrome (NS) presents with symptoms including massive proteinuria, hypoalbuminemia, hyperlipidema, and edema. The disorder can occur as a first primary disease known as idiopathic nephritis, childhood nephritic or minimal change renal syndrome. (MCNS). Second, a secondary defect that occurs as a clinical presentation after or associated with damage to the glomeruli. Third, the congenital type is inherited as an autosomal recessive disorder. This is caused by increased leakage of proteins by the glomeruli into the body, resulting in a significant lossing of urinary protein. The glomerulus is responsible for the first steep in the formation of urine, and the filtration rate depende on the integrity of the glomerular membranes

[1]. The incidence of the nephrotic syndrome (2) per (100,000) children, (9 - 16) per (100,000) between Arab and Asian population and a peak incidence between (1) and (5) years old. Males are commonly affected more than females (2.5:1). Approximately (85%) as major cause children with NS have minimal change type[2]. Morbidity and mortality were high in NS and infection was the leading cause of death in children with nephrotic syndrome, in addition the effects of immunosuppressive therapies which are used as treatment may increase the susceptibility of infection[3].

Nephrotic syndrome often affects the physical and psychosocial condition of children in the short and
long term, particularly the child who suffers severely from frequent relapse, steroid dependence or steroid resistance. Steroids and other immunosuppressive treatments for these conditions have particularly negative effects on the physical and mental health of children [4].

Mothers are assisting health workers, especially younger children, such as weighing diapers, checking the jar for albumin, weighing day after day, measuring the circumference of the abdomen, and secondly, assessing edema by staring at the swelling around the eye and the dependent area, third, it must be restricted Diet such as salt, fluid, and excessive protein at some point from the onset of edema and IV, and protecting the infant with NS from contamination all these responsibilities fall on the mothers, which inevitably affects the quality of life [5].

Methodology

Design of the study:

Using a quantitative data collection approach, the analysis was descriptive (cross-sectional). Knowing and evaluating the quality of life of mothers towards their children with Nephrotic Syndrome in i children’s educational hospitals in Baghdad City during the study period from the period of 19th September 2020 to the 22th June 2021.

Ethical Consideration:

The researcher obtained the approval of the Specialized Nursing Department at the College of Nursing / University of Baghdad. Additional approval was obtained from Al-Karkh Health Directorate / the Child’s Central Teaching Hospital At AL-iskan sector and the children Welfare Teaching Hospital of the Medical City Directorate in Baghdad to meet with each child and mother. Finally, the subject agreement was also from mothers during a cover letter and verbal consent to participate in the current study. They answer the questionnaire, after which the researcher explains to them the purpose of the research. The researcher seeks informed consent and provides respect for members ’privacy as well as volunteer input to answer questionnaire questions . These approvals made it easier for the researcher to enter hospitals and centers and meet the children and their mothers and Ethical ethics in the College of Nursing / University of Baghdad regarding confidentiality and disclosure of participants’ identities.

Settings of the Study:

The study was conducted on a child with Nephrotic Syndrome and mothers of infected children in the city of Baghdad / Al-Karkh Health Directorate / Teaching Housing Hospital and the Medical City Health Department / children Welfare Teaching Hospital

Sample of the study:

Non-probability sampling technique (purposeful sampling) for a sample of (100) mothers having children with nephrotic syndrom (70) from children Welfare Teaching Hospital (30) the Child’s Central Teaching Hospital At AL-iskan sector.

Instrument of the study:

A Self-management questionnaire was created to achieve the objectives of the study. The mothers ’quality of life was assessed using (WHOOol.-BREF Arabic version) that contains 26 items,

Data Collection Methods:

The sample was collected through a questionnaire designed to study the quality of life of the mother of a child with Nephrotic Syndrome, conducting direct interviews with them, asking questions, and leaving them free in educational children’s hospitals for a period of 5 days a week. Mothers who meet the research criteria and who are accepted to participate in the research were included in the study. 100 mothers with sick babies were treated in a face-to-face fashion. Data were collected during the period from 1february to 10 march 2021. The estimated time to complete the questionnaire is 15-20 minutes and data collection is
from 9.00 AM to 1.00 PM every day visiting five days a week.

**Statistical Analysis**

The following statistical data analysis approaches were used in order to analyze and assess the results of the study under application of the statistical package (SPSS) ver. (22.0):

**Results and Discussion**

**The Socio-Demographic Characteristics Related mother and Child of the Child with Nephrotic syndrome. Table (1):**

The results were shown the presence of (38.0%) of mothers in the age group (20-29) years. The results of the study also indicated that (80.0%) of mothers are housewives. This is in agreement with a study by (Zyarah, 2011). For 80 participating samples, (28.8% and 27.5%) of mothers were found in the age group (30-34) years and (25-29) years respectively, and (77.5%) were housewives. Table (1-4) show with regard to mother occupational, the study found that (80.0%) of mothers were housewife, and this is agreement with the results of the study conducted by (Mishra et al., 2015) which found that the majority of mothers are housewife.

The demographic characteristics of mothers in Table (1) show that all mothers participating in this study were 94% from urban areas. This can be explained by the study that was conducted in the city of Baghdad, the hospitals were in the center of the city. This finding was consistent with Malhotra and colleagues (2012) who reported that all participants belonged to an urban habitat.

Table(1) show that 81.0 % of families reported an Barely Sufficient. This result may be due to the fact that children with Renal Syndrome have medical treatments and reviews, which causes a financial burden on the family. The inability of mothers to work may also add to the financial pressure on families. As reported by (Mitra & Banerjee, 2011) The spending on illness was more in families with NS patients, ranging between 30% and 60% of the monthly income depending on the severity of the disease compared, but it caused great psychological and economic stress on families.

During the analysis of the data of the current study, regarding gender, the result shows that more males than females, males (77%) versus (23%) (Table 2-4), as evidenced by some research. Participants study chronic kidney diseases such as (Guha and Gossal, 2009), who reports that the majority of the study subjects were 66% were male and (34%) female.

With regard to the child’s age, the results of their study showed that the age of the study sample is (47%) within (3-4) years and the majority of age of the child at diagnosis (44.0%) after the first year old and the Second year (39.0%) were after Three years (13.0%) It is agree with (Trautmann, et al., 2015). who showed results on( 68%) of patient referred with possible Nephrotic syndrome aged within (1-5 ) years. And It agrees with a study by( Zyarah,2011) (55.0%) at the age of <5 years, onset of nephrotic syndrome takes high percentage at age ≥ 5 years and <2 years (26.2%, 22.5%) respectively, (41.3%) of children have NS at 1 year.

**The Level Mothers’ Quality of Life towards their children with Nephrotic Syndrome. Table (2):**

The present study finding revealed that the overall main domains Table (2) shows the level of physical health ranging from mild, moderate and severe. The majority of the mothers’ physical health (62.1%) was moderate, This result was consistent with (Mitra and Banerjee, 2011) who found that the defect of physical well-being is higher in mothers than in fathers, as it was 65.3% in mothers and the current study showed that mothers of children with nephrotic syndrome had physical health problems.

According to my opinion of researcher, the majority of mothers were between the ages of 20 and
39, and most of them do not have chronic diseases, but suffer from some health diseases as a result of intensive care for the child due to nephrotic syndrome.

Table 2 also shows that 65.8% of mothers reported a moderate level of social interaction. This finding was consistent with what (Tsai et al., 2015) reported that quality of life correlates with higher scores in social relationships and psychological state of caregivers of kidney disease children undergoing dialysis. Peritoneal kidney.

Table (2) reveals the majority of participants (70.1% - 65.8%) have a moderate burden level of psychosocial burden these results have the same agreement with the study of (Zyada, et al., 2013) who reported Parent of the child with Nephrotic Syndrome are more likely to have a psychosocial problem, had less social adjustment and had a poorer quality of life than the healthy parents of healthy children.

Table (2) shows Regarding the environmental domain, a proportion (68.6%) of mothers are moderate level. This result was consistent with (Mungo and colleagues, 2007) who found that mothers scored lower than fathers in the environmental domain of quality of life.

Table (2) shows the level of spirituality / religious beliefs and their relative impact on mothers’ quality of life. 48.6% agree With (WHO’s 2006 Quality of Life Assessment) reporting on spirituality, religion, and personal beliefs about quality of life (QoL). The aspects deal with issues such as inner peace, faith, hope, optimism, and spiritual connection. Where it was found that 52% of them have a negative impact on the quality of life. The results showed that the women still felt a greater sense of spiritual connection and faith.

According to the researcher, this is possible within normal conditions because nephrotic syndrome is the largest cause of the impact on the quality of psychological and social life on patients and their mothers. This may be related to patients and needs that include physical, psychological and emotional support. This requires a lot of knowledge and more skills from mothers that have developed and responsibilities towards them. These responsibilities may lead to a significant impact on the quality of life in terms of social and economic. Therefore, the mother is constantly exposed to the largest impacts on the quality of life and does not care for himself.

The above result is also in agreement with (Tsai, et al., 2006) who reported that mothers play a major role for their child, including maintaining their health, providing professional advice, as well as a typical request for patient upbringing. Therefore, it is not uncommon for mothers to experience a significantly lower quality of life compared to mothers of healthy infants.

The study showed that a child with kidney syndrome has an effect on mothers QoL. This is consistent with a rapid study of the impact of a child’s illness on the family, and on the mother’s quality of life. The results showed the impact of the child with kidney syndrome on the mother’s life. The lowest scores were found in two domains, ‘anxiety’ and ‘emotional function’, although there were other domains of quality of life that were significantly affected.

The Association among study characteristics and Mothers’ QoL. Table (3):-

Table (4-3-2) shows the results of the relationships. The results showed that weak relationships were obtained, as statistically significant levels at P > 0.05 were not calculated with respect to the studied overall evaluation in light of SDCv. , weak relationships are counted, as no significant levels were recorded at P > 0.05 between children (SDCv.) and the main areas studied. This is due to the fact that the child in the advanced stages of life is more responsive and understands his health condition.

Conclusion

The results of the study showed that mothers
of children with nephrotic syndrome suffer from moderate (63.0%) overall level of quality of life in the five domains of, physical, psychological, social, environmental, beliefs and spiritual. All social and demographic characteristics of the sample do not affect the quality of life.

**Recommendations**

Health and educational institutions should hold special meetings and celebrations for mother and child. Nephrotic Syndrome is educational and entertaining at the same time. Availability of multidisciplinary team of supportive care as well as follow-up out-patient clinic that include pediatric nurses, renal physicians, social workers, dietitian, psychotherapists and physiotherapist to assist children and their mother in maintaining near normal lifestyle at highest possible level of emotional feeling.

| Table (1): Distribution of the studied Mothers and child according to (SDCv.) |
|---------------------------------|------------------|-----|-----|
| variables                       | Groups           | No. | %   |
| Mother’s Age Groups             | < 20             | 24  | 24  |
|                                 | 20 _ 29          | 38  | 38  |
|                                 | 30 _ 39          | 23  | 23  |
|                                 | ≥ 40             | 15  | 15  |
| Mother’s Occupational           | House wife       | 80  | 80  |
|                                 | Government employee | 14  | 14  |
|                                 | Earner           | 6   | 6   |
| Residency                       | Urban            | 94  | 94  |
|                                 | Rural            | 6   | 6   |
| Your monthly income             | Sufficient       | 14  | 14  |
|                                 | Barely Sufficient | 81  | 81  |
|                                 | Insufficient     | 5   | 5   |
| Marital Status                  | Separate         | 2   | 2   |
|                                 | Married          | 94  | 94  |
|                                 | Widow            | 4   | 4   |
|                                 | No               | 3   | 3   |
|                                 | Yes              | 97  | 97  |
| Gender                          | Male             | 77  | 77  |
|                                 | Female           | 23  | 23  |
| The educational level of a child (his participation in school like other normal children) | School joint | 10 | 10 |
|                                 | School refused   | 3   | 3   |
|                                 | She/he not yet complete | 87 | 87 |
| When did the symptoms of the begin? | < 1 yr. | 4 | 4 |
|                                 | First yr.        | 44  | 44  |
|                                 | Second yr.       | 39  | 39  |
|                                 | Third & Fourth yr. | 13 | 13 |
Table (2): Mothers’ Knowledge, Attitudes, and Practices related to “Children with Nephrotic Syndrome “ at directorate of military medical affairs unit’s

<table>
<thead>
<tr>
<th>Domains</th>
<th>No.</th>
<th>Min.</th>
<th>Max.</th>
<th>GMS</th>
<th>PSD</th>
<th>GRS %</th>
<th>Ev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>The physical well-being</td>
<td>100</td>
<td>1.556</td>
<td>2.222</td>
<td>1.862</td>
<td>0.135</td>
<td>62.1</td>
<td>M</td>
</tr>
<tr>
<td>The Psychological well-being</td>
<td>100</td>
<td>1.700</td>
<td>2.500</td>
<td>2.104</td>
<td>0.162</td>
<td>70.1</td>
<td>M</td>
</tr>
<tr>
<td>Social Interaction</td>
<td>100</td>
<td>1.714</td>
<td>2.286</td>
<td>1.973</td>
<td>0.120</td>
<td>65.8</td>
<td>M</td>
</tr>
<tr>
<td>The Environment and Place of Residence</td>
<td>100</td>
<td>1.333</td>
<td>2.667</td>
<td>2.057</td>
<td>0.193</td>
<td>68.6</td>
<td>M</td>
</tr>
<tr>
<td>Spirituality / Religious Beliefs</td>
<td>100</td>
<td>1.000</td>
<td>1.833</td>
<td>1.457</td>
<td>0.143</td>
<td>48.6</td>
<td>L</td>
</tr>
<tr>
<td>Overall Evaluation</td>
<td>100</td>
<td>1.723</td>
<td>2.035</td>
<td>1.891</td>
<td>0.071</td>
<td>63.0</td>
<td>M</td>
</tr>
</tbody>
</table>

Ev. : Evaluated (33.33 – 55.55) Low (L) ; (55.56 – 77.77) Moderate (M) ; (77.78– 100) High (H).

Table (3) Relationship between mother quality life and Demographical Characteristics

<table>
<thead>
<tr>
<th>Demographical Characteristics and some related variables</th>
<th>Overall Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C.C.</td>
</tr>
<tr>
<td>Mother’s Age Groups</td>
<td>0.248</td>
</tr>
<tr>
<td>Mother’s Occupational</td>
<td>0.219</td>
</tr>
<tr>
<td>Residency</td>
<td>0.084</td>
</tr>
<tr>
<td>Your monthly income</td>
<td>0.143</td>
</tr>
<tr>
<td>Marital Status</td>
<td>0.175</td>
</tr>
<tr>
<td>Is the father alive</td>
<td>0.059</td>
</tr>
<tr>
<td>Gender</td>
<td>0.048</td>
</tr>
<tr>
<td>The educational level of a child</td>
<td>0.173</td>
</tr>
<tr>
<td>When did the symptoms of the disease begin?</td>
<td>0.219</td>
</tr>
</tbody>
</table>

NS : Non Sig. at P>0.05 ; Testing are based on a Contingency Coefficient test.
Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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References


Assessment of Pregnant women’s Practices Concerning Measures taken by Pregnant Women for Prevention of (Covid19) at Primary Health Care Centers in Baghdad City

Athraa Kadeem Kareem1, Rabea Mohsen Ali2

1MSc. Student, 2Professor, University of Baghdad, College of Nursing, Maternal and Neonate Nursing Department

Abstract
Corona virus is one of the major pathogens that mainly target the respiratory system of humans. Pregnant women considered to be a special population group because of the unique ‘immune suppression’ caused by pregnancy. The immunologic and physiologic changes might make pregnant women at higher risk of severe illness or mortality with Covid-19, compared with the general public.

Objectives: To assess pregnant women’s practices concerning measures taken by pregnant women for prevention of (Covid19) at primary health care centers in Baghdad City. Methodology: A descriptive (cross sectional) study has been carried out on Non-probability sample of (150) of pregnant who attend five Primary health care centers. Study has been conducted for the period of February 8th to 18th March 2020.

Results: The results of the current study indicate that (33.3%) of pregnant women are with age group (21-25) year, (28%) with age group (16-20), and (27.3%) with age group (26 -30) years. (22.7%) of study sample are graduated from intermediate school, (76.7%) of pregnant women are housewives, (51.3%) of sample are resident in a high class neighborhood, (76%) of pregnant women are moderate monthly income, (34.7%) of pregnant women are gravid for (3) times, (81.4%) have (1-2) births, (53.3%) of pregnant women reporting they have normal vaginal delivery, (34%) of pregnant women have good level of practices regarding measures taken by pregnant women for prevention of (Covid19), (34%) have fair levels of practices, and (32%) have poor levels.

Conclusion: The study concludes that half of pregnant women have good level of practices regarding prevention of corona virus, and more than half of pregnant women are showing fair level of health behaviors toward reduction of corona virus spread.

Recommendations: the pregnant women should be supplied with instructional booklets in order to raise practice level of preventive measures of COVID-19 infection at the beginning of their pregnancy.

Keywords: Assessment, Pregnant, Precaution Measures, Practices, COVID-19.

Introduction
Corona virus is a severe disorder that mainly affects the respiratory systems of individuals. Severe acute respiratory syndrome (SARS)-CoV and Middle East respiratory syndrome (MERS)-CoV have both been reported as Corona virus epidemics in the past[1]. The variations are in the genetic make-up, clinical manifestations, case mortality, and global spread rate. SARS-CoV2, also known as the coronavirus virus, is a virus that causes coronavirus disease 2019 (COVID-19), which has become the world’s newest health threat [2]. The most common COVID-19
symptoms include fever, dry cough, dyspnea, headache, sore throat, rhinorrhea, and hemoptysis, which can range from mild (or no symptoms) to severe illness [3].

Pregnancy is a state of partial immune suppression which makes pregnant women more vulnerable to viral infections, and the morbidity is higher even with seasonal influenza. Therefore, the COVID-19 epidemic may have serious consequences for pregnant women. However, information on the effect of COVID-19 on the course and outcome of pregnancy in the first and second trimesters is not available yet. As COVID-19 still appears to be spreading, more infections in pregnant women are likely to be encountered in different regions, countries, and continents. Therefore, it is important that pregnant women and their families, as well as the general public and health-care providers, receive as accurate information as possible. During infectious disease outbreaks most frequently researched preventive behaviors and have proven to the spread of pandemics [4].

**Materials and Methods**

A descriptive (cross sectional) study has been carried out on Non-probability sample of (150) of pregnant women’s Practices Concerning Corona Virus Disease Precaution Measures who attend five Primary health care centers. Study has been conducted for the period of February 8th to 18th March 2020. The questionnaire has been used as a tool of data collection and consist of three main parts; including Socio-Demographic Information, Pregnant Women History, and pregnant women’s practices regarding measures to be taken by pregnant woman to prevent (COVID 19). A pilot study conducted on (15) pregnant women to determine the study reliability, descriptive and inferential statistic approaches are used for data analysis. Data are analyzed through the use of (SPSS) ver.24.

**Results and Discussion**

Discussion their Socio-demographic Characteristics of study (Table 1):

The present study has reported that the highest percentage (33.3) of the study sample is at age group range (21 – 25) years old. This finding is consistent with, Abdulla, Akram, & Mardan Abullah,( 2021) in Iraqi study which indicated that the highest percentage of the participants in the study were between the ages of 20 and 29, accounting for 50.5 percent of the total. And the study found that the age group range (36) years old has the lowest percentage (2.7) of the study sample than any other age group[5].

Regarding the education level refers that the highest percentage among pregnant women are graduated from intermediate school (22.7%), (21.3%) are graduated from primary school, while (20.6%) are graduated from secondary school, and (18.7%) are Institute/college graduate. This finding is consistent with, Ferdous, et al. (2020) which indicates that the level of education for study sample was primary in (35%), secondary in (50%), and tertiary (15%) [6].

Regarding the occupational status indicates that (76.7%) of pregnant women are housewives and only 23.3% of them are working as governmental employee. This result supported by Maharlouei et al [7] who reported in the study that the majority of the sample (House wives) account for approximately 488 (90.4%) of the total sample, and the lowest percentage of the group, 52 (9.6%), was employed (7), and Lee et al,(2020) reported in the study that the majority of the sample are (House wives), accounting for approximately 116 (69.5%) of the total sample, while the lowest level, 51 (30.5%), are employed[8].

Regarding monthly income, (76%) of pregnant women are perceived moderate monthly income. This study agree with yassa et al,( 2020) who reported that (52.9%) of pregnant women are perceived moderate monthly income [9]. This study disagree with Maharlouei et al (2020) also observed that majority of study are high socioeconomic status[7].
Discussion of Reproductive Health Characteristics (Table 2):

Regarding reproductive history the results of present study reveals that pregnant women are gravid for (3) times as referred by high percentage (34.7%), the parity is shows that (81.4%) of pregnant women have (1-2) births, while (14.6%) of them have (3-4) births. Regarding mode of delivery, (53.3%) of pregnant women reporting they have normal vaginal delivery and (46.7%) having cesarean section, (61.3%) have delivered in hospital and only (38.7%) are delivered at home. This result agree with: Nie, et al., 2020 who found that the majority of the participants in the study are pregnant for (1) time, as evidenced by a high number of 1 13 (39.4%), while the most of the participants have (1-2) parity, accounting for 66.7 percent of the total (3.0 percent) (10). Regarding the mode of delivery and delivery place, this study disagree with: Nile, et al., 2020 who found that Twenty-two (81.5%) women delivered via cesarean section, and 5(18.5%) had vaginal deliveries , (74%) of women were worried about being infected with COVID-19; (53%) of women would choose having a caesarean section over a vaginal delivery [10].

Overall Assessment of Measures taken by Pregnant Women for Prevention of Corona Virus among) Table 3):

Regarding the measures taken for prevention of corona virus among pregnant women the study results presents the mean scores which indicate fair level. The overall assessment of measures taken by pregnant women for prevention of corona virus the results reveals that pregnant women have fair (34%), good (34%) , and poor (32%) levels of practices regarding measures taken to prevent corona virus.

The present results study supported by Metwally, & Desoky (2020) Who have reported that all of the women (100%) were aware of ongoing COVID-19 infection. Most of them reported fever and cough to be symptoms of COVID-19 infection (87.3% & 85.1% respectively). More than two thirds of women (70.8%) reported to wear face mask when they had that symptoms, (62.7%) of them reported to stay at home and (52.7%) reported to inform their health care provider before going to hospital. The majority of women (89.2%) reported that the COVID-19 virus spreads via respiratory droplets of infected individuals[11]. While Abdulla, Akram, & Mardan Abdullah (2021) that about 2/3rd of the participants were presented with poor practice (67.25%), while less than 1/3rd (32.75%) with good practice .For the participant practice response for preventing COVID-19 show that 73 (18.25.0%) choose the washing hands frequently as major preventive way, 59 (14.75%) said that a distance for 2 meters and more between the people can prevent the infection with disease, 123 (30.75%) choose wearing mask, (75.16%) choose do not touch eye, nose and mouth common way to prevent the infection, and 78 (19.5%) mentioned staying home was the way to prevent infection with the disease [5].

Correlation among Practices related to Measures for Prevention COVID19 among Pregnant Women with regard to their Socio-demographic Characteristics (Table 4):

The current study results shows that there are significant relationships (positive) among pregnant women practices with regard to their level of education and occupation at p-value= 0.003 and 0.006, and there are high significant relationships (strong positive) among pregnant women practices with regard to their monthly income at p-value= 0.001 respectively. This result of the study agrees with Metwally & Desoky (2020), that reported there were statistically significant relations between the studied women’s level of practice about preventive measures against COVID-19 infection and their education level and parity with p-value <0.05. Women who had university education had higher practice level than other education levels (26.53% & 20.77% respectively), and the women whose income level was inadequate and just meet life expenses had higher positive practice about COVID-19 infection than those who had insufficient income (90.63%,
Correlation among Practices related to Measures for Prevention COVID19 among Pregnant Women with regard to their Reproductive Characteristics Table 5):

Table 1: Distribution of Pregnant Women According to their Socio-demographic Characteristics (N= 150)

<table>
<thead>
<tr>
<th>%</th>
<th>F</th>
<th>Characteristics</th>
<th>List</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>42</td>
<td>16 – 20 year</td>
<td></td>
</tr>
<tr>
<td>33.3</td>
<td>50</td>
<td>21 – 25 year</td>
<td>1</td>
</tr>
<tr>
<td>27.3</td>
<td>41</td>
<td>26 – 30 year</td>
<td></td>
</tr>
<tr>
<td>8.7</td>
<td>13</td>
<td>31 – 35 year</td>
<td></td>
</tr>
<tr>
<td>2.7</td>
<td>4</td>
<td>36 ≤ year</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>Doesn’t read &amp; write</td>
<td>2</td>
</tr>
<tr>
<td>10.7</td>
<td>16</td>
<td>Read &amp; write</td>
<td></td>
</tr>
<tr>
<td>21.3</td>
<td>32</td>
<td>Primary school</td>
<td></td>
</tr>
<tr>
<td>22.7</td>
<td>34</td>
<td>Intermediate school</td>
<td></td>
</tr>
<tr>
<td>20.6</td>
<td>31</td>
<td>Secondary school</td>
<td></td>
</tr>
<tr>
<td>18.7</td>
<td>28</td>
<td>Institute/college +</td>
<td></td>
</tr>
<tr>
<td>76.7</td>
<td>115</td>
<td>Housewife</td>
<td>3</td>
</tr>
<tr>
<td>23.3</td>
<td>35</td>
<td>Employee</td>
<td></td>
</tr>
<tr>
<td>23.3</td>
<td>35</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>114</td>
<td>Moderate</td>
<td>4</td>
</tr>
<tr>
<td>0.7</td>
<td>1</td>
<td>High</td>
<td></td>
</tr>
</tbody>
</table>

f: Frequency, %: Percentage, M: Mean, SD: Standard deviation
Table (2): Distribution of Pregnant Women according to their Reproductive Health Characteristics (N=150)

<table>
<thead>
<tr>
<th>List</th>
<th>Characteristics</th>
<th>%</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gravidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>21.3</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>34.7</td>
<td>52</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>27.3</td>
<td>41</td>
</tr>
<tr>
<td>5+</td>
<td></td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 – 2</td>
<td></td>
<td>81.4</td>
<td>122</td>
</tr>
<tr>
<td>3 – 4</td>
<td></td>
<td>14.6</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Normal vaginal</td>
<td>53.3</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Cesarean section</td>
<td>46.7</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Delivery place</td>
<td>38.7</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Hospital</td>
<td>61.3</td>
<td>92</td>
</tr>
</tbody>
</table>

f: Frequency, %: Percentage, M: Mean, SD: Standard deviation

Table (3): Overall Assessment of Measures taken by Pregnant Women for Prevention of Corona Virus

<table>
<thead>
<tr>
<th>Levels</th>
<th>SD</th>
<th>M</th>
<th>%</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>7.042</td>
<td>13.18</td>
<td>32</td>
<td>48</td>
</tr>
<tr>
<td>Fair</td>
<td></td>
<td></td>
<td>34</td>
<td>51</td>
</tr>
<tr>
<td>Good</td>
<td></td>
<td></td>
<td>34</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

f: Frequency, %: Percentage, M: Mean for total score, SD: Standard Deviation, Poor= 0 – 8; Fair= 9 – 17; Good= 18 – 26
Table (4): Correlation among Practices related to Measures for Prevention COVID19 among Pregnant Women with regard to their Socio-demographic Characteristics (N=150)

<table>
<thead>
<tr>
<th>Sig</th>
<th>p-value</th>
<th>Pearson correlation</th>
<th>Practices Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.S</td>
<td>0.734</td>
<td>-0.028</td>
<td>Age</td>
</tr>
<tr>
<td>S</td>
<td>0.003</td>
<td>0.244</td>
<td>Level of education</td>
</tr>
<tr>
<td>S</td>
<td>0.006</td>
<td>0.223</td>
<td>Occupation</td>
</tr>
<tr>
<td>H.S</td>
<td>0.001</td>
<td>0.296</td>
<td>Monthly income</td>
</tr>
</tbody>
</table>

P: probability, Sig: Significance, N.S: Not Significant, S: Significant, H.S: High significant

Table (5): Correlation among Practices related to Measures for Prevention COVID19 among Pregnant Women with regard to their Reproductive Characteristics

<table>
<thead>
<tr>
<th>Sig</th>
<th>p-value</th>
<th>Pearson correlation</th>
<th>Practices Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.S</td>
<td>0.136</td>
<td>0.122</td>
<td>Gravidity</td>
</tr>
<tr>
<td>S</td>
<td>0.041</td>
<td>0.167</td>
<td>Parity</td>
</tr>
<tr>
<td>N.S</td>
<td>0.191</td>
<td>-0.107</td>
<td>Mode of delivery</td>
</tr>
<tr>
<td>N.S</td>
<td>0.402</td>
<td>0.069</td>
<td>Delivery place</td>
</tr>
</tbody>
</table>

P: probability, Sig: Significance, N.S: Not Significant, S: Significant, H.S: High significant

The study results present that there is significant relationships (positive) between pregnant women practices and their parity at p-value= 0.041, while there is no significant relationship has been reported among pregnant women practices with regard to gravidity, mode of delivery, and delivery place. This study supported by Metwally& Desoky. (2020), Multiparous women had a higher level of practice than primiparous women, according to the study (64.40 % & 6.98%) respectively [11].

Conclusion

The study finding manifests that overall assessment of measures taken by pregnant women for prevention of corona virus, the results reveals that pregnant women have fair to good levels of practices regarding measures taken to prevent corona virus, also there is significant relationship relationships (positive) among pregnant women practices with regard to their level of education and occupation at p-value= 0.003 and 0.006, and there are high significant relationships (strong positive) among pregnant women practices with regard to their monthly income at p-value= 0.001 respectively, and significant relationships (positive) between pregnant women practices and their parity at p-value= 0.041.
Recommendations: The study suggests further research studies are needed to determine the impact of COVID-19 on pregnant women and fetal outcomes.

Acknowledgments: we would like to thank all the pregnant women who participated in this study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


Impact of Normal and Overweight Pregnant Women upon Neonatal Outcomes in Al-Nasiriya Hospitals: Comparative Study

Sara Ahmed Rashed¹, Rabea Mohsen Ali²
¹MSc. Student, University of Baghdad, College of Nursing, Maternal and Neonate Nursing Department, Al-Nasiriya City, Iraq, ²Professor, University of Baghdad, College of Nursing, Maternal and Neonate Nursing Department, Baghdad City, Iraq

Abstract

Background: Maternal obesity is a major risk for both the mother and the fetus, and it is considered an obstetrical risk factor that leads to a high incidence of complications throughout the prenatal period, as well as an increased risk of many adverse pregnancy outcomes.

Objectives: To assess the impact of normal and overweight pregnant women upon Neonatal outcomes at Al-Nasiriya city.

Methodology: A descriptive analytic study was conducted from January 11th, 2021 to March 18th, 2021 at Al-Nasiriya City Hospitals for Maternity and Children on (100) pregnant women who attending labor room, (50) were overweight and (50) normal weight pregnant women with gestational age for 08-40 weeks were included. A non-probability (Purposive sample) was used to select the participants of study sample.

Results: The result of study revealed that (34.1%) (36.4%) of study sample aged (26-30) years in normal, and overweight pregnant respectively. The study results clarifies the previous and current body mass index among normal weight pregnant women (100% & 95%) respectively, while in overweight (63.6%) overweight previously and (65.9%) obesity class (I) at current. The impact of weight upon neonate outcomes; the findings indicates that weight has significant impact up on outcomes related to neonate, at p-value= 0.006, while there is no impact has been reported among group of normal weight pregnant women.

Conclusion: The study concluded that overweight impact upon outcomes related to neonate condition, while there is no impact has been reported among group of normal weight pregnant women.

Recommendations: Increased physical activity and knowledge of healthy eating habits among pregnant women as part of primary health care centre initiatives to reduce obesity in women.

Keywords: Pregnant, Overweight, Normal weight, Adverse neonatal outcomes.

Introduction

Overweight and obesity are steadily growing in all age groups worldwide, especially in countries with low and medium incomes [1]. Given the correlation with negative short- and long-term maternal and child outcomes, pre-pregnancy obesity (body mass index, BMI> 30 kg/m²), excessive gestational weight gain (GWG) and postpartum weight retention (PPWR) are seen as new public health threats [2]. These findings include, in later life, obstetric or neonatal complications, obesity, type 2 diabetes (T2D) and cardiovascular disease (CVD) [3].
Overweight is defined by a BMI of $\geq 25$ and obesity by a BMI of $\geq 30$ \[^4\]. Several maternal, fatal and neonatal complications are associated with overweight and obesity. Maternal complications are associated with maternal obesity as well as fatal and neonatal complications, such as hypertensive disorders, diabetes and venous thrombo-embolism, including miscarriage and stillbirth, foetal abnormalities, macrosomia, preterm birth, extended pregnancy, Caesarean delivery, postpartum haemorrhage and complications in anesthesia \[^5\].

**Materials and Methods**

A descriptive analytic study was conducted to determine the Impact of normal and overweight pregnant women upon neonatal outcomes in Al-Nasiriya city Hospitals: Comparative Study. The study was performed from January 11\(^{th}\), 2021 to March 18\(^{th}\), 2021. Non-probability (purposive sample) used to collect the data from (100) pregnant women. The study sample consists of (50) pregnant women with normal weight and (50) pregnant women with overweight. A pilot study conducted in order to determine the reliability of the questionnaire in a sample of (10) pregnant women. Content validity was determined through a panel of (16) experts who had more than 8 years of experience in their field. The data was collected after obtaining the agreement from women to participate in this study. Data are analyzed through the use of (SPSS) ver. (24.0).

**Results and Discussion**

**Discussion of Socio-demographic Characteristics of study (Table 1):**

The present study results reveals that the highest percentage (34.1\%) of normal weight pregnant are with age group of (26 - 30) years, with mean $\pm$ SD (24$\pm$5) years, and the highest percentage (36.4\%), among overweight pregnant is also refers to (26 - 30) age group, with mean $\pm$ SD of (27$\pm$6) years. Khan & Qianli, (2017) stated that the most important cause for increase in weight gain with age is the activity of the metabolism decreases with increase of age, and this lead to fewer of body burns calories and this ends up accumulating lipid in the body and thus an increase in weight \[^6\].

Regarding the level of education, the highest percentage among pregnant women are graduated from primary school; (47.7\%) among overweight pregnant and (38.6\%) among normal weight. These findings are consistent with Pakniat & Ranjkesh, (2015) and with Stanford et al., (2015) and with Teixeira et al., (2012) they found that lack of appropriate understanding and adequate competence regarding obesity likely contributes to ambivalent belief development and negative attitudes toward obese individuals, who are described as unmotivated, lazy, and lacking self-control \[^7,8,9\].

Regarding Income, the highest percentage among pregnant women are perceived barely sufficient monthly income (70.5\%) among normal weight pregnant and (65.9\%) among overweight pregnant women. The study by Ogden et al., (2017), stated that obesity prevalence decreased with increased levels of income and educational attainment among women \[^10\] and, May et al., (2013) have suggested that obesity prevalence varies by income and educational level, although patterns might differ between high-income and low-income countries \[^11\].

**Discussion of Pregnant Women According to BMI (Table 2):**

The study results clarifies the previous and current body mass index among pregnant women that refer to normal among the group of normal weight pregnant (100\% and 95.5\%), while refer to overweight previously (63.6\%) and obesity I (65.9\%) at current among the group of overweight pregnant women. This result agree with Eren et al., (2015) they found the mean of pre-pregnancy body mass index is (27.18 $\pm$ 5.38), also one third of the study sample are within normal weight status and then followed by overweight and class-I obesity \[^12\], also agree with Machado et al., (2020) pregnancy BMI: (29.6\%) women (n = 918).
were classified as overweight and (27.3%) (n = 846) as obese \(^{(13)}\). Also agree with Subhan et al., (2019) that most women (64%) had a normal pre-pregnancy BMI \(^{(14)}\).

### Table (1): Distribution of Pregnant Women According to their Socio-demographic Characteristics

<table>
<thead>
<tr>
<th>List</th>
<th>Characteristics</th>
<th>Normal weight</th>
<th>Overweight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>f</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 – 20 year</td>
<td>14</td>
<td>31.8</td>
</tr>
<tr>
<td></td>
<td>21 – 25 year</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>26 – 30 year</td>
<td>15</td>
<td>34.1</td>
</tr>
<tr>
<td></td>
<td>31 – 35 year</td>
<td>4</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>36 ≤ year</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>44</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>M±SD</td>
<td>24±5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Level of education</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Doesn’t read &amp; write</td>
<td>2</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Read &amp; write</td>
<td>3</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>Primary school</td>
<td>17</td>
<td>38.6</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>7</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td>Secondary</td>
<td>4</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>Institute</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>College/higher</td>
<td>11</td>
<td>25</td>
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<tr>
<td></td>
<td>Total</td>
<td>44</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>Perceived income</td>
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<tr>
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<td>Insufficient</td>
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<td>13.6</td>
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<td>31</td>
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<td>Sufficient</td>
<td>7</td>
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</table>

f: Frequency, %: Percentage, M: Mean, SD: Standard deviation
### Table (2): Distribution of Pregnant Women According to BMI

<table>
<thead>
<tr>
<th>List</th>
<th>Body mass index</th>
<th>Normal weight</th>
<th>Overweight</th>
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<td>f</td>
<td>%</td>
</tr>
<tr>
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</tr>
<tr>
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<td>44</td>
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<tr>
<td></td>
<td>Overweight</td>
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</tr>
<tr>
<td></td>
<td>Obesity I</td>
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<td>Obesity III</td>
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<td>Obesity III</td>
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f: Frequency, %: Percentage

### Table (3): Assessment the Effect of Weight on Neonate Condition among Pregnant Women

<table>
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<tr>
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<th>Outcomes (Neonate conditions)</th>
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<th>Overweight N=44</th>
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<tr>
<td></td>
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<td>f</td>
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<td>Yes</td>
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<td>2</td>
<td>Child weight less &lt; 2500gm</td>
<td>No</td>
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<td>Yes</td>
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<td>Small gestational age (SGA)</td>
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<td>1</td>
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<td>4</td>
<td>Large gestational age (LGA)</td>
<td>No</td>
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<td>Admission to the neonatal intensive care unit</td>
<td>No</td>
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<td>Umbilical cord problems</td>
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### Cont... Table (3): Assessment the Effect of Weight on Neonate Condition among Pregnant Women

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</table>

f: Frequency, %: Percentage

### Table (4): Simple Linear Regression for Assessment the Impact of Normal and Overweight upon Neonate Outcomes among Pregnant Women

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Dependent Variable: Outcomes</th>
<th>Normal weight Pregnant (N=44)</th>
<th>Overweight Pregnant (N=44)</th>
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</thead>
<tbody>
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<td></td>
<td>Unstandardized Coefficients</td>
<td>Standardized Coefficients</td>
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<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
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<td>Neonate outcome</td>
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<td>0.897</td>
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</table>

Dependent Variable: Outcomes
<table>
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<th>Pregnant Weight Outcomes</th>
<th>Normal weight Pregnant (N=44)</th>
<th>Overweight Pregnant (N=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spearman correlation</td>
<td>p-value</td>
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<tr>
<td>Macrosomia (a child’s weight more than 4000 g)</td>
<td>0.033</td>
<td>0.830</td>
</tr>
<tr>
<td>Child weight less than 2500gm&gt;</td>
<td>0.069</td>
<td>0.656</td>
</tr>
<tr>
<td>Small gestational age SGA</td>
<td>0.033</td>
<td>0.830</td>
</tr>
<tr>
<td>Large gestational age LGA</td>
<td>0.033</td>
<td>0.830</td>
</tr>
<tr>
<td>Admission to the neonatal intensive care unit</td>
<td>0.069</td>
<td>0.656</td>
</tr>
<tr>
<td>Umbilical cord problems</td>
<td>0.033</td>
<td>0.830</td>
</tr>
<tr>
<td>Newborn death</td>
<td>0.033</td>
<td>0.830</td>
</tr>
<tr>
<td>Birth defect / malformations</td>
<td>0.033</td>
<td>0.830</td>
</tr>
<tr>
<td>Hypoglycemia in newborns</td>
<td>0.033</td>
<td>0.830</td>
</tr>
<tr>
<td>Hyperbilirubinemia in newborns</td>
<td>0.048</td>
<td>0.759</td>
</tr>
<tr>
<td>Delayed intrauterine growth (during pregnancy)</td>
<td>0.033</td>
<td>0.830</td>
</tr>
<tr>
<td>Childbirth before 37 weeks of pregnancy</td>
<td>0.059</td>
<td>0.703</td>
</tr>
<tr>
<td>Postdate birth after 42 weeks of pregnancy</td>
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<td>0.830</td>
</tr>
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<td>Shortness of breath</td>
<td>0.059</td>
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<td>Inhalation of amniotic fluid</td>
<td>0.048</td>
<td>0.759</td>
</tr>
<tr>
<td>Apgar score Less than 7 in 5 minutes</td>
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</tr>
<tr>
<td>Neonatal asphyxia</td>
<td>0.033</td>
<td>0.83</td>
</tr>
</tbody>
</table>

P: probability, Sig: Significance, N.S: Not Significant, S: Significant, H.S: High significant
Discussion of Assessment the Effect of Weight on Neonate Condition among Pregnant Women (Table 3):-

Regarding the outcomes related to neonate conditions; the results shows that “macrosomia” is seen among (2.3%) of normal weight and (13.6%) of overweight; the “child weight less than 2500 g” is seen among (9.1%) of normal weight and (22.7%) of overweight; “small gestational age” is reported among (2.3%) of normal weight and (20.5%) of overweight; “Large gestational age” is seen among only (2.3%) of overweight; “admission of neonate to NICU” is associated with (9.1%) of normal weight and (40.9%) of overweight; “umbilical cord problems” is only seen in (2.3%) of overweight; “newborn death” is reported in only (20.5) of overweight; the “birth defect” is seen among only (4.5%) of overweight pregnant; “hypoglycemia” is reported among only (13.6%); “hyperbilirubinemia” is reported among (4.5%) of normal weight and (6.8%) of overweight; “delayed intrauterine growth” is reported among only (2.3%) of overweight; the “childbirth before 37 weeks” is reported among (6.8%) of normal weight and (15.9%) of overweight; the “postdate birth after 42 weeks” is reported among only (4.5%) of overweight; the “shortness of breath” is reported among (6.8%) of overweight and (13.6%) of overweight; the “inhalation of amniotic fluid” is seen among (4.5%) of normal weight and (11.4%) of overweight; the “Apgar score less than 7 in 5 minutes” is seen in only (25%) of overweight; and “neonatal asphyxia” is reported in (2.3%) of normal weight and (9.1%) of overweight.

Simple Linear Regression for Assessment the Impact of Normal and Overweight upon Neonate Outcomes among Pregnant Women (Table 4):-

The results illustrate that there is no significant relationship between pregnant women’ weight with regard to neonate’s outcomes among the normal weight pregnant women, but among the overweight pregnant women, it shows there is significant relationship among weight with regard to neonate’s outcomes of: macrosomia, admission to the neonatal intensive care unit, hypoglycemia in newborns, and shortness of breath at p-value= (0.022, 0.015, 0.022, and 0.022). Bhavadharini et al., (2017) (Ram et al., (2020) they found in their study consistently higher macrosomia which was more frequently present (16.1%) in the obese and overweight group (2.0%) category of woman compared to normal (2.5%) [15,16].

Papazian et al., (2017) found that (LBW) significantly was present in overweight (1.0%) category of women compared to normal (2.0%) [17]. Lamminpää et al., (2016), and Black et al., (2013) found that with overweight and obesity significantly contributing to the growing prevalence of large for gestational age infants and increasing the risk of pregnancy-related complications[18,19]. Liu et al., (2013) found that of neonatal intensive care admission (AOR, 2.07; 95% CI, 1.32–3.23) and Apgar score of under 7 at 5 minutes (95%) in overweight compared with normal weight [20]. Also Hartge et al., (2016) , and Hanneman et al., (2020) found that neonates born to obese women were associated with significantly increased rates of hypoglycemia (95%)[21,22].


Conclusion

The study finding manifests that there is no significant relationship between pregnant women’
weight with regard to neonatal complications among the normal weight pregnant women, but among the overweight pregnant women, it shows there is significant relationship among weight with regard to neonate’s outcomes of: macrosomia, admission to the neonatal intensive care unit, hypoglycemia in newborns, and shortness of breath at p-value= (0.022, 0.015, 0.022, and 0.022).

**Recommendations:** The study suggests that nursing and health-care professionals play a bigger role in educating pregnant women and raising awareness about their health, especially natural weight gain before and during pregnancy.

**Funding:** this research was funded by Author. Moreover,

**Conflict of Interest:** None declared.

**Ethical approval:** clearance was taken from committee in College of Nursing/ University of Baghdad.

**Acknowledgments:** we would like to thank all the pregnant women who participated in this study.

**References**


13) Machado C, Monteiro S, Oliveira MJ. Impact of overweight and obesity on pregnancy outcomes in women with gestational diabetes—results


Instruction Program for Nurses Knowledge about Applying the Communication Strategies (SBAR) in Critical Care Units

Haneen Qahtan Abadi¹, Huda Baker Hassan²

¹MSC Student, University of Baghdad, College of Nursing, Department of Adult Nursing, Iraq,
²Assist. Professor University of Baghdad, Adult Nursing Department, College of Nursing, Iraq.

Abstract

Background: Effective communication can improve patient care and safety by ensuring complete and correct information transmission and preventing misrepresentation and misinterpretation of patient-centered data. Situation, Background, Assessment and Recommendation (SBAR) is one of the most effective communication strategies that are used in transmitted the clinical information between the health professionals.

Objectives of the Study: Present study aims to determine the effectiveness of Instruction program on nurses’ knowledge regarding communication strategy (SBAR) and find out the association between the effectiveness of instruction program and nurses’ level of education, year of experiences, year of experiences in critical units and training course.

Methodology: A pre-experimental study design was carry out in the critical care units of Imam Al-Hussein Medical City at Holy Karbala City between the periods 17th of September 2020 to 25th of May 2021. A non-probability (purposive) sample of (25) nurses were selected based on the study criteria.

Results: The results of the study indicated that there were high statistically differences between the pre and post-test of the instruction program related to the communication strategy. The study found a high statistically significant relationship between the effectiveness of the instruction program related to the communication strategy, educational level, training courses for nursing documentation, years of experience in nursing, and years of experience for nurses in the critical care units.

Conclusion: The study concluded that the instruction program had a positive effect on nurses’ knowledge concerning communication strategy (SBAR).

Recommendations: Testing the current program on a wide group of health-care providers in hospitals.

Key Words: Instruction, Communication Strategy (SBAR), Nurses Knowledge, Critical Care Units.

Introduction

One of the effective communication strategies in nursing services is the SBAR (Situation, Background, Assessment, and Recommendation). SBAR is an easy framework for communicating critical information between health professionals[1]. In hospitals, structured communication techniques have been widely used to improve communication between healthcare staff. These techniques have been demonstrated to improve patient safety, prevent unexpected deaths, and reduce communication-related mishaps[2].

Communication strategy (SBAR) situational awareness has been demonstrated as an inner element in the method for providing patient concern and basic leadership that assists health care practitioners, particularly in critical care settings[3]. Communication
failure may trigger a delay in the rapid response team’s activation, which has related to a rise in in-hospital deaths. The number of SBAR components in the vital message has a direct relationship with in-hospital survival. As a result, nurses’ education on the use of the SBAR tool for transmitting critical information to physicians will increase situation sensitivity and likely improve patient outcomes [4].

Intensive care Nurses play an effective role in patient protection in their workplace. With the SBAR situational awareness approach, they should be able to recognize and interpret patient safety events using procedures, work as a team, learn from mistakes, and recognize behaviors and guidelines on how to avoid communication error incidents [5].

The technique of SBAR has helped nurses to have a focused and easy communication during transition of care during handover. Importance and relevance of capturing information need to be reinforce. An audit to look for reduced number of incidents related to communication failures is essential for long-term evaluation of patient outcomes. Use of standardized SBAR in nursing practice for bedside shift handover will improve communication between nurses and thus ensure enhance and improve patients health conditions [6].

Nurses often take more of a narrative and descriptive approach to explain a situation, while physicians usually want to hear only main aspects of a situation. The SBAR technique closes the gap between these two approaches allowing communicators to understand each other better. It is specially use for communication between a physician and a nurse when there is a change in patient condition or between a nurse and nurse during patients shift to a new department or during shift change. It is a technique used to deliver quality patient care [7].

**Methodology**

**Design and setting of the study:** A pre-experimental study design was carried out in the critical care units at Imam Al-Hussein Medical City of Karbala Holy City from the period of 17th of September 2020 to 25th of May 2021, in order to find out the effectiveness of instruction program on nurses’ knowledge regarding communication strategy (SBAR).

**Sample of the study:** A purposive sample was consists of (25) nurses was selected according to study criteria and after obtains verbal and written consent permission from them.

**The study instrument:** First part, concerned with the nurses’ demographic data. Second part, concerned with nurses’ knowledge regarding communication strategy (SBAR) includes (27) items.

**Statistical Analysis:** The IBM Statistical Package of Social Sciences (SPSS) Version 22 was used to analyze the results. Both descriptive statistical analysis and inferential statistical analysis approaches were used in order to investigate or predicts the relationships between variables.

**Results and Discussion**

1. Discussion demographic information (table 1):-

The characteristics of the participants in the present study was high percentage of them 52% at age group 18-28 years old, (Achrekar et al. 2016) including in their study 80% nurses at age 21-30 years old, 60% of the present study was females [8]. (Nagammal et al. 2016) their sample was high percentage of females which of 87.3% [9]. Regarding the years of experience in nursing, the characteristics of the present study indicated that the majority of nurses have experience from (1-5 years) with percentage 52% and according to years of experience in critical care units, the majority of nurses have experience from (1-5 years) with percentage 76%. (Sankpal et al. 2020) their study sample who included in their study have years of experience in nursing within (1-5 years) which of 83.9% of nurses [10].
nursing institute, (Coolen et al. 2020) reported in their study that the diploma degree have high percentage in their study High percentage of the study sample not included in the nursing documentation training courses which of 72% [11]. (Müller et al. 2018) stated in their study that the most of the participants not having courses of nursing documentation [12].

2. Discussion overall nurses knowledge and difference regarding communication strategy (SBAR) at the pre-test and post-test period of the program( table 2,3):-

The effectiveness of program was clear improved the practical knowledge of nurses regarding communication strategy which of their level was moderate at pretest then improved to high level and the result revealed that were high statistically significant differences between the pre-test and the post-test at (p<0.05). (Dalky et al. 2020) conducted a quasi-experimental approach with a pretest–posttest was adopted. 71 ICU nurses took part in the study as a convenience sample. SBAR effectiveness was examine using the 43-item ICU physician-nurse questionnaire, which included subscales for general relationship and communication, teamwork and leadership, and job satisfaction. The finding indicate that there was a significant improvement in posttest knowledge scores [13]. (Jiang et al. 2020) stated their study that conduct in hospital in Guangzhou research subjects were 100 clinical nurses. To explore and assess the clinical nurses’ understanding of SBAR’s blood glucose management, data were obtain via a questionnaire survey. The finding shows that SBAR strategy in addition to improving the communication between medical staff; it could also optimize the knowledge structure of nurses and make quick and accurate judgment of problems [14]. (Hadi et al. 2021) conduct a cross-sectional study was adopted in the investigation. Purposive sampling was used to choose the 102 nurses who took part in this study. A questionnaire was employed in the study to assess the SBAR’s perceptions, knowledge, attitudes, motivation, and application. The results showed that there was a significant relationship between perception, knowledge, attitude, motivation, and the application of the SBAR method [15].

3. Discussion Association between the effectiveness of the interventional program and Demographic information of study ( table 3):-

The result of present study indicates that there were highly significant association between the effectiveness of interventional program on nurses’ knowledge and years of nursing experience, years of experience in critical care units, levels of education and nursing documentation training courses. (Jeong & Eun, 2020) conduct a single-blind randomized control pretest–posttest approach use 54 nursing students in their third semester at a Korean university were chosen (SBAR group 26, handoff group 28). The finding stated there was no statistically significant difference between the SBAR group and handoff group knowledge with age groups, gender of the participants. However, there is significant association between nurses’ knowledge and levels of education and years of experience in nursing [16]. In the end that led us to accept the alternative hypothesis H₁: The program of communication strategy (SBAR) is improve the nurses’ knowledge, and reject the null hypothesis H₀: The program of communication strategy (SBAR) is not effect on nurses’ knowledge.
Table (1): Distribution of the participants according to their demographic information (n=25).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Categories</th>
<th>F.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Groups / Years</td>
<td>18-28</td>
<td>13</td>
<td>52.0</td>
</tr>
<tr>
<td></td>
<td>29-38</td>
<td>9</td>
<td>36.0</td>
</tr>
<tr>
<td></td>
<td>39-48</td>
<td>3</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td><strong>x ± s. D.</strong></td>
<td></td>
<td><strong>29.6±6.2</strong></td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>15</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>10</td>
<td>40.0</td>
</tr>
<tr>
<td>Years of nursing experience</td>
<td>1-5 years</td>
<td>13</td>
<td>52.0</td>
</tr>
<tr>
<td></td>
<td>6-10 years</td>
<td>4</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>11-15 years</td>
<td>3</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>16-20 years</td>
<td>5</td>
<td>20.0</td>
</tr>
<tr>
<td>Years of experience in critical care units</td>
<td>1-5 years</td>
<td>19</td>
<td>76.0</td>
</tr>
<tr>
<td></td>
<td>6-10 years</td>
<td>6</td>
<td>24.0</td>
</tr>
<tr>
<td>Levels of education</td>
<td>High school nursing</td>
<td>6</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>Nursing Institute</td>
<td>11</td>
<td>44.0</td>
</tr>
<tr>
<td></td>
<td>College of Nursing</td>
<td>7</td>
<td>28.0</td>
</tr>
<tr>
<td></td>
<td>Postgraduate certificates</td>
<td>1</td>
<td>4.0</td>
</tr>
<tr>
<td>Work shift</td>
<td>Morning</td>
<td>19</td>
<td>76.0</td>
</tr>
<tr>
<td></td>
<td>Evening</td>
<td>6</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>25</td>
<td><strong>100.0</strong></td>
</tr>
<tr>
<td>Nursing documentation training courses</td>
<td>Yes</td>
<td>7</td>
<td>28.0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>18</td>
<td>72.0</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>25</td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

F = Frequency; % = Percentage.
Table (2): Comparison among overall nurses knowledge regarding communication strategy (SBAR) at the pre-test and post-test period of the program.

<table>
<thead>
<tr>
<th>Program periods</th>
<th>Rating</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-test</td>
<td>Low</td>
<td>4</td>
<td>16.0</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>14</td>
<td>56.0</td>
<td>72.0</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>7</td>
<td>28.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>25</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Post–test I</td>
<td>High</td>
<td>25</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Low =Low level of knowledge (R.S. <66.6); Moderate = Moderate level of knowledge (R.S.=66.6-83.3); High = High level of knowledge (R.S.=≥83.4)

Table (3): Paired sample t-test of the difference in nurses’ knowledge regarding communication strategy (SBAR) at the pre-test and post-test period of the program.

<table>
<thead>
<tr>
<th>Tests</th>
<th>M.S</th>
<th>S.D.</th>
<th>T-test</th>
<th>df.</th>
<th>p-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-test</td>
<td>1.5</td>
<td>0.24</td>
<td>-9.68</td>
<td>24</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td>Post-test</td>
<td>1.8</td>
<td>0.08</td>
<td>-9.68</td>
<td>24</td>
<td>0.000</td>
<td>HS</td>
</tr>
</tbody>
</table>

M.S = Mean of score; S.D. = Standard Deviation; df. = Degree of Freedom; P-value = Probability value; HS =Highly Significant (p-value ≤ 0.05).

Table (4): Association between the effectiveness of the interventional program on nurses’ knowledge, age groups, gender, years of nursing experience, years of experience in critical care units, levels of education, work shift and nursing documentation training courses.

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Comparative patterns</th>
<th>df.</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Groups</td>
<td>Between Groups</td>
<td>2</td>
<td>14.13</td>
<td>0.06 (NS)</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Between Groups</td>
<td>1</td>
<td>2.600</td>
<td>0.79 (NS)</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years of nursing experience</td>
<td>Between Groups</td>
<td>3</td>
<td>6.402</td>
<td>0.03 (HS)</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Conclusion and Recommendations**

The study concluded that the instruction-nursing program had a positive impact on nurses’ knowledge concerning communication strategy (SBAR). The researcher is recommended; testing the current program on a wide group of health-care providers in hospitals. Use the SBAR formula in all hospital units. All nurses should attend an SBAR training session. Incorporating the SBAR into nursing education at all levels and expanded research to include more hospitals.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


Investigation of the Antibiotic-Resistant ESKAPE Pathogens in Ramadi Hospitals, Iraq

Hamid M. Sharqi¹, Omar M. Hassan¹, A.S. Obaid²
¹MsC Student. ²Ass. Proff. Department of Biology, ³Ass. Proff. Department of Physics, College of Science, University of Anbar, Ramadi 31001, Iraq

Abstract

Bacterial species of the ESKAPE group (Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Escherichia coli) are often resistant to antibiotics. The purpose of this study was to find out the antibiotics resistance formed by ESKAPE pathogens in hospitals in the city of Ramadi, in Iraq. All bacteria within this clinically relevant ESKAPE group were isolated from September 2020 to April 2021. Identification of isolates was performed by Vitek 2 system and the Sensitivity Test was carried out using a disk diffusion method. The majority of pathogens isolated from patients at Ramadi Hospitals belong to the ESKAPE group. The percentage of bacterial isolates was K. pneumoniae (33.96%), E. coli (20.75%), S. aureus (20.13%), P. aeruginosa (11.32 %), E. faecalis (9.43%), and A. baumannii (4.41%). Some isolates of E. coli were Resistance to all antibiotics, while P. aeruginosa and K. pneumoniae were 100% resistant to Vancomycin and Tetracycline. The presence of the ESKAPE group of pathogens is a major problem in Ramadi city hospitals. The results of this study support the implementation of special antimicrobial strategies to specifically target these microorganisms.

Keywords: ESKAPE, MDR, antibiotic, resistance, susceptibility

Introduction

The term ESKAPE includes six genera of pathogens with multi-drug resistance (MDR) and high virulence. ESKAPE pathogens are responsible for the majority of nosocomial infections and are able to “escape” from the biocidal effect of antimicrobial agents [¹]. The World Health Organization (WHO) has also recently included ESKAPE pathogens in the list of 12 bacteria that urgently need new methods and antibiotics [²]. Antimicrobial resistance between both Gram-positive and Gram-negative bacteria has been on the rise in the past few years [³]. The presence of multidrug resistance (MDR) pathogens has become a cause of serious concern with regard to nosocomial infections. In fact, the World Health Organization recently recognized antimicrobial resistance as one of the top three human health concerns [⁴]. The most common and threatening multidrug-resistant pathogens were grouped together under the acronym “ESKAPE”, which stands for E. faecalis, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa, and E. coli. [¹]. Three decades ago, A. baumannii was sensitive to most antibiotics, but today it is exceptionally resistant to most antibiotics, with carbapenem resistance increasing more than 50% in some countries [⁵]. Recently, statistics have been recorded on meropenem resistance (MEM) of 69% [⁶]. A major factor contributing to antibiotic resistance is the production of extended-spectrum β-lactamases (ESBLs) by Enterobacteriaceae species, especially K. pneumoniae [⁷] ESBL producing Gram-negative pathogens in hospitals is an emerging global
problem that deserves special attention [8]. Another important species of the ESKAPE group is \textit{S. aureus}, especially methicillin-resistant \textit{S. aureus} (MRSA), which has an incidence and prevalence that continues to increase rapidly in many regions of the world. Mortality associated with invasive MRSA infections is estimated to be around 20\% [9] and bloodstream infections caused by these bacterial species are associated with high mortality and length of hospital stay [10]. Finally, \textit{E. faecalis} isolates are the third to the fourth most prevalent pathogen in hospitals worldwide. Acquired resistance, in particular to glycopeptides, has been reported for a number of these isolates, which limits the number of therapeutic options [11]. Global and regional surveillance of ESKAPE pathogens is essential to control infections caused by these bacterial species [12]. The purpose of this study was to monitor the occurrence of ESKAPE pathogens and their resistance to diseases in hospitals in Ramadi, Iraq.

**Materials and Methods**

**Sampling**

This study was conducted at Al-Ramadi General Teaching Hospital and Women’s and Children’s Hospital, Iraq. This hospital provides medical care to its patients and residents. This study was conducted in the medical field (from September 2020 to April 2021), 53 clinical isolates were obtained from patients referred to these hospitals. The first private isolate species was recorded for each patient, regardless of a body location. Patient samples taken for diagnostic purposes only are included.

**Identification of isolated bacteria**

Two hundred samples from various sources such as urine, faeces, burns, wounds, and sputum were collected from patients in the Ramadi hospitals. Blood agar and MacConkey agar were used to culture the samples. Culture plates were incubated aerobically at 37 °C for 24 h. After incubation, bacterial isolates were identified by performing Gram staining and using the Vitek-2 Compact system (bioMerieux). A total of 159 bacterial isolates were collected from different clinical samples.

**Antimicrobial susceptibility assays**

The antibiotics susceptibility was determined using the disc diffusion according to Kirby-Bauer method by using Mueller’s solid agar plates [13]. Antimicrobials were tested against both Gram-negative and Gram-positive bacteria including Piperacillin (PRL), Amikacin (AMK), Aztreonam (AZT), Ciprofloxacin (CIP), Imipenem (IPM), Vancomycin (VA), Azithromycin (AZM), Tetracycline (T), Ceftriaxone (CRO), and Tigecycline (TGC). Multidrug resistance MDR has been defined as resistance to three or more classes of antimicrobials. Class definitions used in this study were: Penicillins (PRL), Cephalosporins (CRO), Carbapenems (IPM), Beta-lactam (AZT), Fluoroquinolones (CIP), Aminoglycosides (AMK), Glycopeptide (VA), Tetracyclines (T), Macrolides (AZM), and Glycylcyclines (TGC). Extensive drug resistance XDR was defined as the lack of susceptibility to at least one agent in all but two or fewer classes of antibiotics (for example, bacterial isolates remain susceptible for one or two classes only) and Pandrug resistance PDR was defined as resistant for all agents in all classes of antibiotics [14]. The diameters of the inhibition zone were measured and compared with the susceptibility breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) [15].

**Results**

Two hundred different samples obtained from patients in Ramadi hospitals were processed. A total 159 (79.5\%) of ESKAPE pathogens were included in this study. The ESKAPE pathogens were distributed into 112 (70.4\%) Gram-negative isolates. The pathogens belonging to the family Enterobacteriaceae (\textit{K. pneumoniae} \& \textit{E. coli}) were 78 (54.7\%) and non-intestinal (\textit{A. baumannii} \& \textit{P. aeruginosa}) 25 (15.7\%), and the Gram-positive isolates (\textit{S. aureus} \& \textit{E. faecalis}) were 47 (29.6\%). \textit{K. pneumoniae} 54
(33.96%) was the most common isolate, followed by E. coli 22 (20.75%), S. aureus 32 (20.13%), P. aeruginosa 18 (11.32%), E. faecalis 15 (9.43%), and A. baumannii 7 (4.41%). The isolates were recovered mostly from feces (78 isolates), followed by urine samples 33 isolates, wounds 25 isolates, sputum 15 isolates, and finally burns 8 isolates. The distribution of ESKAPE pathogens isolated from different samples is shown in Table 1.

Table (1) The ESKAPE pathogens profile based on specimen type

<table>
<thead>
<tr>
<th>Bacteria spices</th>
<th>source of bacteria</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>urine</td>
<td>faeces</td>
<td>wounds</td>
</tr>
<tr>
<td>E. coli</td>
<td>15</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>S. aureus</td>
<td>18</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>-</td>
<td>36</td>
<td>9</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>-</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>-</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>78</td>
<td>25</td>
</tr>
</tbody>
</table>

**Antibiotic Resistance levels**

The results of the current study showed the existence of a common resistance of ESKAPE groups toward classes of antibiotics such as penicillins, cephalosporins, fluoroquinolones, and aminoglycosides, which makes it have multi-resistance to the antibiotics, as the term multi-resistance to this group of bacteria is called (Multidrug resistance), which means the ability of bacteria to resist three or more antibiotics used, provided that the bacteria have the ability to resist at least one antibiotic within the class. The isolates were distributed according to Table (2), the types of resistance, and their proportion to the isolates of the (ESKAPE) group. Among all 159 isolates, 146 were found to be MDR (100% from E. coli, 94.4% from K. pneumoniae, 88.9% from P. aeruginosa, 87.5 from S. aureus, 86.6% from E. faecalis, and 71.4 from A. baumannii). There were only 13 isolates that were found to be sensitive to most antibiotics. E. coli isolates of the ESKAPE group that were understudy has an Extensive drug resistance (XDR) of 21.2%, followed by K. pneumonea of 18.5%, as the danger of Extensive resistance strains lies in their endemicity within hospitals, their rapid spread, the high rate of resistance and their transformation into strains resistant to all drugs. The term (Pandrug resistance) is applied to the recorded resistance of all classes of antibiotics studied. The isolates of the (ESKAPE) group were recorded with resistance to the ten classes of antibiotics, represented by three isolates of E. coli (9.1%), also three isolates of K. pneumoniae (5.5%), and one isolate of P. aeruginosa (5.5%) Table (2).
### Table (2) Antibiotic resistance levels of ESKAPE isolates during the study period.

<table>
<thead>
<tr>
<th>Bacteria species</th>
<th>Resistance type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDR %</td>
</tr>
<tr>
<td>E. coli</td>
<td>100.0</td>
</tr>
<tr>
<td>S. aureus</td>
<td>87.5</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>94.4</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>71.4</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>88.9</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>86.6</td>
</tr>
</tbody>
</table>

**Antibiotic Susceptibility Pattern**

Antibiotic sensitivity testing showed that the rate of resistance of *E. faecalis* to Amikacin and Tetracycline was 100%. It was noted that the lowest resistance was in the case of ciprofloxacin (6.67%), while *S. aureus* showed the highest resistance rate of Aztreonam which was (75%). The results also showed that Imipenem was the most successful antibiotic in the treatment of Gram-positive ESKAPE pathogens (Table 3).

### Table (3). Antibiotic susceptibility pattern of Gram-positive ESKAPE isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>S. aureus (n=32)</th>
<th>E. faecalis (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S%</td>
<td>I%</td>
</tr>
<tr>
<td>Amikacin</td>
<td>21.88</td>
<td>25.00</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0.00</td>
<td>37.50</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>0.00</td>
<td>25.00</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>56.25</td>
<td>0.00</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>62.50</td>
<td>6.25</td>
</tr>
<tr>
<td>Imipenem</td>
<td>81.25</td>
<td>18.75</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>68.75</td>
<td>31.25</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>6.25</td>
<td>21.88</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>43.75</td>
<td>25.00</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>15.63</td>
<td>43.75</td>
</tr>
</tbody>
</table>
The frequency rate of resistance of *E. coli* to vancomycin, piperacillin, and tetracycline was 100%. As well, the resistance frequency rate of *K. pneumoniae* against vancomycin and piperacillin was 100%. Imipenem was again effective against (81.82%) of the tested *E. coli* isolates, while Ceftriaxone was effective against (81.48%) of *K. pneumoniae* (Table 4). None of the isolates were 100% sensitive to all antimicrobials tested. Furthermore, some *E. coli* and *K. pneumoniae* isolates were found to be highly drug-resistant as they showed resistance to all antimicrobial agents evaluated.

### Table (4). Antibiotic susceptibility pattern of Enterobacteriaceae ESKAPE isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th><em>E. coli</em> (n=33)</th>
<th></th>
<th><em>K. pneumoniae</em> (n=54)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S%</td>
<td>I%</td>
<td>R%</td>
<td>S%</td>
</tr>
<tr>
<td>Amikacin</td>
<td>69.70</td>
<td>0.00</td>
<td>30.30</td>
<td>9.26</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>9.09</td>
<td>9.09</td>
<td>81.82</td>
<td>0.00</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>0.00</td>
<td>15.15</td>
<td>84.85</td>
<td>16.67</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>12.12</td>
<td>0.00</td>
<td>87.88</td>
<td>81.48</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>45.45</td>
<td>0.00</td>
<td>54.55</td>
<td>25.93</td>
</tr>
<tr>
<td>Imipenem</td>
<td>81.82</td>
<td>6.06</td>
<td>12.12</td>
<td>74.07</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>63.64</td>
<td>27.27</td>
<td>9.09</td>
<td>3.70</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

The susceptibility pattern of non-intestinal Gram-negative ESKAPE isolates displayed high resistance of *P. aeruginosa* to Vancomycin (100%), Tigecycline (100%), Tetracycline (83.33%), and Azithromycin (72.22%). Also, high resistance to Vancomycin (100), Tetracycline (100%), and Aztreonam (71.43%) was observed in *A. baumannii*. Otherwise, Imipenem and Ceftriaxone have the best antibacterial effect against *A. baumannii* and *P. aeruginosa* (Table 5).

### Table (5). Antibiotic susceptibility pattern of non-intestinal Gram-negative ESKAPE isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th><em>A. baumannii</em> (n=7)</th>
<th></th>
<th><em>P. aeruginosa</em> (n=18)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S%</td>
<td>I%</td>
<td>R%</td>
<td>S%</td>
</tr>
<tr>
<td>Amikacin</td>
<td>14.29</td>
<td>28.57</td>
<td>57.14</td>
<td>64.44</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>42.86</td>
<td>14.29</td>
<td>42.86</td>
<td>16.67</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>28.57</td>
<td>0.00</td>
<td>71.43</td>
<td>83.33</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>71.43</td>
<td>0.00</td>
<td>28.57</td>
<td>88.89</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>57.14</td>
<td>42.86</td>
<td>0.00</td>
<td>77.78</td>
</tr>
<tr>
<td>Imipenem</td>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
<td>94.44</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>28.57</td>
<td>28.57</td>
<td>42.86</td>
<td>77.78</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
<td>11.11</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>71.43</td>
<td>28.57</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Discussions

The emergence of isolates resistant to a wide range of antibiotics poses a threat to health and this is a result of the wide and indiscriminate use of these wide groups of antibiotics and the lack of care for the emergence of such resistant isolates, the increase of which poses a danger to patients, and this situation remains uncontrolled at the hospital and community levels, and this is the reason for the emergence of multidrug-resistant strains, especially the broad-spectrum Multidrug-resistant strains [16-17]. In a hospital environment, there may be different bacterial species that are the causative agents of infectious diseases. For them, with a high level of proliferation and association with antibiotic-resistant microorganisms, the ESKAPE group of pathogens deserves special attention. To control the incidence of infection caused by ESKAPE pathogens, site-by-site observational studies are necessary to establish hospital-specific guidelines for effective empirical therapy [18]. In this study, ESKAPE pathogens were followed up and the incidence of K. pneumoniae and the presence of resistance to Vancomycin in Ramadi hospitals were described. The organisms most often recovered from our bodies were intestinal infections from ESKAPE pathogens (54.61%), with a predominance of Gram-negative bacteria. The most common organisms in ESKAPE are K. pneumoniae, E. coli, and S. aureus. These common bacterial pathogens were found to be similar to other predominant pathogens reported in other countries [19]. In general, an elevated MDR was observed in E. coli deserves special attention because it showed a high MDR (100%). The reason is that these bacteria in the (ESKAPE) group possess many resistance mechanisms, such as the production of aminoglycoside-modifying enzymes and beta-lactamase enzymes, and their exogenous pumping mechanism and other mechanisms that work together causing the phenomenon of multiple resistance [20]. The noticeable rise in the percentage of isolates of the (ESKAPE) group with multiple resistance in Al-Ramadi Teaching Hospitals is an indication of the outbreak of multi-resistant strains of antibiotics, which leads to the possibility of failure of the treatments currently used to treat infections caused by these bacteria.

Conclusion

Rapid identification and susceptibility testing of ESKAPE pathogens with the compact VITEK-2 system helps treat these pathogens with appropriate antibiotics and leads to a reduction in total antibiotic consumption. It was found that the Enterobacteriaceae ESKAPE group (K. pneumoniae & E. coli) are the highest prevalence among other bacterial species, and all ESKAPE pathogens have shown multi-drug resistance (MDR). Also, most ESKAPE species have Extensive resistance to antibiotics, in addition to the occurrence of resistance to all classes of antibiotics in some isolates of E. coli. In this study. Most ESKAPE isolates were found to be sensitive against Imipenem and Ceftriaxone. These antibiotics can be good treatment options for infections due to the etiology of ESKAPE.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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[18] De Oliveira DM, Forde BM, Kidd TJ, Harris PN, Schembri MA, Beatson SA, Paterson DL,


Outcomes of Laparoscopic Surgery of Liver Hydatid CYST

Nasser M. Meazher¹, Fadhil Abbas Al-Janabi², Bashar Mohammed Hamzah³, Haider Nadum Obaid³
¹FICMS, Dr., Dept. of Surgery, College of Medicine / Kufa University, Iraq, Nasserm, ²CABS, FACS, Dr., ³FICMS, Dr., Dept. of Surgery, AL Sadder Medical City, Al-Najaf Health Care Center /Iraq, ⁴CABS, FICMS, Dr., Dept. of Surgery, Al-Khadymia Teaching Hospital,Baghdad/ Iraq

Abstract

Objective: To evaluate the outcomes of laparoscopic management of liver hydatid disease regarding intra-operative and post-operative advantage and disadvantage.

Patients & Methodology: The study conducted from October 2014 to April 2018 at Alsader teaching hospital by management of liver hydatid cysts laparoscopically. All patients with four or less liver hydatid who underwent laparoscopic surgery for liver hydatid were included. Evaluation of intra-operative and post-operative outcomes regarding: hospital stay, pain, complications, cost effectiveness and recurrence of hydatid cyst were been studied.

Results: Forty eight patients had laparoscopic operation for liver hydatid cysts. The mean age was 37.89 ± 17.03 years (ranging from 8 to 85 years). There were 21 male (43.75%) and 27 female (56.25%). The most common cause of presentation was pain, which occurring in 38 (79.16%) of patients, and the second cause was abdominal mass, found in 9 (18.75%) of patients, and there is no conversion to open surgery. The complications were occurred in 17 (35.41%) of patients, such as ports site infection in 3 (6.25%) of patients, and bile leak in 5 (10.41%) of cases. Other complication include, collection in residual cavity in 3 (6.25%) and fever in 5 (10.41%). Neither recurrence nor mortality happened in our study. The mean operation time was 56.27 ± 13.84 minutes.

Conclusion: The laparoscopic approach for liver hydatid cysts is safe and effective in selected patients.

Key words: hydatid cyst, laparoscopic surgery.

Introduction

Echinococcal disease is caused by tapeworm infestation in its larval stage. In humans, four Echinococcus species cause infestation. The most common are E. granulosus (cystic echinococcosis) and E. multilocularis, (alveolar echinococcosis). The other two species, E. vogeli and E. oligarthrus, are less common [1].

The echinococcus life cycle includes a definitive (dogs or its related species) and an intermediate host (sheep, cattle, camels, goats, etc). The typical hydatid cyst has a three-layer wall cavity containing fluid. The outer layer is the pericyst, a thin fibrous tissue layer representing an adventitial reaction to the parasitic infestation. Over time, the pericyst may calcified.

The outer layer of the cyst itself is the ectocyst (laminated membrane) and is bluish-white, gelatinous. The inner layer or endocyst is the germinal membrane, responsible for the production of ectocyst, clear fluid, scoleces, brood capsules and daughter cysts (endogenic vesiculation). [3] The diagnosis of liver hydatid is based on laboratory test represented ELISA test (enzyme-linked immune sorbent assay) for antigens and are positive in approximately 85% of cases. If the
parasite is no longer viable or does not contain scolices, or the cyst has not leaked, the test results may be negative. In approximately 30% of cases, eosinophilia is seen. CT scanning and Ultrasonography are both sensitive for detecting liver hydatid. The cysts appearance on images depends on the stage of development. Typically, hydatid cysts are well-defined hypo-dense lesions with a distinct wall. Ring-like calcifications of the pericysts are present in 20% to 30% of cases. Daughter cysts generally occur in a peripheral location within the main cyst and are typically slightly hypo-dense compared with the mother cyst [4].

The specificity of ultrasound in hydatid diseases around 90%. The classification proposed by Gharbi and associates provides a morphologic description [5]. Abdominal MRI of the may be useful for evaluation of the cyst characteristics [6].

Clinically, the hydatid cyst is mostly asymptomatic until complications developed. The most common symptoms are dyspepsia, abdominal pain and vomiting. Hepatomegaly is most frequent sign. Fever and Jaundice are present in approximately 8% of patients [7].

Treatment is indicated to prevent progressive cysts enlargement and rupture. A course of albendazole or mebendazole may be tried perioperatively. Many articles mention that percutaneous treatment (PAIR) of hydatid cysts is effective and safe [8].

The surgical options range from deroofing with evacuation of the contents to liver resection or local excision of the cysts.

Intrabiliary rupture of liver hydatids may result in acute cholangitis or obstructive jaundice. This may be managed endoscopically by ERCP prior to cyst removal from the liver [8].

**Patients and Methods**

This study designed as case series conducted in the General Surgical department in Alsader Medical City at Al-Najaf, Iraq, from October 2014 to April 2018, with acceptance and permission of scientific council of board center and patients consents for surgery, video recording and taking pictures.

**Exclusion criteria for laparoscopic intervention include:**

1. The site of hydatid cyst within the liver parenchyma or segment VII and VIII.
2. If the number of cyst is more than 4.
3. The diameter of cyst less than 4 cm
4. Those were treated in the emergency due to ruptured hydatid cyst.
5. Dead cyst (calcified)
6. If laparoscopic surgery was refused.

The diagnosis of the liver hydatid cysts based on:-

a- History
b- Clinical examination.
c- Imaging (abdominal ultrasound and Computerized tomography scan (CT scan).

We start oral albendazole(15 mg/kg/day or 400 mg orally twice daily to all patents for two weeks before surgery and continue after surgery for 28 days followed by a 14-day drug-free period. Repeat for 2 more cycles.

Intra-operative and post-operative outcome regarding, hospital stay, pain, complications, cost effectiveness and recurrence of hydatid cysts were evaluated.

The type of anesthesia was general anesthesia for all patients. At the time of induction, intra venous prophylactic antibiotics was given. Prophylactic injection Hydrocortisone before surgery also given. Inflation of abdomen by CO2, using veress needle to make pneumoperitoneum, then insert a zero or thirty degree telescope (10mm) through infra-umbilical port, this is the 1st port, after that Hydatid cyst was identified on the liver surface. The 2nd port (10mm) was inserted.
either at 1 cm below xiphoid process (if the cysts on the right lobe of liver) or at left mid-clavicular line, about 2 cm below costal margin (if the cysts at left lobe of liver). The last port (5 mm) inserted about 2 cm below costal margin at right mid clavicular line placed, for making access and mobilization of hydatid cysts.

At first, we do packing of the peritoneal cavity with 10% povidone iodine-soaked packs to control spillage if happened.

Then we puncture hydatid cyst wall and aspirate the fluid from the cysts, followed by instillation of 10% povidone iodine solution inside the cavity (scolicidal agent), lastly we do re-aspiration, sometime followed by deroofing of the hydatid cysts wall. The cyst contents were evacuated by using 10 mm sucker and irrigated with povidone iodine then 0.9 Normal saline. The endocyst extracted to outside by endo bag. Any remaining contents in the cavity should be examined (daughter cysts and bile leak). A tube drain placed inside the residual cavity for drainage later on.

In most cases, the drain was removed after 4-5 days, but in case of bile leak, the drain was kept longer. Usually the patients were discharged home two days after surgery post-operatively, after three months the cyst cavity was monitored by ultrasound. CT scan was performed if the ultrasound report was inconclusive or suspicious.

Results

Forty eight patients with liver hydatid were treated by laparoscopic surgery. The age range 8 to 85 years. The males were 21 (43.75%) while 27 (56.25%) of them were females.

The most common cause of presentation was pain, occur in 38 (79.16%) of cases, while 9 (18.75%) of patients complained from a mass in addition to pain. One of series has jaundice (2.08%) treated by ERCP preoperatively. It was 4 (8.33%) of cases incidental diagnoses, by abdominal ultrasound examination performed for another causes (Table I).

In 37 (77.08%) of cases were present with single liver hydatid cyst, two cysts in 7 (14.58%) of cases, three and four cysts in 2 (4.16%) of cases. (Table II). The most common site of liver hydatid cysts was the right lobe, 40 (83, 33%). Left lobe of liver were 5 (10.41%), lastly in bilateral liver lobes were reported in 3 (6.25%) of cases.

Mean time of surgical duration 56.27 minutes, if the patient has single or small hydatid cyst the surgery will take shorter time. Anaphylactic cyst the surgery will occur in one of patient during surgery.

However, Complications were occurred in this study to 17 patients (35.41%), listed in Table III.

The Port site infection happened in 3 (6.25%) of patients and bile leak in 5 (10.41%) they stopped by conservative treatment in 4 to 7 days, except in one case, the leak not stopped until underwent ERCP. Other complications were reported in 3 (6.25%) with residual cavity collection, and fever in 5 (10.41%).

All patients’ procedure was completed laparoscopically, with no conversion to open surgery. Recurrence of hydatid cysts were not reported in this series after follow-up for 6 months to one year. No mortality in this study. In table IV, there were 28 (58.33%) of patients need only 2 days hospital stay, 8 (16.66%) of patients 3 days stay, others (4 – 5) days. Regarding to return to normal activity, after 5 days in 33 (68.75%) of patients, 7 days in 12 (25%) and 12 days in 3 (6.25%) of patients.
Table 1: Clinical presentation of liver hydatid cyst.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>38</td>
<td>79.16%</td>
</tr>
<tr>
<td>Incidentally diagnosed</td>
<td>4</td>
<td>8.33%</td>
</tr>
<tr>
<td>Abdominal mass</td>
<td>9</td>
<td>18.75%</td>
</tr>
<tr>
<td>Obstructive jaundice</td>
<td>1</td>
<td>2.08%</td>
</tr>
</tbody>
</table>

Table 2: No. and sits of cysts

<table>
<thead>
<tr>
<th>No. of cysts</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solitary cyst</td>
<td>37</td>
<td>77.08%</td>
</tr>
<tr>
<td>Multiple cysts (2,3,4)</td>
<td>11</td>
<td>22.19%</td>
</tr>
<tr>
<td>Right Lobe</td>
<td>40</td>
<td>83.33%</td>
</tr>
<tr>
<td>Left Lobe</td>
<td>5</td>
<td>10.41%</td>
</tr>
<tr>
<td>Bilateral Lobes</td>
<td>3</td>
<td>6.25%</td>
</tr>
</tbody>
</table>

Table 3: Surgical Complications

<table>
<thead>
<tr>
<th>Complications</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaphylactic shock</td>
<td>1</td>
<td>2.08%</td>
</tr>
<tr>
<td>port site infection</td>
<td>3</td>
<td>6.25%</td>
</tr>
<tr>
<td>Biliary leak</td>
<td>5</td>
<td>10.41%</td>
</tr>
<tr>
<td>Collection inside residual cavity</td>
<td>3</td>
<td>6.25%</td>
</tr>
<tr>
<td>Fever</td>
<td>5</td>
<td>10.41%</td>
</tr>
</tbody>
</table>

Table 4: Hospital stay

<table>
<thead>
<tr>
<th>Hospital stay</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3 days</td>
<td>36</td>
<td>75%</td>
</tr>
<tr>
<td>4-5</td>
<td>12</td>
<td>25%</td>
</tr>
</tbody>
</table>
Discussion

Our study is for evaluation of results of minimal invasive technique in the treatment of liver hydatid cysts in a selected patients group.

The mean age was 37.89 ± 17.03 years (ranging from 8 to 85 years), which is near other series age of presentation [9, 10].

In fact, all age are affected equally, in other areas, usually with the average age being older at presentation [7]. Female’s gender was affected predominantly in our study look like in some others [7, 10]. However other studies have predominance in male’s gender or equally reported in both gender [9, 11].

The most common cause of presentation was abdominal pain, occurred in 38 (79.16%) of patients, this also previously reported by another authors [7, 9, 10]. Liver hydatid cysts should be in differential diagnosis to all patients with abdominal pain, fever, abdominal mass and jaundice, however, a lot of cases are asymptomatic, mostly in non-endemic area, and they diagnosed incidentally [9, 12].

In our study, the pathology was single liver hydatid cyst most commonly, and in the right lobe of liver. Which was similar reported in other studies, from India in Uruguayan community [9, 11]. Abdominal ultrasonography and CT scan are very effective for identification and diagnosis of liver hydatid cysts. Ultrasound is clearly identify the membranes of hydatid cysts, its septa in addition to hydatid sand, while calcified cyst wall and infected cysts are best identified by CT scan [8]. Specific criteria on US or CT scan may indicate biliary communication and this confirmed on MRI. US and CT scan both were done to all patients in this study. Surgery is the corner stone for treatment of liver hydatid cysts. Laparoscopic treatment of hydatid cysts disease was reported in 1994 for the first time [9]. However, the reports regarding long-term results are limited in this technique [13]. Many different laparoscopic techniques are present, total pericystectomy, marsupialization following puncture and aspiration, deroofing then drainage, deroofing then omentopexy [14, 15, 16].

In our study, 48 patients with liver hydatid cysts were managed with laparoscopic technique, we applied the principles of usual surgery including scolices deactivation with 10% povidone iodine solution (scolicidal agent), aspiration of contents inside hydatid cysts, deroofing the cavity and entire cysts contents were evacuated. Similar studies in Turkey from University Hospital, they reported cysts drainage simply with a specific trocar and cannula in 18 patients and deroofing with drainage in other 22 patients and have good results and low recurrence [16]. Other study, in Pakistan from Khyber Teaching Hospital in 2010, also report low recurrence rate and complications such as, recurrence in 4.65% of patients, bile leak in 9.3% and conversion 6.97% of patients [17], Ertem et al. has successful result in laparoscopic cystectomy and partial cystectomy with drainage in 33 patients along with omentopexy in 15 cases and have 2 cases converted to open surgery [13]. In another study from India, evacuation and marsupialization, trans-cystic fenestration and lobectomy were performed by lap using a Palanivelu Hydatid System (PHS), especially trocar design for control contamination and spillage from hydatid cysts [9].

The disadvantage of the laparoscopic surgery is difficulty to control spillage; different tools have been designed to evacuate the hydatid cysts contents without spillage. Bicket et al. used a beveled transparent cannula, Al-Sharif et al. used cannula of liposuction, however, Bickel et al. described the isolated hypobaric technique, he has attempted to deal with spillage problem [18, 19]. PHS not only for preventing spillage, but also for assisting complete evacuation of the hydatid cysts in addition to allows magnified intra cystic visualization of biliary communication to it [9]. Unfortunately these specific devices not available in the our hospital. In our study, no conversion to open surgery happened in comparison to others study have conversion rates of 3% and 23% mainly due to difficult access, anatomy and exposure [13-16]. Also we have no mortality.
Complications included, port site infection, fever, collection in residual cavity, bile leak, all treated by conservative management, except one treated by ERCP for bile leakage. In other study, they report infection in 5 (4%), bile leak in 10 (14.5%) cases and peritonitis, also their complication managed conservatively [9].

Open surgery studies reported recurrence rate much higher (ranges from 3% to 10%), following liver hydatid cysts surgery [13].

So, with laparoscopic surgery, the morbidity and recurrence rate decrease versus in open surgery.

**Conclusion**

Laparoscopic surgery of liver hydatid cyst was effective and safe treatment in selected patients, with decreased risk of spillage in the abdominal cavity, lower rate of complications and recurrence. It also given to patients the advantages of minimal invasive surgery.

**Recommendation**

- New study involves larger number of cases.
- Using new technologies to control of spillage such as Palanivelu Hydatid system.
- Long term follow up.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** “All experimental protocols were approved under the college of medicine and carried out in accordance with approved guidelines”.

**References**


Study the Behavior of Activity and Express for Enzymes

Antesar Rheem Obead1, Anmar Hameed Bloh2, Haider Rasol Abbass Alwahami3

1Lecture, Babylon University, College of Education, Science Dept., Hilla, Iraq, 2Lecture Department of Radiology and Sonography Technique, AL-Rafidian University College, Pediatric Teaching Hospital in samawah

Abstract

Enzymes are be defined as soluble, colloidal, organic catalysts which are produced by living cells. Enzymes are biologic catalysts to accelerate the rate of biochemical reactions.

in this study Quantification of the Enzyme Activity, Where: E = enzyme concentration; S = substrate concentration; ES = enzyme-substrate complex concentration; k1, k3= 1st order reaction rate constants (l-1); P = product concentration; k2= 2nd reaction rate constant (M-1.t-1).At first, it must be stressed that the formation of ES (enzyme-substrate complex) is an obligatory step to any type of enzyme-catalyzed reaction. The ES, depending on the reaction conditions, can form the product (forward reaction) or not (reward reaction). The immobilization technique allowed a more frequent use of biomaterials in industry, therapeutics (confection of subcutaneous capsules for controlled delivering of hormones), and lab analysis (automatic dosing equipments, enzyme electrode, biosensors and enzyme immunoasays). In industry, the use of glucose isomerase for the conversion of glucose into fructose (high fructose corn syrup), the aminoacylase for the separation of racemic mixture of amino acids, microbial lipase in triglyceride hydrolysis, and the lactase for removing lactose from milk and/or whey are stressed.

Key word : enzyme , activity , immobilization, Specificity, factors, thyromodynamic

Introduction

Enzymes are be defined as soluble, colloidal, organic catalysts which are produced by living cells. Enzymes are biologic catalysts to accelerate the rate of biochemical reactions. All enzymes are protein in nature; they are water soluble, precipitated by usual reagents like alcohols, ammonium sulfate and alkaloid reagents.

Most enzymes are present in cells at much higher concentrations than in plasma.

Normal plasma levels reflect the balance between the synthesis and release of enzymes during ordinary cell turnover and their clearance from the circulation, therefore, enzymes can be used as markers to detect and localize cell damage proliferation. The enzyme (holo-enzyme) composed of inactive protein (apo-enzyme) with cofactor which may be organic (coenzyme) or inorganic (elements like Mn, Mg, Fe). If the cofactor linked forced to the protein portion of enzyme then it called prosthetic group (1).

Enzyme unit: the amount of enzyme required for transformation one micromole of substrate (reactant molecules) in one minute. Turnover number: the number of substrate molecules transformed per one minute by a single enzyme molecule

Specificity

Enzymes catalyze a reaction accepting only one substance as substrate. When it accepts two or more substrates, one ofthem is always preferred .The enzyme specificity is determined by the active site, a particular domain of the peptide molecule. There are two main theories seeking to explain the enzyme-substrate interaction. One –proposed by Fischer in the beginning of the 20th century –states that the
substrate-enzyme interaction would resemble a key-
lock mechanism, in which the key (substrate) fits into
the lock (active site), since two conditions are satisfied:
the substrate and the active site have complementary
structures and compatible polarity and size(2). The
other –proposed by Koshland in the 1960–states that
the substrate nearest the enzyme molecule induces on it
some structural modifications that favor the enzyme-
substrate fitting

Nowadays, it is accepted that the active site can
be subdivided into two particular regions, one called
bond site (responsible for enzyme specificity) and
the other called catalytic site (in which a sequence
of well-defined 1st and 2nd order reactions occur
transforming the substrate into the product or vice-
versa) (3).

Enzyme Activity

When enzymes are used in industrial processes
and analytical procedures, the precise evaluation of
the enzyme activity becomes important. Focusing
on the industrial scale, the decision to use or not an
enzyme in a process must be based on issues such as1)
the amount of enzyme required for carrying out the
process properly, 2) the duration of the reaction, 3) the
amount of substrate to be converted, 4) the conditions
under which the reaction occurs, and 5) the overall
cost of the process. Once the decision for using an
enzyme is taken, and considering that the enzyme
has a cost, the impact of this cost on the overall
process must be evaluated (4). Thereby, the increase
in product yield and/or the value added to the final
product, as well as the reduction of the overall energy
consumption, could minimize the effect of enzyme
cost on the overall process cost. Thereby, it is clear
that the success of the enzyme process depends on the
optimization of three factors, i.e., amount of enzyme
needed, operation conditions (pH, temperature,
agitation etc.), and reaction yield. Theoretically, as
the enzymes are catalysts not consumed during the
reaction, a small amount could transform into huge
amount of substrate. However, an optimized and finite
correlation among the amount of catalyst, its initial
activity and the amount of substrate to be converted
to product must be found. Moreover, the duration
of industrial processes can take one hour (such as
the mashing of malted barley in brewing), three
hours (such as the saccharification of the liquefied
starch with glucoamylase in syrup production), or
about twenty hours (such as the hydrolysis of lactose
by lactase in milk or whey (5). Of course, the enzyme
technology aims to perform quick reactions at a low
cost, albeit respecting the restrictions imposed by the
operational conditions and the scale of the process.

Quantification of the Enzyme Activity

Let us consider the simplest model for an enzyme-
catalyzed reaction: (6)

\[ E+S\overset{E}{\rightarrow}E+P \]

\[ k_1 \ ] \ [ k_2 \ ] \ [ k_3 \ ] \]

Where: \( E \) = enzyme concentration; \( S \) = substrate
concentration; \( ES \) = enzyme-substrate complex
concentration; \( k_1, k_3 \) = 1st order reaction rate
constants (t\(^{-1}\)); \( P \) = product concentration; \( k_2 \) = 2nd
reaction rate constant (M\(^{-1}\).t\(^{-1}\)). At first, it must be
stressed that the formation of \( ES \) (enzyme-substrate
complex) is an obligatory step to any type of enzyme-
catalyzed reaction. The \( ES \), depending on the reaction
conditions, can form the product (forward reaction)
or not (reward reaction). An enzymatic reaction can
be divided in three phases (Figure 1). During phase I
(reaction onset), there is the accumulation of the
enzyme-substrate complex (ES) without product
formation and significant substrate consumption.
The existence of the ES was predicted by Brown in 1892,
reinforced by Henry in 1902 and experimentally
demonstrated in 1936 (3). The presumption of
ESexistence allowed Michaelis and Menten (1913) to
postulate the first mathematical model for quantifying
enzyme activity. Briggs and Haldane improved it in
1926. Phase II begins as soon as the ES concentration
reaches a maximum value, remaining invariable for
a period. The duration of the steady stateregarding
the ES concentration depends on the relative concentrations between the enzyme and the substrate. Along phase II, the substrate is consumed and the product accumulates in the reaction medium. Along the phase III—when the ES concentration is no longer constant—the substrate consumption and product formation occur slowly. The quantification of the enzyme activity considers the conditions observed in phases II and III.

![Figure 1: Variation of enzyme (E), substrate (S), product (P) and enzyme-substrate complex (ES) concentrations in relation to reaction time](image)

The starting point for establishing the enzyme kinetic equation is the determination of the variation in the initial concentration of substrate in relation to the reaction time at a fixed amount of enzyme (E0). This is made by measuring the amount of substrate consumed along the period, followed by plotting the data in a \( S = f(t) \) graph (Figure 2). The inclination of the linear part of each curve related to a determined initial substrate concentration \( S_1, S_2, S_3, \ldots, S_n \) represents the initial reaction rate of the enzymatic reaction \( v_1, v_2, v_3, \ldots, v_n \). By plotting \( v \) versus \( S \), the result is a hyperbola, whose curvature tends asymptotically to a maximum \( v \) (Vmax), i.e., the reaction rate becomes invariable in relation to the substrate concentration (Figure 3). Under controlled reaction conditions, the Vmax becomes a kinetic constant, which describes the enzyme catalysis quantitatively. The phenomenon of saturation is seldom observed in non-enzymatic reactions. The asymptotic zone of the hyperbola would correspond to the condition in which the amount of substrate is enough to saturate the enzyme molecules present into the reaction medium completely, i.e., the ES concentration into the reaction medium is constant.
Figure 2: Variation of the substrate concentration versus time. The initial enzyme concentration ($E_0$) was constant. At the interval $T$, the substrate consumption varies linearly with time, i.e., the reaction rate is constant. Therefore, the initial substrate concentration ($S_1, S_2, \ldots, S_n$) was enough to saturate all the enzyme ($E_0$).

Figure 3: Reaction rate ($v$) versus initial substrate concentration. At the substrate concentration over $S_2$, the curve tends to a maximum value of reaction rate ($V_{\text{max}}$), which indicates that all enzyme ($E_0$) is saturated with substrate.
Considering \((ES \to E + P)\) as the limiting step of the overall reaction, then

\[
(-dS/dt) = v = k3.(ES) \quad (1)
\]

However, remembering that at any time along the reaction, the whole enzyme is distributed as

\[E0 = (E) + (ES) \quad (2)\]

Where \(E0\) = total amount of enzyme in the reaction medium; \(E\) = amount of enzyme not bound to the enzyme-substrate complex \((ES)\).

When all enzyme molecules are bound to substrate molecules, then \((E) = 0\), and the equation \(2\) becomes

\[E0 = (ES) \quad (3)\]

Replacing \(3\) in \(1\):

\[(-dS/dt) = v = k3.E0 \quad (4)\]

Equation \(4\) leads to the conclusion that when the enzyme is saturated with substrate, the reaction follows \(^{(4)}\) a “pseudo-zero order” kinetic, i.e., apparently the reaction rate is independent from the substrate concentration. In other words, the reaction rate is directly proportional to the whole enzyme concentration. Under this condition, the \(k3\) is called “turnover number” \((kcat)\), which indicates the number of substrate molecules transformed per unit of time by a single enzyme molecule when the enzyme is the rate-limiting factor\(^{(5)}\). Under the saturation condition, \(kcat.E0\) represents the maximum rate of the reaction catalyzed by the enzyme \((Vmax)\). Therefore,

\[
(-dS/dt) = v = Vmax \quad (5)
\]

Integrating Eq. \(5\):

\[
(S) = (S0) - Vmax.t \quad (6)
\]

Therefore, when the enzyme is saturated by the substrate, the substrate consumption decreases linearly along the period, i.e., the reaction occurs at a constant and maximum rate\(^{(6)}\).

Figure 3 – at low substrate concentration \((0 \to S1)\) clearly shows that \(v\) varies linearly in relation to \(S\), or in algebraic terms:

\[
(-dS/dt) = v = k'.(S) \quad (7)
\]

Where \(k'\) is a 1st order rate constant.

Integrating Eq. \(7\):

\[
LnS = LnS0 - k'.t \quad (8)
\]

Therefore, Eq. \(8\) shows that the substrate concentration decreases exponentially with time, when the reaction becomes under unsaturated and substrate concentration decreases \(^{(7)}\).

Along phase II, the \((ES)\) remains invariant for a time interval (Figure1). This means that

\[
d(ES)/dt = 0 \quad (9)
\]

Consequently,

\[
k1.(E).(S) = k2.(ES) + k3.(ES) \quad (10)
\]

Rearranging Eq. \(10\):

\[
(E) = \frac{KM.(ES)}{(S)} \quad (11)
\]

Where \(KM = (k2 + k3)/k1\).

Replacing Eq. \(11\) in Eq. \(2\) [this is valid insofar as the conditions under which the reaction is carried out preserve the full enzyme catalytic activity]:

\[
(ES) = \{[(S).E0] / [(S) + KM]\} \quad (12)
\]

Replacing Eq. \(12\) in Eq. \(1\):

\[
v = Vmax.(S)/[(S) + KM] \quad (13)
\]

Eq. \(13\) describes completely the hyperbolic curve shown in Figure 3. The terms \(Vmax\) and \(KM\) – the so called kinetic constants – characterize an enzyme\(^{(8)}\), when the catalysis is carried out under defined conditions (pH, temperature, agitation etc.). These constants are often calculated by the Eq. \(14\):

\[
1/v = (1/Vmax) + (1/S).(KM/Vmax) \quad (14)
\]

Equation \(14\) represents a straight line from
which the kinetic constants are calculated.

Regarding KM, three aspects must be stressed: 1) from Eq. (13), when KM = (S), then v = 0.5Vmax; 2) it is considered a referential for setting the operational substrate concentration. When (S) is at least 100 times lower than KM, the reaction occurs in an unsaturated substrate concentration, whereas when (S) is at least 100 times higher than KM, the reaction occurs in substrate saturation; 3) it is considered a characteristic of an enzyme when the catalysis occurs under defined conditions(9).

Along phase III, the (ES) is not constant, but the substrate continues to be transformed into product, although at a lower rate (Figure 1). To evaluate this phase, Eq. (13) must be integrated, resulting in

\[ t = \left[ (S_0 - S) - KM \cdot \ln\left(\frac{S}{S_0}\right) \right] \frac{V_{max}}{V_{max}} \ (15) \]

Defining substrate conversion (Y) as:

\[ Y = \frac{(S_0 - S)}{S_0} \ (16) \]

Replacing Eq. 16 in Eq. 15:

\[ t = \left[ Y \cdot S_0 - KM \cdot \ln \left(1 - Y\right) \right] \frac{V_{max}}{V_{max}} \ (17) \]

The practical aspect of Eq. (17) is that the duration of the reaction can be estimated from a desired substrate conversion.

**Expression of the Enzyme Activity**

The enzyme activity can be expressed by several manners. For example, the hydrolysis of sucrose by invertase is expressed as total-reducing sugars formed per minute, the collagen hydrolysis by collagenase is expressed as the viscosity diminution of a standard collagen solution per minute, and the amylase activity expressed as a SKB unit(10),(11),(12). However, it is recommended to express the enzyme activity using International Unit (U), which is defined as the amount of enzyme that catalyzes the formation of 1 mole of product per minute in fixed conditions. The specific activity is defined as U/mg protein (if the enzyme preparation is impure), or U/mol enzyme (for pure enzymes) (15).

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**Ethical standard:** The study was formally approved the research plan by the ethical committee board at the Babylon health directorate.

**Informed consent** was taken from all the participant patients before being enrolled in the study

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Frequency and Determinants of Neurological Symptoms of COVID-19 Patients

Mohammed Radhi Radeef, Fatimah Mustafa Mohammed Alattraqchi, Ahmed Abbas Shlaka, Shaymaa Abdul Lateef Alfadhul

1Lecturer, Department of Neuro Medicine, Faculty of Medicine, University of Kufa, Iraq. 2Doctor, Department of Community and Family Medicine, Ministry of Health, Iraq. 3Doctor, Al Najaf Health Directorate, Ministry of Health, Iraq. 4Assistant Professor, Department of Family and Community Medicine, Faculty of Medicine, University of Kufa, Iraq

Abstract

Objective: To determine the frequency, type, and associated determinants of neurological symptoms among COVID-19 patients.

Methods: A cross-sectional study was conducted among 161 doctors from different Iraqi cities using online questionnaires. They were provided information about their documented case. Data were collected from the 1st to 30th of October 2020. The questionnaire consists of 2 parts, first part concerns sociodemographic data of patients (age, gender, and severity of COVID-19 infection), while the second part deals with details of COVID-19 neurological symptoms. Data were stored and analyzed using SPSS version 24.

Results: A total of 161 COVID-19 cases were described in this study. Headache was found in 120 patients (74.5%); loss of smell in 108 patients (67.1%); and loss of taste in 86 patients (53.4%). It was found that ataxia more predominant in females (34.0%) than males (17.9%), statistically, there was a significant difference (P=0.024). Sever COVID-19 disease showed a significantly higher presentation of vertigo, hearing loss, loss of smell, disturbance of level of consciousness, sensory symptoms, motor symptoms, and stroke (p<0.05). Statistical analysis showed a significantly higher presentation of disturbance in level of consciousness, and stroke symptoms in patients older than 40 years (P<0.05).

Conclusion: Different neurological presentations are reported from COVID-19 cases. Headache, loss of smell, and taste are the most frequent symptoms. Some neurological manifestations are found more predominant in females, older age, and severe cases.

Keywords: SARS-CoV2 · COVID-19, neurological symptoms.

Introduction

Coronavirus disease 2019 (COVID-19) is an infectious acute respiratory disease. The first patient was confirmed to have COVID-19 on 1st of March 2020 in Wuhan, China (1).

The pathogen, later on, was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). Globally, until the 16th of August 2020, there have been 21,260,760 confirmed cases of COVID-19, including 761,018 deaths, reported to WHO [1]. Severe COVID-19 can cause progressive respiratory failure and death. Susceptibility is high among elderly
patients and those with comorbidities, recent surgery, and intrinsically or iatrogenically compromised immunity (2).

Neurological symptoms were reported in 0.04% of SARS and 0.2% of MERS, also, 28.2 million people of COVID-19 cases have neurological symptoms (3).

The most frequent question of neuroscientist is that if a virus direct infects CNS so need treatment with antiviral or virus is cleared from the body then treated with anti-inflammatory therapy, Michael said the is difficult to find the virus in the brain, compared with other organs. The polymerase chain reaction (PCR) often do not detect it there, despite their high sensitivity, and other studies have failed to find any virus particles in the cerebrospinal fluid, this is maybe due to that the ACE2 receptor, a protein on human cells that the viruses use to gain entry, is not much found in brain cells (3).

Coronaviruses may cause Central nervous system manifestations (Headache, dizziness, consciousness disorder, acute brain disease, seizures, and ataxia) (4), and peripheral nervous system symptoms (loss of smell, loss of taste, visual impairment, and nerve pain) (5).

Coronaviruses are thought to cause neurological manifestation either directly through neuroinvasive capacity (ACE2 receptors on neuronal tissues) or indirectly through the response of the immune system (an inflammatory mechanism). Also both SARS-CoV-2 and COVID-19 have neurological manifestation, so the diagnosis of SARS-CoV-2 infection should be kept in mind when patients presented with neurological symptoms during the pandemic (6).

The current study aimed to determine the types, and frequency of neurologic manifestations of COVID-19 infection, and to find its association with age, gender, and severity of COVID-19 infection.

Methods

An internet-based cross-sectional survey was conducted. The survey was distributed among doctors who deal with covid19 patients. The participants’ doctors were provided with an internet link to the survey created with the Google Forms application. After opening the invitation link, the respondent needs to agree to participate in research, before answering the questions. The data were collected from the 1st to 30th of October 2020. The questionnaire consists of 2 parts, of which part 1 concerns sociodemographic data (age, gender, and severity of COVID-19 infection). Part 2 deals with COVID-19 neurological symptoms. Data were analyzed using SPSS version 24, Mean and standard deviations were used for numerical variables, while frequency and percentages were used for categorical variables. A Chi-square test was used to test the significance of the association between variables.

Results

A total of 161 COVID-19 cases were described in this study. More than half of cases (94, 58.4%) are females. Their mean age (standard deviation) is 38(14.8) years. The higher number of cases (71, 44.1%) are in the age group 18-29 years, followed by (42, 26.1%) in the age group 30-39 years. According to COVID-19 Severity, 116 (72 %) patients had a mild infection, 37 (23%) had a moderate infection, the rest had a severe infection, table1.

Considering neurological symptoms, the headache was found in 120 patients (74.5%); loss of smell in 108 patients (67.1%); and loss of taste in 86 patients (53.4%). Other symptoms like generalized weakness, vertigo, unsteadiness, and drowsiness were reported from (54.2%), (28.6%), (27.3%), and (34.2%) of patients, respectively, table1.

Table 2 described the gender differences in neurological symptoms. It was found that ataxia more predominant in females (34.0%) than males (17.9%), statistically there is a significant difference(P=0.024).
Meanwhile, loss of taste sensation is more in females than males (74.5% vs 23.9%), statistical analysis shows a highly significant difference (P = 0.001).

Considering association of severity with presence of neurological symptoms, current results showed significantly higher percentages of neurological manifestation including vertigo, hearing loss, loss of smell, disturbance of level of consciousness, sensory symptoms, motor symptoms, and stroke in severe COVID19 disease (P < 0.05), table (3).

Table 4 demonstrated variation of neurological symptoms according to age groups, disturbance of level of consciousness is significantly higher in older (≥40 years) than younger age group (<40 years) (P = 0.009), also, four patients (12.9%) of older age recorded stroke symptoms in comparison to only two (1.5%) of younger age group. Statistical analysis shows a highly significant difference (P = 0.003).

Table 1: distribution of COVID-19 patients according to age, gender, severity, and neurological symptoms.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
<th>Percentage(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age In Years</strong></td>
<td>Mean (S.D)</td>
<td>38 (14.8)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>67</td>
<td>41.6</td>
</tr>
<tr>
<td>Female</td>
<td>94</td>
<td>58.4</td>
</tr>
<tr>
<td><strong>Age Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less Than 18</td>
<td>5</td>
<td>3.1</td>
</tr>
<tr>
<td>18-29</td>
<td>71</td>
<td>44.1</td>
</tr>
<tr>
<td>30-39</td>
<td>42</td>
<td>26.1</td>
</tr>
<tr>
<td>40-49</td>
<td>12</td>
<td>7.5</td>
</tr>
<tr>
<td>50-59</td>
<td>16</td>
<td>9.9</td>
</tr>
<tr>
<td>≥60</td>
<td>15</td>
<td>9.3</td>
</tr>
<tr>
<td><strong>COVID-19 Infection Severity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>116</td>
<td>72.0</td>
</tr>
<tr>
<td>Moderate</td>
<td>37</td>
<td>23.0</td>
</tr>
<tr>
<td>Sever</td>
<td>8</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>Ataxia Unsteadiness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>117</td>
<td>72.7</td>
</tr>
<tr>
<td>Yes</td>
<td>44</td>
<td>27.3</td>
</tr>
<tr>
<td><strong>Headache</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>41</td>
<td>25.5</td>
</tr>
<tr>
<td>Yes</td>
<td>120</td>
<td>74.5</td>
</tr>
<tr>
<td><strong>Vertigo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>115</td>
<td>71.4</td>
</tr>
<tr>
<td>Yes</td>
<td>46</td>
<td>28.6</td>
</tr>
<tr>
<td><strong>Hearing Loss</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>154</td>
<td>95.7</td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>4.3</td>
</tr>
<tr>
<td><strong>Loss Of Smell</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>53</td>
<td>32.9</td>
</tr>
<tr>
<td>Yes</td>
<td>108</td>
<td>67.1</td>
</tr>
<tr>
<td><strong>Seizure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>157</td>
<td>97.5</td>
</tr>
<tr>
<td>Yes</td>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Disturbance In Level Of</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Consciousness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drowsiness</td>
<td>55</td>
<td>34.2</td>
</tr>
<tr>
<td>Confusion</td>
<td>9</td>
<td>5.6</td>
</tr>
<tr>
<td>Delirium</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Coma</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>Stupor</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Lethargy</td>
<td>25</td>
<td>15.5</td>
</tr>
<tr>
<td>No</td>
<td>24</td>
<td>14.9</td>
</tr>
<tr>
<td>All</td>
<td>43</td>
<td>26.7</td>
</tr>
</tbody>
</table>
Table 1: Distribution of COVID-19 patients according to age, gender, severity, and neurological symptoms.

<table>
<thead>
<tr>
<th>Variable(s)</th>
<th>Male</th>
<th>Female</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia(Unsteadiness)</td>
<td>No</td>
<td>55(82.1%)</td>
<td>62(66.0%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>12(17.9%)</td>
<td>32(34.0%)</td>
</tr>
<tr>
<td>Headache</td>
<td>No</td>
<td>17(25.4%)</td>
<td>24(25.5%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>50(74.6%)</td>
<td>70(74.5%)</td>
</tr>
<tr>
<td>Vertigo</td>
<td>No</td>
<td>51(76.1%)</td>
<td>64(68.1%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>16(23.9%)</td>
<td>30(31.9%)</td>
</tr>
<tr>
<td>Hearing Loss</td>
<td>No</td>
<td>65(97.0%)</td>
<td>89(94.7%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2(3.0%)</td>
<td>5(5.3%)</td>
</tr>
<tr>
<td>Loss Of Smell</td>
<td>No</td>
<td>21(31.3%)</td>
<td>32(34.0%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>46(68.7%)</td>
<td>62(66.0%)</td>
</tr>
<tr>
<td>Seizure</td>
<td>No</td>
<td>65(97.0%)</td>
<td>88(93.6%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2(3.0%)</td>
<td>6(6.4%)</td>
</tr>
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</table>

Cont. Table 2: Distribution of neurological symptoms according to gender

<table>
<thead>
<tr>
<th>Variable(s)</th>
<th>Gender</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Sensory Symptoms</td>
<td>Paresthesia</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Anesthesia</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Burning Sensation</td>
<td>16</td>
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<tr>
<td></td>
<td>Allodynia</td>
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<tr>
<td></td>
<td>Hyperalgesia</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>110</td>
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<tr>
<td>Motor Symptoms</td>
<td>Weakness</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Cramp</td>
<td>12</td>
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<td></td>
<td>Dystonia</td>
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<tr>
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<tr>
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<td>Tremor</td>
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<tr>
<td></td>
<td>Imbalance</td>
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</tr>
<tr>
<td>Stroke Symptoms</td>
<td>No</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td>Loss of taste sensation</td>
<td>No</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>86</td>
</tr>
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</table>
### Table 2: Distribution of neurological symptoms according to gender

<table>
<thead>
<tr>
<th>Disturbance In Level Of Consciousness</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>P_Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drowsiness</td>
<td>15(22.4%)</td>
<td>19(20.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confusion</td>
<td>5(7.5%)</td>
<td>6(6.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delirium</td>
<td>1(1.5%)</td>
<td>1(1.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coma</td>
<td>2(3.0%)</td>
<td>2(2.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleepy</td>
<td>4(6.0%)</td>
<td>3(3.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lethargy</td>
<td>10(14.9%)</td>
<td>15(16.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13(19.4%)</td>
<td>11(11.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drowsiness</td>
<td>Stupor</td>
<td>Sleepy</td>
<td>Lethargy</td>
<td>3(4.5%)</td>
</tr>
<tr>
<td>All of above</td>
<td>14(20.9%)</td>
<td>29(30.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensory Symptoms</td>
<td></td>
<td></td>
<td></td>
<td>0.905</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>4(6.0%)</td>
<td>6(6.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anesthesia</td>
<td>4(6.0%)</td>
<td>4(4.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor Symptoms</td>
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<td></td>
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<td>0.178</td>
</tr>
<tr>
<td>Weakness</td>
<td>32(47.8%)</td>
<td>55(58.5%)</td>
<td></td>
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</tr>
<tr>
<td>Cramp</td>
<td>9(13.4%)</td>
<td>3(3.2%)</td>
<td></td>
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</tr>
<tr>
<td>Dystonia</td>
<td>0(0.0%)</td>
<td>1(1.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>21(31.3%)</td>
<td>26(27.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tremor</td>
<td>4(6.0%)</td>
<td>8(8.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imbalance</td>
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<td>1(1.1%)</td>
<td></td>
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<tr>
<td>Stroke Symptoms</td>
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</tr>
<tr>
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<td>1(1.5%)</td>
<td>5(5.3%)</td>
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</tr>
<tr>
<td>Loss Of Taste Sensation</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16(23.9%)</td>
<td>70(74.5%)</td>
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</table>

### Table 3: Distribution of neurological symptoms according to severity of COVID-19 infection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>P_Value</th>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
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<td>24(64.9%)</td>
<td>5(62.5%)</td>
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</tr>
<tr>
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<td>28(24.1%)</td>
<td>13(35.1%)</td>
<td>3(37.5%)</td>
<td></td>
</tr>
<tr>
<td>Vertigo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>90(77.6%)</td>
<td>21(56.8%)</td>
<td>4(50.0%)</td>
<td>0.020</td>
</tr>
<tr>
<td>Yes</td>
<td>26(22.4%)</td>
<td>16(43.2%)</td>
<td>4(50.0%)</td>
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</tr>
<tr>
<td>Hearing Loss</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>112(96.6%)</td>
<td>36(97.3%)</td>
<td>6(75.0%)</td>
<td>0.013</td>
</tr>
<tr>
<td>Yes</td>
<td>4(3.4%)</td>
<td>1(2.7%)</td>
<td>2(25.0%)</td>
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</tr>
<tr>
<td>Loss Of Smell</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>31(26.7%)</td>
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<td>Yes</td>
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<td>17(45.9%)</td>
<td>6(75.0%)</td>
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</table>
**Cont... Table 3: Distribution of neurological symptoms according to severity of COVID-19 infection.**

<table>
<thead>
<tr>
<th>Seizure</th>
<th>No</th>
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<td>113(97.4%)</td>
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<td>4(10.8%)</td>
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</table>

<table>
<thead>
<tr>
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<th>No</th>
<th>Yes</th>
<th>0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drowsiness</td>
<td>24(20.7%)</td>
<td>10(27.0%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>Confusion</td>
<td>2(1.7%)</td>
<td>8(21.6%)</td>
<td>1(12.5%)</td>
</tr>
<tr>
<td>Delirium</td>
<td>1(0.9%)</td>
<td>1(2.7%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>Coma</td>
<td>0(0.0%)</td>
<td>1(2.7%)</td>
<td>3(37.5%)</td>
</tr>
<tr>
<td>Sleepy</td>
<td>6(5.2%)</td>
<td>0(0.0%)</td>
<td>1(12.5%)</td>
</tr>
<tr>
<td>Lethargy</td>
<td>21(18.1%)</td>
<td>3(8.1%)</td>
<td>1(12.5%)</td>
</tr>
<tr>
<td>No</td>
<td>20(17.2%)</td>
<td>3(8.1%)</td>
<td>1(12.5%)</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>10(8.6%)</td>
<td>1(2.7%)</td>
<td>0(0.0%)</td>
</tr>
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</tr>
<tr>
<td>Lethargy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>10(27.0%)</td>
<td>1(12.5%)</td>
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</table>

<table>
<thead>
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</thead>
<tbody>
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<td>4(10.8%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>Anesthesia</td>
<td>3(2.6%)</td>
<td>2(5.4%)</td>
<td>3(37.5%)</td>
</tr>
<tr>
<td>Burning Sensation</td>
<td>8(6.9%)</td>
<td>7(18.9%)</td>
<td>1(12.5%)</td>
</tr>
<tr>
<td>Allodynia</td>
<td>8(6.9%)</td>
<td>4(10.8%)</td>
<td>1(12.5%)</td>
</tr>
<tr>
<td>Hyperalgesia</td>
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<td>2(5.4%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>No</td>
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</table>

<table>
<thead>
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<th>0.001</th>
</tr>
</thead>
<tbody>
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<td>Weakness</td>
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<td>23(62.2%)</td>
<td>2(25.0%)</td>
</tr>
<tr>
<td>Cramp</td>
<td>10(8.6%)</td>
<td>1(2.7%)</td>
<td>1(12.5%)</td>
</tr>
<tr>
<td>Dystonia</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>1(12.5%)</td>
</tr>
<tr>
<td>No</td>
<td>37(31.9%)</td>
<td>9(24.3%)</td>
<td>1(12.5%)</td>
</tr>
<tr>
<td>Tremor</td>
<td>7(6.0%)</td>
<td>4(10.8%)</td>
<td>1(12.5%)</td>
</tr>
<tr>
<td>Imbalance</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>2(25.0%)</td>
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</table>

<table>
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<th>No</th>
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<th>0.003</th>
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<tbody>
<tr>
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<td>37(100.0%)</td>
<td>6(75.0%)</td>
</tr>
<tr>
<td>Yes</td>
<td>4(3.4%)</td>
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<td>2(25.0%)</td>
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<table>
<thead>
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<th>0.253</th>
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<tbody>
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<td>17(45.9%)</td>
<td>6(75.0%)</td>
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<td>64(55.2%)</td>
<td>20(54.1%)</td>
<td>2(25.0%)</td>
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</table>
Table 4: distribution of neurological symptoms according to age group

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Age ≥40 years</th>
<th>P Value</th>
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<td>No</td>
<td>95(73.1%)</td>
<td>22(71.0%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>35(26.9%)</td>
<td>9(29.0%)</td>
</tr>
<tr>
<td>Headache</td>
<td>No</td>
<td>30(23.1%)</td>
<td>11(35.5%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>100(76.9%)</td>
<td>20(64.5%)</td>
</tr>
<tr>
<td>Vertigo</td>
<td>No</td>
<td>92(70.8%)</td>
<td>23(74.2%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>38(29.2%)</td>
<td>8(25.8%)</td>
</tr>
<tr>
<td>Hearing Loss</td>
<td>No</td>
<td>125(96.2%)</td>
<td>29(93.5%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>5(3.8%)</td>
<td>2(6.5%)</td>
</tr>
<tr>
<td>Loss Of Smell</td>
<td>No</td>
<td>40(30.8%)</td>
<td>13(41.9%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>90(69.2%)</td>
<td>18(58.1%)</td>
</tr>
<tr>
<td>Seizure</td>
<td>No</td>
<td>124(95.4%)</td>
<td>29(93.5%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>6(4.6%)</td>
<td>2(6.5%)</td>
</tr>
<tr>
<td>Disturbance In Level Of Consciousness</td>
<td>Drowsiness</td>
<td>33(25.4%)</td>
<td>2(6.5%)</td>
</tr>
<tr>
<td></td>
<td>Confusion</td>
<td>6(4.6%)</td>
<td>5(16.1%)</td>
</tr>
<tr>
<td></td>
<td>Delirium</td>
<td>2(1.5%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td></td>
<td>Coma</td>
<td>1(0.8%)</td>
<td>3(9.7%)</td>
</tr>
<tr>
<td></td>
<td>Sleepy</td>
<td>5(3.8%)</td>
<td>2(6.5%)</td>
</tr>
<tr>
<td></td>
<td>Lethargy</td>
<td>21(16.2%)</td>
<td>4(12.9%)</td>
</tr>
<tr>
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<td>No</td>
<td>20(15.4%)</td>
<td>4(12.9%)</td>
</tr>
<tr>
<td></td>
<td>Drowsiness</td>
<td>Stupor</td>
<td>Sleepy Lethargy</td>
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<td>10(32.3%)</td>
</tr>
<tr>
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<td>Paresthesia</td>
<td>8(60.4%)</td>
<td>2(6.5%)</td>
</tr>
<tr>
<td></td>
<td>Anesthesia</td>
<td>5(3.8%)</td>
<td>3(9.7%)</td>
</tr>
<tr>
<td></td>
<td>Burning Sensation</td>
<td>15(11.5%)</td>
<td>1(3.2%)</td>
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<td>Allodynia</td>
<td>12(9.2%)</td>
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</tr>
<tr>
<td></td>
<td>Hyperalgesia</td>
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<td>1(3.2%)</td>
</tr>
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<td>23(74.2%)</td>
</tr>
<tr>
<td>Motor Symptoms</td>
<td>Weakness</td>
<td>71(54.6%)</td>
<td>16(51.6%)</td>
</tr>
<tr>
<td></td>
<td>Cramp</td>
<td>11(8.5%)</td>
<td>1(3.2%)</td>
</tr>
<tr>
<td></td>
<td>Dystonia</td>
<td>0(0.0%)</td>
<td>1(3.2%)</td>
</tr>
<tr>
<td></td>
<td>No Symptoms</td>
<td>36(27.7%)</td>
<td>11(35.5%)</td>
</tr>
<tr>
<td></td>
<td>Tremor</td>
<td>11(8.5%)</td>
<td>1(3.2%)</td>
</tr>
<tr>
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<td>Imbalance</td>
<td>1(0.8%)</td>
<td>1(3.2%)</td>
</tr>
<tr>
<td>Stroke Symptoms</td>
<td>No</td>
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<td>27(87.1%)</td>
</tr>
<tr>
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<td>Yes</td>
<td>2(1.5%)</td>
<td>4(12.9%)</td>
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<tr>
<td>Loss Of Taste Sensation</td>
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<td>61(46.9%)</td>
<td>14(45.2%)</td>
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<td>Yes</td>
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</table>
Discussion

Recent evidence suggests that COVID-19 patients commonly had neurological symptoms manifested as acute stroke (6%), consciousness impairment (15%), and skeletal muscle injury (19%) (5). Therefore, the current study conducted to demonstrate the types, and frequencies of neurological manifestations in COVID-19 patients through online reports of physicians dealing with laboratory documented COVID-19 cases.

In this study, different neurological symptoms were reported by participants, the highest frequencies were headache (74.5%), loss of smell (67.1%); and loss of taste (53.4%). The cause of headache in COVID-19 could be explained by trigeminal vascular activation, in addition to systemic inflammation (increased cytokines) that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binding to ACE2 on trigeminal nerve endings within the nasal cavity. This is also most likely the cause of the loss of the sense of taste and smell (7).

Consistent with other findings (7,8) muscle symptoms like generalized weakness were highly reported in this study. These symptoms could be explained by muscle injury caused by SARS-CoV2 as recognized by high creatine kinase levels (7). Other symptoms like vertigo, unsteadiness, and drowsiness were reported in more than a quarter of cases. These presentations could be due to viral vestibular neuritis or due to damage to nerve tissue from hypoxia or hypercoagulopathy. A review of 14 studies that investigated dizziness as a symptom in COVID-19 cases reported dizziness in 4 to 30 percent of patients which was similar to the present finding (9).

Regarding gender differences in the presence of neurological symptoms, this study found that females reported ataxia and loss of taste sensation significantly higher than males(P<0.05). Females are more significantly affected by gustatory dysfunction (10). The higher susceptibility of females to develop gustatory dysfunctions could be attributed to the gender-related differences in the inflammatory reaction process (11).

Similar to previous findings (5), severe cases of COVID-19 infection were more likely to develop neurologic manifestations including vertigo, hearing loss, loss of smell, disturbance of level of consciousness, sensory symptoms, motor symptoms, and stroke. Therefore, close attention should be taken to the neurologic manifestations of COVID-19 patients, especially for those with severe infections, which may have contributed to their death. Also, during the epidemic period of COVID-19, physicians should consider SARS-CoV-2 infection as a differential diagnosis when seeing patients with these neurologic manifestations, to avoid misdiagnosis or delayed diagnosis.

In this study, disturbance of level of consciousness and stroke symptoms are significantly higher in older (≥40 years) than younger age group (<40 years). Whereas other neurological symptoms are not significantly differed between the two age groups. A previous study conducted in Tunisia among patients with COVID19 reported no significant differences in neurological manifestation according to age groups (12). Acute stroke is a commonly reported neurologic complication of COVID-19, particularly in the elderly population (13,14). Researchers have reported increasing trends of double-positive ACE2+TMPRSS2+ cell proportions with increasing age which could be a factor for disease severity in the elderly. These receptors had been found in tissues beyond the respiratory system, including oligodendrocytes in the brain. Additionally, neurologic autoimmunity had been suggested as a cofactor through the invasion of ACE2+TMPRSS2+ cells in organs such as the lungs and gut (15).

A limitation of this study is that all data were obtained through an online survey of physicians depending on their recall for cases, hence additional associated risk factors cannot ascertain. Besides, laboratory data and imaging studies were not available for comparison with the current results. However, this
is one of the earlier studies that focus on neurological manifestations in COVID-19, and documented reports of the physicians were dependent.

**Conclusion**

Different neurological symptoms have been reported from patients with COVID-19 infection. The most frequent neurological symptoms are headache followed by loss of smell and loss of taste. Some neurological manifestations are found more frequent in females, older age, and severe cases.

**Ethical Clearance:** Taken from University of Kufa ethical committee.

**Conflict of Interest:** The authors declare no conflicts of interest.

**Financial Statement:** The authors did not receive any financial support from any source

**References**


Original Article

Evaluation of Improvement in Knowledge and Attitude of Primary School Teacher’s Towards Various Aspects of Paediatric Dentistry after Education Intervention

Merin Sara Sojan¹, Swapnil Taur², N. D. Shashikiran³, Savita Hadakar², Namrata Gaonkar⁴, Sachin Gugwad⁴, Dhirajkumar Mane⁵

¹BDS Student, ²Senior Lecturer, ³Prof. and HOD, ⁴Reader, Department of Paedodontology and Preventive Dentistry, School of Dental Sciences, Karad, ⁵Statistician, Directorate of Research, Krishna Institute of Medical Sciences “Deemed To Be University”, Karad, Maharashtra (INDIA)-415539.

Abstract

Background: Dental Health Education in teachers has prominently invested the knowledge about various aspects of paediatric dentistry. Oral health theories and implementations can be augmented and reinforced by teachers who can work allied with dental professionals thereby bringing noteworthy improvement in oral health status of children. Therefore it is analytical to ascertain knowledge and positive attitude towards various aspects of paediatric dentistry of primary school teachers who are a primary care giver and mentor to children.

Material and Methods: The interventional study was outlined by two stage cluster sample. A total of four thirty seven primary school teachers took part in the research where the data was first collected by a close ended questionnaire. Re-evaluation was conducted after the education intervention and the results were statistically examined.

Results: Pre tests revealed that only 52% primary school teachers had sufficient knowledge and positive attitude towards various aspects of paediatric dentistry. After the education intervention, post tests revealed 75.5% primary school teacher’s good knowledge and positive attitude towards various aspects of paediatric dentistry which was appreciated. It was found statistically significance after post intervention with p<0.05.

Conclusion: The study observes that there is a notable innocence and knowledge gap concerning the knowledge about oral health and various aspects of paediatric dentistry that a primary school teacher holds and this has accentuated more the requirement of education intervention among primary school teachers to bring out better oral health status in children.

Keywords- Attitude, Primary school teachers, Knowledge, Education intervention, Dental Health Education.

Introduction

Dental Health Education in teachers has prominently invested the knowledge about various aspects of paediatric dentistry. Oral health theories and implementations can be augmented and reinforced by teachers who can work allied with dental professionals thereby bringing noteworthy improvement in oral health status of children. Therefore it is analytical to ascertain knowledge and positive attitude towards various aspects of paediatric dentistry of primary school teachers who are a primary care giver and mentor to children.
Teacher’s awareness about dental trauma, fluoride application and elimination of dental decay, their point of view and blockade of execution of Dental Health Education programmes can be assessed through surveys. Inadequate training and productions and time within the syllabus were pinpointed as major roadblock to application of a Dental Health Education programme in primary school. Expanding teacher’s training programme that inserts oral health knowledge and evidence based proposal to Dental Health Education within the academic world could authorize primary school teachers to play a remarkable role in oral health encouragement for children in Trinidad. [4]

Inspirational method using ‘the smiling robot’, through demonstration, exhibition and macro models have reduced the risk in dental decal and plaque accumulation. [5] A teacher should be instructed broadly concerning the consciousness of oral health advancement and significance of oral health for their students in amalgamation with health care assistants. [6]

Oral health standard results from degree of information available, attitude, routine and subsistence. [7] Origin of details about oral can be acquired through dental office (82%) accompanied by books and magazines (74%). When grading the ten methods of averting dental caries in children, teacher did methods such as fluoridated water and pit and fissure sealants lower than making methodical dental visits and lessening the ingestion of sugared foods. [8]

Despite of immense possible work force resources and growing economy, India stand behind in expression of education, quality of livelihood and peculiarly health even though there are different plan of action of National oral health programme that are used in up skilling the teachers, children and health care workers about oral health. [9]

There are numerous researches revealing about poor knowledge teachers have in dental education in western countries. [10] In numerous formerly school educational programmes have seemed to fail to bring any transition in children’s behaviour quantifiable improvement in oral hygiene. [11]

Few studies have been carried out to demonstrate the impact and effectiveness of school based Dental Health Education programmes in India and to evaluate the efficacy and feasibility of using primary school teachers for imparting Dental Health Education to school children. Thus, we intend to access the improvement in knowledge and attitude of primary school teacher’s towards various aspects of paediatric dentistry after education intervention.

Need of the Study

In today’s scenario, ideas of prevention, maintenance and awareness of dental health education have become topmost rime concern as per suggestions attained. In this study, three education intervention in the form of power point presentation, play act and exhibition will be used to improve the overall knowledge and attitude of primary school teacher’s towards various aspects of paediatric dentistry.

Aim:

To evaluate and compare the improvement in knowledge and attitude of primary school teacher’s towards various aspects of paediatric dentistry after education intervention.

Objectives:

1. To evaluate the knowledge and attitude of primary school teacher’s towards various aspects of paediatric dentistry before education intervention.

2. To evaluate the improvement in knowledge and attitude of primary school teacher’s towards various aspects of paediatric dentistry after education intervention.

3. To compare the knowledge and attitude of primary school teacher’s towards various aspects of paediatric dentistry with education intervention.
Material and Methods:

This was an interventional study conducted among 437 primary school teachers in Karad city, Maharashtra, India. The range of subject were selected by two stage cluster sampling. Two out four sector were selected in first stage. All the schools were then drafted out from the selected sector. This was used as a sampling figure in which 20% of school in the sector was selected using random sampling tactic. The teachers who teach age groups of 8 to 14 years in the selected school agreed to participate in the study after written consent were inserted.

The collection of data was done by a single trained investigator to avoid inter examiner changes. Firstly, a close ended questionnaire and survey per forma was handed over to the primary school teachers to collect the knowledge on attitude towards deciduous teeth problems and its importance, necessity of pulpectomy, dental caries and diet correlation, dental and mental health correlation, awareness about paediatric dentistry, oral habits and jaw growth correlation, attitude towards child first dental visit, fluoride treatment and dental caries correlation, dental health and overall development correlation and need of awareness programme for paediatric dentistry.

The primary school teachers were then given education intervention through power point presentation, play act and exhibition concerning various aspects of paediatric dentistry. The data is collected again after the education intervention for post interventional analysis.

To compare the knowledge and attitude of primary school teachers towards various aspects of paediatric dentistry after education intervention,

Ethical Consideration- This is an interventional study conducted after due approval from ethical committee (protocol number)

Statistical Analysis: First the data was transformed into MS-Excel. Frequency and Percentage was done by using MS-Excel itself. Comparison of knowledge, attitude in pre and post test score was done by using Paired t-test. It was found significant when p<0.05. The SPSS (Statistical Packages for Social Sciences) 20.0 was used to do analysis for Paired t-test.

Result

The demographic characteristics of all 437 teachers who participated in the study are primary school teachers from Karad, For getting this 437 primary school teachers we found to be Out of the 21 schools in each group, 15 (71.4%) from the Marathi medium and 6 (28.6%) from the English medium responded. Out of 437 school teachers 298 (68.1%) were males, 139 (31.8%) were females. The average age of school teachers were 43±6.7 years.
Table 1: Comparison of knowledge and attitude of primary school teachers towards various aspects of paediatric dentistry

<table>
<thead>
<tr>
<th>Questionnaire Regarding Knowledge and Attitude with its Correlation</th>
<th>Correct Answers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre test</td>
</tr>
<tr>
<td>1. Attitude towards Deciduous teeth problems?</td>
<td>277</td>
</tr>
<tr>
<td>2. Importance of deciduous teeth?</td>
<td>299</td>
</tr>
<tr>
<td>3. Time to change tooth brush?</td>
<td>247</td>
</tr>
<tr>
<td>5. Correlation of Diet and dental caries?</td>
<td>359</td>
</tr>
<tr>
<td>6. Correlation of Extraction and jaw growth?</td>
<td>163</td>
</tr>
<tr>
<td>7. Knowledge of Dental trauma management?</td>
<td>81</td>
</tr>
<tr>
<td>8. Correlation of Oral habits and jaw growth?</td>
<td>226</td>
</tr>
<tr>
<td>9. Awareness about Paediatric dentistry?</td>
<td>181</td>
</tr>
<tr>
<td>10. Correlation of Dental and mental health?</td>
<td>212</td>
</tr>
<tr>
<td>11. Attitude towards Child first dental visit?</td>
<td>180</td>
</tr>
<tr>
<td>12. Correlation of Dental health &amp; over all development?</td>
<td>311</td>
</tr>
<tr>
<td>13. Correlation of Fluoride treatment and dental caries?</td>
<td>101</td>
</tr>
<tr>
<td>14. Need of Awareness programme for paediatric dentistry?</td>
<td>293</td>
</tr>
</tbody>
</table>

Table 1 shows the knowledge and attitude of teachers prior to the educational intervention, and also compares it after the educational intervention as a post-test response. The understanding was consistently lacking in almost all questions prior to the invasion. The level of correct answer varied from 18.54 to 89.93%. In contrast, the attitude was seen to be more positive, and most teachers wanted to learn proper management skill for handling the dental paediatric intensity. Significant differences were found in knowledge and attitude score concerning
teachers work experience and gender (p < 0.05). After the health education, significant improvement was observed in knowledge about the correct management of dental injuries. The level of correct answers increased significantly which varied from 62 to 87%. Improvement in positive attitude was also seen in teachers after receiving the intervention.

### Table 2: Comparison of knowledge and attitude Score of primary school teachers towards various aspects of paediatric dentistry.

<table>
<thead>
<tr>
<th>Knowledge and Attitude Score</th>
<th>Pre- Test</th>
<th>Post-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>225.21</td>
<td>330.64</td>
</tr>
<tr>
<td>SD</td>
<td>94.76</td>
<td>30.33</td>
</tr>
<tr>
<td>Paired t-test</td>
<td>22.15</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001*</td>
<td></td>
</tr>
</tbody>
</table>

*Significant When p<0.05

Table 2 shows that comparison of knowledge and attitude Score by transforming responses of 437 of primary school teachers towards various aspects of paediatric dentistry it was found that mean score of before intervention was 225.21±94.76 and after intervention the mean score was 330.64±30.33. We had used the paired t-test for comparison and found statistically significant with p<0.05.

**Discussion**

In today’s framework or storyline, schools are in necessity of having perfect acknowledgement about maintenance, application and importance of oral hygiene. Most of the oral hygiene practice do come from home but since a child spends most of the time in school, it has been a prime prerequisite for their teacher to have knowledge about paediatric dentistry and its various aspects. A teacher can strongly create an impact about oral hygiene practice, various treatment in emergency situation like dental trauma, diet and dental caries correlation to children in order to bring a good oral health standard in them. So, it is important to educate the teachers in today’s academic setting about various aspects of paediatric dentistry to ensure a better outcome of the clinical treatment.

There are many researches and studies done to evaluate the knowledge and attitude of school teachers about dental education. However, numerous researches done in Zanzibar12, Trabzon13, Saudi Arabia 14-15 and Nigeria16 has shown the lack of knowledge and poor attitude of teacher’s towards dental education and its benefits. Various researches from different part of India in Davengiri6, Mumbai17 and Manglore18 have also concluded similar results regarding school teacher’s knowledge and attitude regarding the same. In the present study too we have observed the insufficiency of knowledge in primary school teachers about various aspects of paediatric dentistry, importance of oral health and its importance was indistinguishable to the national and international data.

In the first section of the questionnaire for the study was about attitude towards deciduous dentition problems dental trauma, dental caries and gingival infection. A study done in Pondicherry by Vidya Shekhar et al19 has revealed that 47% of school teachers were aware the reason of dental caries due bacteria and high intake of sugar and 42% of school teachers were aware of the reason of plaque and
calculus accumulation causing gingival diseases. Similarly, studies conducted by Ehizele et al\textsuperscript{15}, Amjad H Wyne et al\textsuperscript{13}, Khalid Almar et al\textsuperscript{14} has revealed that 60\%, 97.4\% d and 80\% of male school teachers and 90\% female school teachers knew that the reason for dental care was due to intake of sugar, poor oral hygiene and wrong brushing technique respectively. In the present study, only 63.39\% primary school teachers knew about deciduous dentition problems prior to the education intervention which was observed that there is increase of 78.72\% in post-test after the education intervention.

In the second section of the questionnaire for the study was about the importance of deciduous dentition. In the present study, 68.42\% primary school teachers do have knowledge about the importance of deciduous dentition compared to the study done by Ankita Mota et al\textsuperscript{17} where the results showed that 53.2\% of primary school teachers knew about deciduous dentition. In the third section of the questionnaire for the study was about the dental trauma and its management which included the knowledge about management of avulsion and fractured tooth. In the present study, the results revealed that primary school teacher had prior knowledge about dental trauma management. These results were compared with studies by Neha Nashine et al\textsuperscript{9} where 26.6\% of school teachers had knowledge on management of fractured tooth and 3.2\% on management of avulsed tooth. Management of avulsed tooth and their storage are not widely believed due to the common acceptance that they are not restorable.

Fourth section of the questionnaire was about the knowledge on dental visit of the child. In the present study, 41.19\% were aware about the importance of regular dental visit for maintenance of oral hygiene during the pre-test and was increased to 77.35\%. Studies by Vidya Sekhar et al\textsuperscript{19}, Amjad H Wyne et al\textsuperscript{13} and Ezgi Baltari et al\textsuperscript{12} has revealed 32\%, 100\% and 83\% responses respectively about the need of regular dental visit of children. In the fifth section of questionnaire, general knowledge on fluorides were asked and it was shown in the present study that primary school teachers had 23.11\% knowledge prior to the education intervention which was increased afterwards to 73\%. Similarly, a Trabzon based study by Ezgi Baltari et al\textsuperscript{12} obtained 65.8\% knowledge on pre-school teachers on the same subject.

It was clearly seen in the present study a knowledge gap in all five sections of questionnaire which suggested the innate importance to recognize the education on oral health and various aspects of paediatric dentistry and the implementation of the same to the children to bring out more significant oral health status.

The last section of questionnaire for the study was featured on the need of awareness programme for paediatric dentistry where the present study shows that many primary school teachers had prior knowledge and positive attitude of various Dental Health Education programmes and their importance in the curriculum before education intervention. Present study emphasised 89.93\% of primary school teachers are having knowledge and positive attitude about need of awareness programmes for paediatric dentistry. All teachers participated in the study with variable job experience had similar attitude towards the subject which gave no statistical difference compared to their job experience. The primary school teachers participated in the study also felt the need for informing the parent about the oral health of their children. A study done in Mumbai by Ankita Mota et al\textsuperscript{17} has specified that 78.9\% of primary school teachers were unaware about the school water fluoridation programme and 54.8\% of primary school teachers had never discussed about the oral health of the children to their parents during the parent’s meet. Whereas, a study done in Trabzon by Ezgi Baltari et al\textsuperscript{12} has revealed that 35.1\% of pre-school teachers has appositive attitude on taking up activities and programmes in educating the children. In another study of Jaya Naidu et al\textsuperscript{20} have also exhibited about how different Dental Health Educational programmes has as impact on schoolchildren on a regular basis.
To observe the effect of education intervention on primary school teachers, power point presentations, play act and exhibition was conducted which drastically improved the knowledge and attitude of primary school teachers towards various aspects of paediatric dentistry. There has not been an Indian study according to our knowledge which emphasis the- bring about of education intervention with pre and post-test scheme that had a significant effect of educating the primary school teachers. A similar study done in Dubai by Al Sari S et al\textsuperscript{21} has shown that knowledge attained by physical education teachers and school nurses had increased after the education intervention and improvement was seen in pre-test (26%) and post-test(95%). In the present study, there is improvement in knowledge and attitude of primary school teachers in some questions 18.54% to 70.94% substantial increase was noted. The use education intervention by the mode of power point presentation, play act and exhibitions has been first study in India where there was significant difference shown in knowledge and positive attitude attained in post-test compared to pre-test. This study pointed more on the importance of educating primary school teachers about various aspect of paediatric dentistry so that it helps in inculcating more knowledge about the same and imparting the knowledge to children. However, power point presentation, play act and exhibitions was agreeable for the presenter in presenting the study and the importance oral health education to primary school teachers.

**Conclusion**

In today’s academic setup, it is elementary need for the primary school teacher to be educated in oral hygiene, dental health programmes and awareness of the same. In the present study, it calls attention for the importance of dental education. There is a big necessity of more studies, education intervention and implementation of these in both urban and rural arts of India. Through the present study, it is understood that more dental health programmes should be enhanced in the current curriculum of teacher’s in order to improvise healthy status and good oral practices in children.

However, parents and primary school teachers should be given more opportunities and privileges to attend more oral health education programmes and to work more associated with health care professionals. Having a positive overview about conservation, prevention of tooth related diseases by giving a healthier environment of understanding the importance of dental education, reinforcing the importance of dentition and topmost guidance given to children would bring a better outcome of oral health in society.

**Ethical Clearance:** Taken from Institutional Ethics Committee, KIMSDU, Karad.

**Source of Funding:** KIMSDU, Karad.

**Conflict of Interest:** None.

**References**


The Effect of Red Sirih Leaf Drinking (Piper Crocatum) Towards Resin Violence Composites of Nanofiller in Laboratory of Makassar Working Center

Masriadi¹, Andy Fairuz Z, Eva², Sarahfin Aslan³, Chusnul Chotimah⁴, Nurasisa Lestari⁵, Isma Pebrianti³

¹Professor, Departemen of Epidemiology, Faculty of Public Health, Universitas Muslim Indonesia, ²Lecturer, Department of Dental Material, Faculty of Dentistry, Universitas Muslim Indonesia, ³Lecturer, Department of Conservative Dental, Faculty of Dentistry, Universitas Muslim Indonesia, ⁴Lecturer, Department of Prostodontic, Faculty of Dentistry, Universitas Muslim Indonesia, ⁵Researcher, Department of Oral Surgery, Faculty of Dentistry, Universitas Muslim Indonesia, Makassar

Abstract

Background. To determine the effect of soaking red betel leaves against the hardness of nanofiller composite resins. Materials and Methods. The research applied True Experimental Laboratory method with one group pretest posttest. The 18 samples of nanofiller composite resin were obtained through purposive sampling. The samples were light cured then soaked in red betel leaf solution for 28 minutes. Then, the hardness measurements were performed using VickerHardness Tester. Results. The results of paired sample t-test indicated the effect of soaking red betel leaf (p 0.002 <0.05) and sterile aquadest (p 0.026 <0.05) on the surface hardness of nanofiller composite resin. Conclusion. The effect of soaking red betel leaves on the surface hardness of nanofiller composite resin was obtained.

Keywords: Nanofiller Composites, Composite Surface Hardness, Red betel leaves

Introduction

In dentistry, the term composite resin refers to polymer systems in hard tissues such as enamel and dentin. Where composites are also commonly used to improve dental and aesthetic contours.¹ Initially the composite consisted of two components and used chemical polymerization for anterior dental lifts. Since then composites have developed rapidly, mainly to improve their clinical performance.² Composite resins are mixtures of two or more different materials with superior or better properties than each material itself.³ This composite resin consists of four main components, namely the organic polymer matrix, inorganic filler particles, coupling agent, and the initiator-accelerator system. [⁴] Particle filler nanosized causes more even distribution of particles, so the particle content of fillers increases followed by reduced polymerization shrinkage and increased mechanical properties of the material. [⁵]

The filler component in nanofiller composite resins Contains a unique combination of individual nanoparticles and nanocluster. Nanoparticles are separate, non-grouped particles that are 20 nanometers in size. A nanocluster consists of nano-sized particles that easily bond to form groups of particles. This group of particles acts as a single unit that allows high filler loading and strength in this composite.⁶ Absorption of fluids in the oral cavity can affect color stability and durability of composite resin. This composite resin

Corresponding author:  
email: arimasriadi@gmail.com  
Adress: Jl. Abd. Dg. Sirua No. 155B Makassar, Indonesia
undergoes a color change that is associated with the level of water absorption and hydrophilicity of the resin matrix. [6] Color change in composite resin is caused by two factors, namely internal factors and external factors. Color changes caused by external factors are caused by liquids or color carriers surrounding the composite resin restoration environment. [6]

In Indonesia various types of plants have been widely used as traditional medicine, one of which is betel plants, various types of betel plants have been found in Indonesia, but in Central Sulawesi there are only two types of betel plants, namely green betel and red betel. [7] Betel leaf is a plant that has been widely used as medicine. There are several types of betel leaves which are distinguished based on the shape of the leaves, taste and aroma, namely green betel, betel betel, betel betel, black betel, and red betel. These alkaloids function as antibacterial. [8] where people have long used red betel leaf as a toothache medicine, mouth ulcer medication and eliminate bad breath, as a mouthwash and also has antioxidant and antiseptic properties. [9]

According to Handayani et al, in 2016 in his study said that the acidity of the pH of red betel leaf decoction water was around 6 and would experience the release of small amounts of H+ and would break the siloxane bond in addition to the presence of phenol content in the red betel leaf decoction water could also reduce the hardness of composite resin. Composite resins soaked in red betel leaf (Piper crocatum) and sterile aquadest are caused by the acidity (PH), where the red betel leaf (Piper crocatum) is classified as more acidic than sterile aquadest resulting in a decrease in the hardness of the composite resin. [10] Where the red betel leaf (Piper Crocatum) contains poly phenols which can reduce the hardness of nanofiller composite resins. Polymers in composite resins contain unstable bonds, so they can be easily degraded by acids or low pH. [11]

### Materials and Methods

This research is an experimental laboratory with the design of The One Group Pratt Posttest. The sampling technique used was purposive sampling. The samples used were 18 pieces which were divided into 2 groups, namely soaking red betel leaves and sterile distilled water. criteria for sterile aquadest immersion. Inclusion criteria for fine nanofiller composite resin surface. While the exclusion criteria were box-shaped nanofiller composite resin, red betel leaf.

Preparation of nanofiller composite resins prepare 18 nanofiller composite resin specimens, by layer-by-layer inframental technique. where the composite resin is applied in the straw, where the straw is 6 mm high, and the straw diameter is 4 mm. then applied in a straw about 2 mm in diameter. then illuminated light cure for 40 seconds. Then the composite was reapplied about 2 mm in diameter and then reapplied again up to 6 mm, and exposed to light cure for 40 seconds until it was finished, and put it into a plastic drug in each sample.

Measurement of sample hardness before treatment, Measure the surface hardness of nanofiller composite resins by using a microhardness tester to determine the surface hardness value of nanofiller composite resins before being treated with Sample Treatment. In group 1 9 samples were soaked with red betel leaf decoction and in the control group were group II using 9 samples which were immersed using sterile aquadest. The treatment process takes 28 minutes then the sample is carefully removed using tweezers in an immersion container. Measurement of composite hardness after treatment. The analysis used is the Paired Sample T-test.

### Results

This research was conducted with two times the data collection, namely before and after immersion (The one group pretest post test design and control group design), to see the effect of soaking the red betel leaf (Piper crocatum) on the hardness of nanofiller composite resins. The researchers’ results are shown in the following table.
Table 1 Average hardness of nanofiller composite resins in a solution of red betel leaf (piper crocatum) at Makassar Laboratory of Work Training Center

<table>
<thead>
<tr>
<th>Soaking Aquadest is sterile</th>
<th>Before soaking</th>
<th>After soaking</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQ1</td>
<td>93,86</td>
<td>44,19</td>
</tr>
<tr>
<td>AQ2</td>
<td>99,40</td>
<td>85,35</td>
</tr>
<tr>
<td>AQ3</td>
<td>84,05</td>
<td>69,50</td>
</tr>
<tr>
<td>AQ4</td>
<td>107,3</td>
<td>100,02</td>
</tr>
<tr>
<td>AQ5</td>
<td>74,62</td>
<td>82,77</td>
</tr>
<tr>
<td>AQ6</td>
<td>96,96</td>
<td>65,01</td>
</tr>
<tr>
<td>AQ7</td>
<td>75,17</td>
<td>74,64</td>
</tr>
<tr>
<td>AQ8</td>
<td>69,52</td>
<td>62,78</td>
</tr>
<tr>
<td>AQ9</td>
<td>118,32</td>
<td>76,83</td>
</tr>
<tr>
<td>Mean</td>
<td><strong>91,02</strong></td>
<td><strong>73,45</strong></td>
</tr>
</tbody>
</table>

Table 1 explains that the hardness value of nanofiller composite resins that were measured before and after soaking for 28 minutes in a solution of decoction of red betel leaf (Piper crocatum) in each of the 9 samples. The average value before immersion was 89.70 kg / mm² whereas, the average value after soaking the red betel leaf solution decreased the average hardness value of the nanofiller composite resin 71.43 kg / mm². Table 1 decreased nanofiller composite resin due to soaking of red betel leaf, in which the red betel leaf contains phenol, so that the red betel leaf decoction water

Table 2 Average hardness of nanofiller composite resins with sterile aquadest immersion (control group) at Makassar Laboratory of Work Training

<table>
<thead>
<tr>
<th>Soaking red betel leaf</th>
<th>Before soaking</th>
<th>After soaking</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS1</td>
<td>64,49</td>
<td>63,21</td>
</tr>
<tr>
<td>DS2</td>
<td>76,82</td>
<td>74,11</td>
</tr>
<tr>
<td>DS3</td>
<td>83,42</td>
<td>65,79</td>
</tr>
<tr>
<td>DS4</td>
<td>85,38</td>
<td>44,68</td>
</tr>
<tr>
<td>DS5</td>
<td>104,6</td>
<td>77,97</td>
</tr>
<tr>
<td>DS6</td>
<td>95,38</td>
<td>78,55</td>
</tr>
<tr>
<td>DS7</td>
<td>104,6</td>
<td>84,05</td>
</tr>
<tr>
<td>DS8</td>
<td>88,07</td>
<td>70,50</td>
</tr>
<tr>
<td>DS9</td>
<td>104,6</td>
<td>84,05</td>
</tr>
<tr>
<td>Mean</td>
<td><strong>89,70</strong></td>
<td><strong>71,43</strong></td>
</tr>
</tbody>
</table>
Table 2 explains that the hardness values of nanofiller composite resins were measured before and after immersion for 28 minutes in a sterile Aquadest (control group) in each of the 9 samples. The average value before immersion was 91.02 kg/mm² while the average value after immersion in a sterile Aquadest decreased the average hardness value of nanofiller composite resin is 73.45 kg/mm².

Table 3 Effect of red betel leaf decoction (piper crocatum) on decreasing hardness of nanofiller composite resins at Makassar Vocational Training Center Laboratory

<table>
<thead>
<tr>
<th>Solution</th>
<th>Change in Mean Std. Deviation</th>
<th>P(value)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red betel leaf</td>
<td>18.27±11.81</td>
<td>0.002</td>
</tr>
<tr>
<td>Aquadest sterile</td>
<td>17.56±19.38</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Table 3 explains that the results of the Paired Sample t-test on the reduction in the hardness value of nanofiller composite resins based on immersion of nanofiller composite resins in a solution of red betel leaf (Piper crocatum) and sterile Aquadest (control group) at Makassar vocational training center in 2018. Paired test results t-test sample showed p of 0.002 (p <0.05) and on the immersion of red betel leaf decoction (Piper crocatum), and p value of 0.026 (p <0.05) sterile aquadest (control group) meant that there was an influence significant between soaking of red betel leaf and Aquadest sterile (control group), based on the value of the reduction in hardness of nanofiller composite resins before and after immersion in the test solution. The results obtained showed a decrease in the mean hardness of nanofiller composite resins by soaking the red betel leaf decoction (Piper crocatum) from 9 samples with a mean change of 18.37 kg/mm² with a standard deviation of (± 11.81). The average value of hardness of nanofiller composite resin in sterile aquadest immersion (control group) with a mean change of 17.56 kg/mm² with a standard deviation of (± 19.38). In table 3 shows the value of the effect of soaking the red betel leaf (piper crocatum) which causes a decrease in the surface hardness of the nanofiller composite resin which shows a p value of 0.002 and a sterile aquadest p value of 0.026.

Discussion

Research has been conducted by researchers using the True experimental laboratory method to see the effect of soaking the red betel leaf decoction (Piper crocatum) on the hardness of nanofiller composite resins at the Makassar Work Training Center Laboratory.

Betel leaf is a plant that has been widely used in Indonesia, where the red betel leaf (Piper Crocatum) where the red betel leaf is widely used in the community for a long time has used red betel leaf as a toothache medicine, mouth ulcers and eliminate bad breath, as a mouthwash and also has antioxidant and antiseptic properties based on the shape of the leaves, taste and aroma and there are several kinds of betel leaves, namely red betel leaf (Piper Crocatum) green betel leaf.[12]

Red betel leaf (Piper Crocatum) has phenol content which can reduce the hardness of nanofiller composite resins in this research. Immersion on red betel leaf (Piper crocatum) The decrease in hardness of nanofiller composite resin is caused by red betel leaves containing polyphenols.

Bonding materials have more hydrophilic properties compared to composite resins. This causes
the bonding material to have greater water absorbing properties compared to composite resin. Water absorption will cause plasticization which results in weak mechanical strength of the bonding material. The solubility of composite resin reflects the amount of unreacted monomer (residual monomer) released into water.\textsuperscript{[13]} The phenol hydroxyl group will release and bind to the siloxan bond which results in the breakdown of the Si-O-Si siloxane to Si-OH, but degradation runs very slowly so that the decrease in violence goes very slowly and takes a long time to experience a decrease in violence.\textsuperscript{[14]}

This study is in line with Handayani’s research which states that composite resins immersed in sterile aquadest have smaller surface hardness differences compared to composite resins soaked with red betel leaves (Piper Crocatum), this is due to differences in pH of the acidity of the cooking water Red betel leaf which ranges from 6 and sterile Aquadest around 7. Besides the presence of phenol content in boiled water, red betel leaf can also reduce the hardness of nanofiller composite resin

The nanofiller composite resin is a universal restoration material that is activated by visible-light which is designed for the purposes of restoring anterior and posterior teeth. Nanofiller composite resins have good physical properties, especially in the results of polishing and strength. Nanofiller composite resin restoration material that has a very small filler, so it can improve physical properties and abrasion resistance Polymers in composite resins contain unstable bonds, so they can be easily integrated by acids or low pH. Acid causes degradation of the polymer and filler components which can affect the surface roughness of the composite resin.\textsuperscript{[15]}

Composite resins exposed to acidic solutions will affect surface roughness. This happens due to degradation in the filler component caused by acid particles which will result in a decrease in physical properties and strength of the nanofiller composite resin. Composite resins exposed to acidic solutions will cause surface roughness. This occurs due to degradation in the filler component caused by acid particles which will result in a decrease in physical properties and strength of the nanofiller composite resin.\textsuperscript{[15]}

Based on the research of the red betel leaf immersion (Piper Crocatum) on the hardness of nanofiller composite resin, it was concluded that the red betel leaf can reduce the hardness of the nanofiller composite resin. with a hardness value after immersion with a mean value of 71.43 kg / mm\textsuperscript{2}, because the red betel leaf (Piper Crocatum) has an acidic pH and phenol content which can reduce the hardness of nanofiller composite resin.

The acid has many H\textsuperscript{+} ions which diffuse into the composite resin and bind the negative ions contained in the matrix so that the composite resin becomes damaged and a residual monomer of methylmethacrylate will form. This results in unstable chemical bonds so that the matrix dissolves and breaks down.

This study is in line with research conducted by June, 2017 where red betel leaves contain polyphenols which can reduce the hardness of nanofiller composite resins. Polymers in composite resins contain unstable bonds, so they can be easily integrated by acids or low pH. Acid causes degradation of the polymer and filler components which can affect the surface roughness of the composite resin.\textsuperscript{[16]}

Research has been conducted by researchers using the True experimental laboratory method to see the effect of immersion of sterile aquadest on the hardness of nanofiller composite resins in the Makassar Work Training Center Laboratory in 2018. The number of samples 18 each sample was divided into 2 groups and each group had 2 trials namely pretest and posttest. In the table shows the average level of violence before immersion in sterile aquadest is higher than after immersion. This shows that sterile aquadest can cause a decrease in hardness of nanofiller composite resin.
Water absorption is a physical property of composite resins that can affect the surface hardness of composite resins [17]. A decrease in the surface hardness of the composite resin results in wear and tear on the composite resin. A decrease in the hardness of the composite resin can occur when the polymer material absorbs water then causes hydrolytic breakdown and loss of bond between the resin matrix and the filler. [18,19]

Water absorption by composite resin can also occur because the resin contains Bis-GMA which has several disadvantages including high viscosity, thus requiring the addition of diluent, TEGDMA. This diluting monomer is added to the resin matrix to reduce viscosity and serves to make composite resin easy to apply. The solubility of the Bis-GMA and TEGDMA matrices is greater than UDMA and BisEMA. HEMA in bonding material has a very large solubility due to the low degree of conversion of monomers so that there are many remaining monomers that will dissolve in water. Water absorption and solubility events affect the effectiveness of the composite resin binder and bonding material in the composite resin restoration.

Based on this research, sterile aquadest immersion against hardness of nanofiller composite resins concluded that the hardness value of nanofiller composite resins before immersion had an average of 91.02 kg/mm² and after soaking for 28 minutes in a sterile Aquadest (control group) in 9 samples, the average value after sterile Aquadest immersion, the average hardness of the nanofiller composite resin decreases to 73.45 kg/mm². This shows that sterile Aquadest also has little effect on nanofiller composite resins which occur due to water absorption due to the process of water diffusion into the composite resin. Composite resins and bonding materials that are immersed in water will experience two different mechanisms, namely water absorption and solubility of the material in water. Water absorption between the matrix and the filler will result in a weakening of the bond between the matrix and the filler. Hardness changes in composite resins have the tendency to absorb water. Water absorption processes in the resin matrix and filler material occur simultaneously. Bis-GMA contained in the resin matrix has a hydroxy group (-OH) which is negatively charged on its lat metraki compound, so it is able to attract and absorb water into the composite by diffusion. In the 2013 study of Erlinawati et al. Composite resins containing zinc fillers and barium glass showed easier absorption of water than those containing quartz fillers. nanofil composite resins contain sirconia / silica fillers. Which is easier to absorb water. [20,21]

Research has been conducted by researchers using the True experimental laboratory method to see the effect of soaking red betel leaf on the hardness of nanofiller composite resins in the Makassar Work Training Center Laboratory in 2018. The total sample of 18 was divided into 2 groups, namely the test group and the control group, where the nanofiller composite resin test group was soaked using red betel leaves and the composite resin control group was soaked with sterile aquadest. Each group has 2 trials namely pretest and posttest. Based on the results of research conducted in the group obtained composite resin soaked with a solution of red betel leaf (Piper crocatum) has more influence on the hardness of nanofiller composite resin compared with sterile aquadest, this shows the cause is due to the pH acidity where red betel leaves classified as more acidic in bandigkan with sterile aquadest. As it is known that composite resin material immersed in acidic liquid has a higher solubility and a higher solubility can result in the erosion of the surface of the composite resin. In addition, the phenol content found in red betel leaves can reduce the hardness of nanofiller composite resins.

Concluded in the research conducted in Table 3 shows the value of the effect of soaking the red betel leaf (Piper crocatum) which results in decreased surface hardness of the nanofiller composite resin which shows a p value of 0.002 and a sterile aquadest p value of 0.026 p <0.05 is significant. This research shows that red betel leaf (Piper crocatum) has more effect on reducing the hardness of nanofiller composite
resin than sterile aquadest.

According to Hengtracol et al in 2011, acidic drinks resulted in surface damage and the aesthetic quality of the composite resin soaked in several types of drinks experienced a decrease in hardness. The greatest decrease in hardness is in drinks that contain acid. That immersion of composite material in an isotonic solution that is a drink with acidic ph can cause erosion of the spilled material and can cause degradation of the polymer matrix matrix composite network that does not react due to acidic drinks compared to aquadest. The process of water absorption is influenced by several factors, namely the type of resin sorted from the most soluble in water, namely TEGDMA, Bis-GMA, and UDMA. The filler content also affects the absorption of water in the material, the higher the filler content the less water absorption. Dental fillings or shades from composite resin also affect the water absorption process. [22,23,24]

Conclusion

It was concluded that there was a decrease in the hardness of nanofiller composite resin in the soaking of betel leaf (Piper Crocatum) and in sterile aquadest

Finacial support and sponsorship: Own cost

Ethical Considerations: Ethical clearance was obtained from Universitas Muslim Indonesia, Indonesia; with number” 093/H.20/ KEPK-UMI/ IX/2020. Just before the interview, written consent was obtained from each participant in Universitas Muslim Indonesia guidelines.

Conflicts of Interest: The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated.

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Targeting Autophagy Induction as a possible Protective Mechanism by Verapamil Compared to Rapamycin (Sirolimus) Against Gentamicin-Induced Ototoxicity in Guinea Pigs

Eman Elzayat¹, Amany A. Abdin², Sabiha Hedya², Mona A. Kotait³, Alshimaa Aboalsoud⁴
¹Demonstrator of Pharmacology, ²Professor of Pharmacology, ³Assistant Professor of Audio Vestibular Medicine, ⁴Lecturer of Pharmacology, Faculty of Medicine, Tanta University, Egypt

Abstract

Background: Ototoxicity is a harmful feature of the cochlea or auditory nerve and sometimes vestibular apparatus. Gentamicin is known to cause irreversible bilateral ototoxicity. Autophagy induction has been proposed as a target for prevention of gentamicin ototoxicity. Aim of the work: to investigate the possible protective effect of autophagy induction by verapamil compared to rapamycin. Methods: This experiment was conducted on 32 male guinea pigs. At the beginning each animal’s hearing status was assessed using auditory brainstem response (ABR) audiometry. Then, they were divided into 4 equal groups: Group 1: control group, Group 2: untreated gentamicin-induced ototoxicity group. Group 3: gentamicin-induced ototoxicity treated concomitantly with rapamycin. Group 4: gentamicin-induced ototoxicity treated concomitantly with verapamil. At the end of the experiment, ABR was repeated then the animals were sacrificed, and blood samples were obtained for assaying of reduced glutathione and malondialdehyde levels. The left cochlea was processed for scanning electron microscope, while the right cochlea was processed for histopathology and LC3-II immunohistochemistry. Results: Verapamil revealed superiority compared to rapamycin proved by significant improvement in ABR, histopathological results, in addition to its antioxidant effect. Conclusion: verapamil could be suggested as a potential therapeutic approach to decrease gentamicin ototoxicity.

Keywords: Autophagy, Gentamicin, Ototoxicity, Rapamycin, Verapamil.

Introduction

The most common sensory condition in humans is hearing loss [1]. Ototoxicity is the functional damage of the inner ear contributing to hearing and/or vestibular function loss. Aminoglycosides, cisplatin, macrolides, loop diuretics are the most common ototoxic drugs [2]. Gentamicin is one of the most frequently used aminoglycosides, but it causes nephrotoxicity and ototoxicity [3]. Gentamicin ototoxicity is frequently bilateral, symmetrical. It affects mainly the basal hair cells of organ of corti. The incidence of aminoglycoside hearing loss is up to 25% [4, 5]. There are multiple mechanisms of aminoglycosides ototoxicity: mutation in mitochondrial DNA [6], oxidative stress [7], phosphoinositide lipids involvement [8], and autophagy has recently been proposed to play a crucial role [9]. There are two pathways for autophagy induction: rapamycin (mTOR)-dependent mammalian target, and mTOR-independent pathway [10]. Rapamycin is established as an autophagy inducer and approved clinically as immunosuppressive drug,
but it has several side effects\textsuperscript{[11, 12]}. Verapamil is a calcium channel blocker that was suggested to be an autophagy inducer through mTOR-independent pathway. Moreover, verapamil might have antioxidant properties\textsuperscript{[13, 14]}, besides having considerable safety profile.

**Materials and Methods**

The study protocol has been approved by the research ethics committee, Quality Assurance Unit, Faculty of medicine, Tanta University, Egypt (Approval no. #32511/08/18).

**Drugs and chemicals**

Gentamicin sulphate (80 mg /2ml ampoule; Memphis Co. for Pharm. & Chem. Ind. Egypt). Rapamycin (1 mg tablet; a product of Wyeth Medica Ireland. United Kingdom). Verapamil hydrochloride (240mg Sustained-Release tablet; a product of Kahira Pharmaceuticals & Chemical Industries Co). Ketamine (50 mg/ml solution; Rotexmedica GmbH, Germany). Xylazine (100 mg/ml vial; BIMEDA Company).

**Animals and study design**

32 male guinea pigs (350-500 gm) were allowed one week of acclimatization and unrestricted access to food and water was provided \textit{ad libitum}. At the start of the research and after its completion, each guinea pig’s auditory brainstem response audiometry (ABR) was assessed under anesthesia. Only guinea pigs with normal ABR were included and divided randomly into four equal groups (n = 8 for each) where group 1; served as control group, received vehicle of 0.5% CMC orally by gastric gavage daily. Ototoxicity was induced in the other three groups using gentamicin by intraperitoneal (ip) injection at a dosage of 120 mg/kg/day\textsuperscript{[15]} where group 2; served as untreated gentamicin ototoxicity group, group 3; was treated concomitantly with rapamycin suspended in 0.5% CMC\textsuperscript{[16]} in a dose of 0.13 mg/kg/day orally by gastric gavage, group 4; was treated concomitantly with verapamil hydrochloride dissolved in distilled water\textsuperscript{[17]} in a dose of 30mg/kg/day orally by gastric gavage. Rapamycin and verapamil doses were calculated according to human equivalent dose equation\textsuperscript{[18]}. Treatment protocol in all groups started from 1st day of the experiment (zero point) and continued for 14 consecutive days. The ABR was reevaluated after completion of the experiment. The animals were ketamine and xylazine anesthetized by intraperitoneal (ip) injection in a dose of (40 mg/kg, ip) and (5mg/kg) respectively\textsuperscript{[15]} and blood was obtained through intracardiac blood sampling and processed for assay of reduced glutathione and malondialdehyde levels. The animals were sacrificed, and each cochlea was extracted and infiltrated with a fixative solution immediately. Left cochlea was prepared for scanning electron microscopy, while right cochlea was prepared for histopathology and immunohistochemistry.

**ABR audiometry**

ABR was achieved while animals were anesthetized through two channel tracking using three electrodes connected to preamplifier: two electrodes were placed as negative electrode on the left and right mastoid and high frontal Fz site as a positive electrode. In response to click stimuli introduced at 90 dBnHL, ABR was registered ipsilaterally, and when response was recorded, the strength was decreased in steps of 10 dB until a threshold was detected. Using alternating polarity, 19.3 / second repeat rate, 150–1500 Hz filter setting, 50,000 gain factor, and 0–10 ms time span, ER3A-insertphone transmitted click stimuli.

**Biochemical assays**

**Blood level determination of reduced glutathione (GSH) (mg/dl)**

GSH level was performed using Biodiagnostic supplied Kit (Cat. No TA 2511.), based on the Beutler spectrophotometric process\textsuperscript{[19]}.

**Serum level determination of malondialdehyde (MDA) (nmol / ml)**

Lipid peroxidation was assessed by calculating
serum (MDA) levels according to the Ohkawa et al. method [20] using Biodiagnostic supplied kit (Cat. No. MD 2529).

**Histopathological examinations of hematoxylin and eosin (H&E) stained tissues**

The right cochlea of each animal was fixed by EDTA formalin 10% for 4 weeks [21]. Paraffin sections (4μm) were examined by light microscope for histopathology.

**Scanning electron microscope (SEM)**

Fenestration was made at left cochlear apex for infiltration with 2.5% glutaraldehyde. The cochlea was processed for examination then dried using critical point dryer then examined with a JEOL (SEM) [22].

**Immunohistochemical examination of LC3-II**

It was carried out in the right cochlea using a rabbit polyclonal antibody (purchased from Sun Red Bio Laboratories Cat. No. 201r-1501) according to scoring method of Schläfli et al [23].

**Statistical Analysis**

Values are interpreted as mean ± standard error of mean (SEM), while in immunohistochemical (IHC) expression of LC3-II scores, median was used. Differences were found to be significant at p < 0.05.

**Results**

**Results of ABR measures**

**Detectability of ABR waves**

At zero point all waves of the ABR were detectable in 100% of animals. Then at the end point ABR showed these results: group 1: ABR was present in all animals (n=8) and all waves of ABR were detectable in 100% of animals while, in group 2 ABR was detectable in 25% of the animals (n=2) and absent in 6 animals indicating severe hearing affection. In group 3 and 4, drug treatment by rapamycin and verapamil respectively led to detection of ABR was in 75% of the animals (n=6) and its absence in 2 animals (Fig.1).

![Fig.1. Results of ABR (A) control group, (B) gentamicin induced ototoxicity group, (C) gentamicin induced ototoxicity group treated with rapamycin, (D) gentamicin induced ototoxicity group treated with verapamil. Results of ABR latencies](image-url)
Regarding latency of waves (I, III, V); there was non-significant difference between all groups at the start point of the study. Moreover, group 1 showed non-significant difference in all latency of waves (I, III, V) at the start and the end point of the study. Regarding Group 2, latency of wave (I) showed non-significant difference comparing zero and end points of the experiment, while latency of wave III showed significant increase at end point compared to zero point (P=0.02), and similarly latency of wave V (P=0.001). On comparing latency of the three waves in group 2 with control group at the end point; there was significant increase in latency of both waves III (P=0.004), and V (P=0.001). Group 3 showed significant increase in latency of wave I and wave V at the end point compared to zero point (P=0.02) and (P=0.05) respectively, Moreover, latency of wave III showed a significant decrease on comparing group 3 with group 2 (P=0.03). On verapamil treatment in group 4 there was a significant increase in latency of wave I (P=0.002), and latency of wave V at the end point compared to the zero point (P=0.003) (Table 1).
Table (1): Latencies in different studied groups at zero point and the end point.

<table>
<thead>
<tr>
<th>Group</th>
<th>Latency (Mean ± SEM)</th>
<th>F (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latency of wave I</td>
<td>F (3.02)</td>
</tr>
<tr>
<td></td>
<td>Latency of wave III</td>
<td>F (6.09)</td>
</tr>
<tr>
<td></td>
<td>Latency of wave V</td>
<td>F (13.83)</td>
</tr>
<tr>
<td></td>
<td>(Mean ± SEM)</td>
<td>(P NS)</td>
</tr>
<tr>
<td></td>
<td>(Mean ± SEM)</td>
<td>(P=0.006)</td>
</tr>
<tr>
<td></td>
<td>(Mean ± SEM)</td>
<td>(P=0.001)</td>
</tr>
<tr>
<td>Group 1</td>
<td>8 (100%)</td>
<td>1.3 ± 0.0</td>
</tr>
<tr>
<td>At zero point</td>
<td>8 (100%)</td>
<td>1.3 ± 0.0</td>
</tr>
<tr>
<td>At end point</td>
<td></td>
<td>Ppaired NS</td>
</tr>
<tr>
<td>Group 2</td>
<td>8 (100%)</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>At zero point</td>
<td>8 (100%)</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>At end point</td>
<td>2 (25%)</td>
<td>Ppaired NS</td>
</tr>
<tr>
<td>Group 3</td>
<td>8 (100%)</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>At zero point</td>
<td>8 (100%)</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>At end point</td>
<td>6 (75%)</td>
<td>Paired=0.02</td>
</tr>
<tr>
<td>Group 4</td>
<td>8 (100%)</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>At zero point</td>
<td>8 (100%)</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>At end point</td>
<td>6 (75%)</td>
<td>Paired=0.002</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM, n: number, NS: non-significant (P>0.05).

Ppaired: P value of paired sample t- test.

F (P value): F value (P value) of one-Way ANOVA.

Post- hoc Tukey test:
- P1: group 2 vs group 1
- P2: group 3 & group 4 vs group 2
- P3: group 4 vs group 3

Results of ABR threshold

Regarding threshold there was no significant difference between all the studied groups at zero point and in group 1 till the end of the study while, in group 2 gentamicin administration induced significant increase in the threshold at the end point compared to zero point (P=0.02). On rapamycin treatment ABR threshold elevated significantly at the end point (P=0.001) and the same change was found on treatment with verapamil (P=0.002). In addition, group 2 showed significant increase in the threshold.
in comparison to group 1 at the end point (P=0.001). Co-treatment with both rapamycin and verapamil exhibited decrease in ABR threshold when compared to group 2 treated with gentamicin alone. This decrease was significant only in case of verapamil (P=0.004) which also exhibited a significant decrease in the threshold compared to group 3 (P=0.05) (Table 2).

Table (2): Threshold in different studied groups at zero point and the end point

<table>
<thead>
<tr>
<th>Threshold</th>
<th>n (% detectability)</th>
<th>Mean ± SEM</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P value of different groups at the end point</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At zero point</td>
<td>8 (100%)</td>
<td>32.5 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>At end point</td>
<td>8 (100%)</td>
<td>32.5 ± 1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P paired NS</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At zero point</td>
<td>8 (100%)</td>
<td>40.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>At end point</td>
<td>2 (25%)</td>
<td>80.0 ± 5.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P paired = 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P1 = 0.001</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At zero point</td>
<td>8 (100%)</td>
<td>32.0 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>At end point</td>
<td>6 (75%)</td>
<td>72.0 ± 3.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P paired = 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P2 NS</td>
</tr>
<tr>
<td>Group 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At zero point</td>
<td>8 (100%)</td>
<td>35.0 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>At end point</td>
<td>6 (75%)</td>
<td>60.0 ± 2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P paired = 0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P2 = 0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P3 = 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F (45.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P value = 0.001</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM, n: number, NS: non-significant (P>0.05).

P paired: P value of paired sample t-test.

F (P value): F value (P value) of one-Way ANOVA.

Post-hoc Tukey test:
- P1: group 2 vs group 1
- P2: group 3 & group 4 vs group 2
- P3: group 4 vs group 3

GSH levels in the blood (mg/dl)

GSH level declined significantly in group 2 as compared to group 1 (P<0.001) while rapamycin
treatment revealed non-significant increase in GSH level as compared to group 2. On the other hand, group 4 revealed significant increase as compared to group 2 and group 3 (p<0.05) (Table 3).

Table (3): GSH levels (mg/dl) in the blood in different studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group 1 (n=8)</th>
<th>Group 2 (n=8)</th>
<th>Group 3 (n=8)</th>
<th>Group 4 (n=8)</th>
<th>One-way ANOVA F value (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(GSH) mg/dl</td>
<td>39.37±3.59</td>
<td>13.49±1.23</td>
<td>15.67±1.13</td>
<td>28.14±4.88</td>
<td>14.64 (P&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>P1&lt;0.001</td>
<td>P1&lt;0.001</td>
<td>P2 NS</td>
<td>P2&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

-Values expressed as mean ± SEM, n: number, NS=non-significant

-Post-hoc Tukey test:

- P1: group 2 vs group 1
- P2: group 3 & group 4 vs group 2
- P3: group 4 vs group 3

MDA levels in the serum (nmol/ml)

In group 2 there was significant increase in MDA level as compared to the control (P<0.01). Group 3 showed non-significant increase as compared to group 2. However, Group 4 showed significant decrease as compared to both groups 2 and 3 (P<0.05) (Table 4).

Table (4): MDA (nmol/ml) level in the serum in different studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group 1 (n=8)</th>
<th>Group 2 (n=8)</th>
<th>Group 3 (n=8)</th>
<th>Group 4 (n=8)</th>
<th>One-way ANOVA F value (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(MDA) (nmol/ml)</td>
<td>6.77±1.23</td>
<td>15.53±1.44</td>
<td>16.51±1.79</td>
<td>9.72±1.41</td>
<td>9.870 (P&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>P1&lt;0.01</td>
<td>P1&lt;0.01</td>
<td>P2 NS</td>
<td>P2&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

-Values expressed as mean± SEM, n: number, NS=non-significant

-Post-hoc Tukey test:

- P1: group 2 vs group 1
- P2: group 3 & group 4 vs group 2
- P3: group 4 vs group 3

**H&E-stained histopathology sections of right cochlea**

Group 1 showed normal outer, inner hair cells (IHCs), normal straight stria vascularis without vacuolations and normal spiral ganglion. Vestibular membrane was intact without vacuolations. In group 2 there was loss or total absence of organ of corti (OC). Others showed hydropic degenerations in outer hair cells (OHCs), broken vestibular membrane, vacuolations and cracking in the stria vascularis and spiral ganglion. Group 3 revealed preservation of hair cells with mild hydropic degeneration, waved basilar membrane in addition to focal degeneration in spiral ganglion. Group 4 showed preservation of normal OC, stria vascularis, spiral ganglion neurons with less degeneration (Fig.2).

**SEM of left cochlea**

Group 2 revealed complete fusion of stereocilia, and massive damage of the normal u or v shaped order. While group 3 showed to some extent preservation of stereocilia with focal fusion or complete loss. Group 4 showed minimal loss of stereocilia with preservation of normal shape (Fig.2).

---

**Fig. 2.** H&E-stained cochlea photomicrographs (A: H) and micrographs of scanning electron microscope SEM (I: T) of different studied groups:
**H&E:** Group 1 (A, B) where (A) showing normal (OHCs) & (IHCS), stria vascularis, straight intact basilar membrane (H& E X40) and (B) normal spiral ganglion with no degenerations (H& E X100). Group 2 (C, D) where (C) showing complete destruction of HCs and basilar membrane with hydropic degeneration, edema in stria vascularis (H& E X40) and (D) showing hydropic degeneration in spiral ganglion (H&E X100). Group 3 (E, F) where (E) showing preservation of HCs with waved basilar membrane, hydropic degeneration in stria vascularis (H&E X40) and (F) showing focal degeneration in spiral ganglion (H&EX100). Group 4 (G, H) where (G) showing preservation of OC architecture and waved basilar membrane with mild hydropic degeneration in stria vascularis (H&E X40) and (H) showing no degeneration of the spiral ganglion (H&E X100).

**SEM:** Group 1 (I: K) where (I) showing panoramic overview of the three and half turns of the cochlea (SEM x35), (J) showing normal stereocilia of HCs of OC (SEM x200), (K) showing (u or v) shaped rows of HCs (SEM x350). Group 2 (L: N) where (L, M) showing complete fusion of stereocilia of HCs (SEM x500-1000), and (N) showing complete damage of the normal order (SEM x350). Group 3 (O: Q) where (O, P) showing to some extent preservation of stereocilia with focal loss of stereocilia in others (SEM x350), (Q) showing preservation to less extent of (U or V) shaped in some areas (SEM x150). Group 4 (R: T) showing minimal loss of stereocilia with preservation of appearance of normal order of HCs (SEM x350).

**LC3-II Immunohistochemical scores**

Group 2 revealed significant increase in LC3-II score as compared to group1 (P=0.001) while, in both groups 3 and 4 a significant decline in LC3-II scoring as compared to group 2 was found (P=0.03) and (P=0.006) respectively (Fig.3, Table 5).

---

Fig.3. A photomicrograph of cochlea from studied groups, (A) group 1 showing barely seen staining for LC3-II expression score (0) (IHC X100). (B, C) group 2 showing destruction of hair cells of OC with expulsion of contents and high expression of the LC3-II with score (2) (IHC X 200). (D) group3 showing mild expression of the LC3-II with score (1) (IHC X 100). (E) group 4 showing mild expression of the LC3-II
with score (1) (group IHC X200).

Table (5): Immunohistochemical (IHC) expression of LC3- II scores in different studied groups

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>LC3-II score (median)</th>
<th>Kruskal–Wallis test &amp; Mann-Whitney test (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Group2</td>
<td>2**</td>
<td>P&lt; 0.001</td>
</tr>
<tr>
<td>Group3</td>
<td>1*</td>
<td></td>
</tr>
<tr>
<td>Group4</td>
<td>1*</td>
<td></td>
</tr>
</tbody>
</table>

Values expressed as median, Kruskal–Wallis and Mann-Whitney test:

** Significant; group 2 vs group 1.

*Significant; group 3, group 4 Vs group 2.

**Discussion**

Autophagy may be double edged sword. Meanwhile in many diseases, it plays an important role in cell survival, but it is still being debated if its activation increases or reduces cell death [24]. It has a protective effect in many diseases [25]. On the contrary it has been reported to cause pathological changes [26]. In addition to that, too much autophagy activation was believed to cause ischemia/reperfusion cardiac cell death [27] and promotion of hepatic fibrosis [28]. Our research aims to examine autophagy role in gentamicin- induced ototoxicity. At the start point, there was non-significant variation between groups with normal ABR in all animals, while at the end point there was significantly increased ABR threshold compared to the start one. Our results in group 2 compared to group 1 showed absence of ABR response in 75% of animals indicating sever hearing loss, while others showed significant increase in wave III latency, wave V latency and significant ABR threshold increase which coincides with many studies [5, 22, 29]. The ABR results are matched with histopathology in light and SEM where there are variable degrees of HCs death, pathological changes in stria vascularis and degeneration of spiral ganglion which are in line with many studies [22, 30, 31]. ROS role in gentamicin ototoxicity is documented in different studies [32, 33]. When there is imbalance between ROS and intrinsic antioxidants, apoptotic cell death is activated [34]. This is represented by marked elevation of MDA and marked reduction of GSH which coincide with Draz et al. [22]. To investigate the role of autophagy, LC3-II immunohistochemistry is determined using Schläfli et al. scoring (0-3) [23]. Our results in group 2 exhibited accumulation of autophagosomes represented by heavy LC3-II staining compared to group1 which shows presence of low basal level of autophagy that coincides with Kim et al. [12] who suggested that this accumulation is due to reducing lysosomes autophagosomes fusion. To investigate HCs survival autophagy role, rapamycin has been used [11, 12]. We used rapamycin orally according to Saegusa et al. who reported effectiveness of oral low dose rapamycin to induce autophagy [33]. There is no definitive oral rapamycin dose in guinea pigs was investigated to induce autophagy, so we depended on the human equivalent dose [35]. Rapamycin group as compared to group 2, it showed significant decrease regarding wave III latency, non-significant difference in wave V latency and ABR threshold. SEM and light
microscope showed to some extent preservation of normal OC with focal vacuolations or loss of some stereocilia. While there are non-significant differences in GSH, and MDA level. Regarding LC3II level there is significant reduction which indicates reduction in autophagosomes suggesting induction of autophagic flux which coincides with Cui et al. [36]. Whether rapamycin enhances HCs preservation or causes cell death, it is a matter of debate because the role of mTOR in cochlea is still unclear. Our results coincides with Leitmeyer et al. [37] who found that mTOR inhibition by rapamycin treatment led to dose dependent (HCS) death due to elevation of oxidative stress [37]. On the other hand, these findings are contradictory to He et al. [38] who documented elevated survival rates of HCs after rapamycin autophagy activation, decreased levels of ROS and decreased cell death, in addition to Kim et al. [12] who found that hearing thresholds were significantly better in rapamycin injected ear group, and reduction of ABR threshold via alleviating cisplatin ototoxicity [39]. This variability can be explained by that the efficacy of rapamycin can depend upon cellular type and mTOR target. Also, it is affected by the level of phosphatidic acid (PA) which is a competitive antagonist to rapamycin. The level of rapamycin which is needed to inhibit mTOR is affected by PA level in the cell [40]. Another explanation is occurrence of excessive autophagy which may lead to cell death as reported in different tissues [27]. So, the concentration of rapamycin in the cochlea is critical for cell survival. Away from the mTOR dependent pathway, verapamil was suggested in previous studies to be an autophagy inducer by decreasing the intracellular Ca\textsuperscript{2+} and increasing autophagosome formation, so help in clearance of mutant aggregate-prone proteins [13, 14]. It was also found to increase fusion between autophagosome and lysosome in liver, so it was tried for autophagic flux restoration and treatment of obesity [41]. It was also reported to have cytoprotective effect via induction of autophagy [42], in addition to recent study that suggested that verapamil extends lifespan by promoting autophagy [43]. As far as we know there is no definitive dose for verapamil to induce autophagy in guinea pigs, so we depended on human equivalent dose (FDA label dose is 240: 480 mg. The average daily dose is 360 mg/day)[44]. Verapamil treated group showed significant decrease in ABR threshold compared to group 2, significant reduction in MDA and significant elevation in GSH which are in line with Kedziora-Kornatowska et al. [13]. SEM and light microscope showed decline in HCs death and less pathological changes compared to group 2 and group 3. Regarding LC3-II expression there is significant decrease as compared to group 2 suggesting induction of autophagic flux. These are in consistence with previous reports which suggested that verapamil allowed lysosomes and autophagosomes to re-associate leading to autophagosome accumulation reduction [41]. Verapamil demonstrated superiority in protection of HCs against gentamicin induced ototoxicity compared to rapamycin due to its antioxidant and significant improvement ABR results.

**Conclusion**

Autophagy induction via Ca\textsuperscript{+2} channel antagonist verapamil may be a new therapeutic target to decrease gentamicin ototoxicity by its autophagy inducer effect and antioxidant properties.

**Recommendations**

More autophagy markers such P62 and different doses of rapamycin could be used to better present autophagy activity in gentamicin ototoxicity.

**Conflict of Interest:** None

**Funding:** None

**Acknowledgement:** Special appreciation to Prof. Dr. Naglaa Sarhan Head of Histology Department, Faculty of Medicine, Tanta university, Egypt

**References**


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34. Li H, Steyger PS. Systemic aminoglycosides are trafficked via endolymph into cochlear hair cells. Scientific reports. 2011;1:159.


Evaluate the Prevalence, Natural Course, and Final Visual Acuity in Patients with Stages III or IV of Diffuse Lamellar Keratitis Following Laser in Situ Keratomileusis

Afshin Mansourian¹, Bahman Sharifi²

¹Assistant Professor, Department of Anesthesiology, School of Medicine, Yasuj University of Medical Science, Yasuj, Iran, ²Assistant Professor, Department of Ophthalmology, School of Medicine, Yasuj University of Medical Science, Yasuj, Iran

Abstract

Background: The prevalence of DLK has been reported differently in different centers based on its severity. Hence, the aim of present study was evaluate the prevalence, natural course, and final visual acuity in patients with stages III or IV of diffuse lamellar keratitis following laser in situ keratomileusis.

Methods: In the present descriptive study, 3941 eyes files from 2125 patients underwent LASIK surgery; were examined. All patients had a complete ophthalmologic examination before LASIK and had suitable conditions for LASIK.

Result: Among the studied cases, 39 eyes (0.98%), out of 16 patients (0.75). The mean time interval between LASIK and DLK diagnosis was 3±2.4 days. A history of allergy was negative in all patients. The mean post-operative spherical refractive error in all eyes was in the range of 1 diopters and the average cylindrical refractive error in 33 eyes was -0.66±1.2 diopters. The mean of final spherical refraction in 34 eyes was 0.5±1.2 diopter and the mean of final cylindrical refraction in 35 eyes was -0.75±1.3 diopter. Severe turbidity at the flap surface in the first week decreased over time in all patients with stage III and IV DLK. So that only in four eyes (10.25%)

Conclusion: The prevalence of stages III and IV in DLK is negligible compared to the large number of LASIK procedures and all patients respond well to topical and systemic corticosteroid therapy.

Keywords: laser, diffuse lamellar keratitis, visual outcome

Introduction

Lamellar diffuse keratitis (DLK) is a non-infectious accumulation of inflammatory cells on the submandibular surface that often occurs within the first week after LASIK surgery. Numerous causes have been implicated in the occurrence of DLK, including the presence of particles and debris during surgery, glove powder, red blood cells, tissue particles, endotoxins, secretions, and infiltration of betadine solution.

Corresponding Author:
Bahman Sharifi,
Email: b.sharifi131@gmail.com

Treatment with corticosteroids before and after surgery reduces the severity of DLK, but treatment with fabric membrane stabilizers and nonsteroidal anti-inflammatory drugs have less effect on the DLK process after surgery. Increased intraocular pressure (IOP) following surgery can create a clinical picture similar to DLK (false DLK). Therefore, IOP control
is necessary in patients with suspected DLK\textsuperscript{10}.

The prevalence of DLK has been reported differently in different centers based on its severity. Hence, the aim of present study was evaluate the prevalence, natural course, and final visual acuity in patients with stages III or IV of diffuse lamellar keratitis following laser in situ keratomileusis.

**Method**

In the present descriptive study, 3941 eyes files from 2125 patients underwent LASIK surgery; were examined. All patients had a complete ophthalmologic examination before LASIK and had suitable conditions for LASIK.

In none of the subjects was there a major systemic disease that precluded LASIK. Follow-up examinations were performed in all patients on days one, two, three and seven, and in the absence of symptoms indicating the onset of DLK, in months one, three and six, and then annually. Accepted of these patients with stage III and IV DLK, in terms of age, sex, history of previous underlying disease (except for LASIK, history of drug use, history of allergies, refraction, topography, type of treatment, duration of treatment, duration of follow-up The response to treatment and the best corrected vision were assessed before LASIK and at the end of follow-up.

**Result**

Among the studied cases, 39 eyes (0.98%) (Figure1), out of 16 patients (0.75) including 12 women and 4 men had stage III or IV of DLK (Figure2). None of the unilateral cases had stage III or IV DLK, depending on whether LASIK was unilateral or bilateral. The mean time interval between LASIK and DLK diagnosis was 3± 2.4 days. A history of allergy was negative in all patients. Four patients had a history of taking non-ophthalmic drugs such as sedatives, antihypertensives and dermal drugs. The mean of preoperative spherical error in these 39 eyes was -2/1±1.1 and the mean of preoperative cylindrical error was -0.96±1.3 diopter.

![Figure1. Prevalence of Stage III or IV of DLK](image-url)
Figure 1. Prevalence of patients had stage III or IV of DLK

Table 1. Comparison of vision and refraction indices before and after surgery in patients with III or IV of DLK

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Spherical refraction</th>
<th>Cylindrical refraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before the operation</td>
<td>-2/1±1.1</td>
<td>-0.96±1.3</td>
</tr>
<tr>
<td>The day after surgery</td>
<td>1</td>
<td>-0.66±1.2</td>
</tr>
<tr>
<td>At the end of the follow-up</td>
<td>0.5±1.2</td>
<td>-0.75±1.3</td>
</tr>
</tbody>
</table>

The mean final spherical refraction in 36 eyes was 0.4±1.8 diopters and the mean refraction of the final cylinders in 38 eyes was -0.76±1.2 diopters.

The mean post-operative spherical refractive error in all eyes was in the range of 1 diopters and the average cylindrical refractive error in 33 eyes was -0.66±1.2 diopters. The mean of final spherical refraction in 34 eyes was 0.5±1.2 diopter and the mean of final cylindrical refraction in 35 eyes was -0.75±1.3 diopter. Severe turbidity at the flap surface in the first week decreased over time in all patients with stage III and IV DLK. So that only in four eyes (10.25%) at the end of the follow-up period, the location of turbidity was barely visible.

DLK was in 32 eyes (82.05%) in stage II and III, in five eyes (12.82%) in stage III and in the other two eyes (12.5%) in stage IV.

No corneal epithelial defect was seen in any eye with stage IV DLK. All patients in the acute inflammatory phase at the end of the first week had a camera higher than +1.9 diopters with astigmatism. The best initial corrected vision of patients before
surgery according to LogMAR was zero on average (equivalent to 20/20). In none of the patients did the reduction of more than two lines in the corrected vision occur. None of the patients underwent refractive surgery during the follow-up period.

**Discussion**

LASIK is the most common surgical procedure for correcting myopia, hyperopia and astigmatism in recent years. Diffuse layer keratitis, especially in advanced stages III and IV, is an important complication that has been reported in many studies as a potentially threatening visual complication if left untreated\textsuperscript{11}. Although many factors have been implicated in the development of DLK, the clinical appearance and findings are all the same. The first and second stages of DLK are mild and transient and may be diagnosed only on the basis of findings and clinical signs\textsuperscript{5, 12, 13}.

In this study, the prevalence of DLK in stages III and IV in patients undergoing LASIK was 0.98%. In the present study, none of the patients had a history of allergies. In a study conducted by Scot et al.,\textsuperscript{14} Conflicting results were reported 46% of patients with a history of atopic dermatitis, severe allergies and asthma in childhood. Noda et al.,\textsuperscript{15} Showed that the prevalence of DLK in stages III and IV was 0.8%, which were almost similar to the present study. Reinstein, et al.,\textsuperscript{7} also reported in a study that the prevalence of DLK in stages III and IV was 0.7%, which was lower than in our study. In an animal-based study, the results showed that corticosteroids were recognized as the best drug for the prevention and treatment of this complication\textsuperscript{16}. In other studies, BCVA and ultimate refraction in these patients have not been reported in the long term. Studies conducted in the short and medium term (up to 2 months) confirm the improvement of camera changes with irregular astigmatism\textsuperscript{7, 17}.

Although post-LASIK refraction stabilization is delayed by the development of the third and fourth advanced stages of the DLK, two major results can be obtained; First, with timely initiation of appropriate treatment, along with inviting patients to be patient and hope for recovery, only the necessary measures are needed. Prescribing a contact lens in a situation where the corneal center is very flat due to DLK. Another conclusion is that in the third and fourth stages of DLK, at least one year delay is required to perform any secondary and corrective surgery, and the results of the intervention earlier than this time are uncertain due to continuous refractive changes and lack of stabilization\textsuperscript{18, 19}.

Although initially hoping to reduce the risk of DLK due to not using a surgical blade, its occurrence has not yet been completely ruled out. On the other hand, re-approach of surgeons to laser ablation methods in possible cases, will eliminate the risk of this complication.

**Ethical Clearance**- Not applied.

**Source of Funding**- No funding was received.

**Conflict of Interest** – The authors declare that there is no conflict f interest.

**Conclusion**

The prevalence of stages III and IV in DLK is negligible compared to the large number of LASIK procedures and all patients respond well to topical and systemic corticosteroid therapy. The patient’s final vision due to this complication will not decrease compared to the best preoperative vision, if treated in a timely and appropriate manner. Although a camera with astigmatism (often irregular) in these patients in the short and medium term causes changes in refraction and visual impairment, but due to the slow recovery of this complication, these patients can be promised improvement in vision and refraction in the long run.
References


Prognostic Significance Association of Neutrophil-To-Lymphocyte Ratio and Platelets-To-Lymphocyte Ratio with Mortality in COVID19 Patients

Hoda Elsayed Mohammed¹, Sama Mohammed N. Attiyah²

¹Assistant Professor of Immunology and Parasitology, Biology Department, Faculty of Sciences and Arts, Al Kamel Branch, University of Jeddah, Jeddah, Saudi Arabia, ²Assistant Professor of Immunology And Cardiology, Biology Department, Faculty of Sciences and Arts, Al Kamel Branch, University of Jeddah, Jeddah, Saudi Arabia

Abstract

Background: Inflammation is a key factor in COVID-19 progression and is potentially an important predictive factor. The ratios of neutrophils to lymphocytes and platelets to lymphocytes may show significant inflammatory progression, which may contribute to the development of some major complications and mortality problems as observed in COVID-19. Objective: To examine and investigate the correlation of neutrophil lymphocytic ratio (NLR) and platelets to lymphocytes ratio (PLR) with increased mortality risk.

Methods: 124 patients admitted to King Abdulaziz hospital with confirmed COVID-19 were enrolled in our study. The blood cell count was used to obtain NLR and PLR. The receiver operating characteristic (ROC) curve was used to determine the sensitivity and specificity of NLR and PLR for severity and mortality of admitted patients with COVID-19. The logistic regression model was used to explore the risk factors associated with mortality and severity.

Results: The mortality rate was 23.4%. Among non-survivors’ patients’ lymphocyte significantly declined with 96.5%, while neutrophil counts increased. The Empirical optimal cut point of NLR correlated with mortality is 4.647, with a sensitivity of 93% and a specificity of 61% (AUC: 0.847, 95% CI 0.774-0.921; P = 0.001). Moreover, the optimal cut point of PLR associated mortality is 17.358, with a sensitivity of 90% and a specificity of 64% (AUC: 0.791, 95% CI 0.703-0.878; P = 0.005).

Conclusions: NLR and PLR variables were significantly correlated with severity and mortality. NLR and PLR can be considered independent biomarkers in COVID-19 patients which play a significant role to predict mortality cases.

Keywords: coronavirus, inflammation, mortality, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio.

Introduction

COVID-19 is a disease caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a virus thought to start in late December 2019 in Wuhan ¹. The number of infected people around the world has been raised significantly now a day. Patients diagnosed with COVID-19 showed different symptoms vary from asymptomatic illnesses to death ². Consequently, it is important to define risk factors linked to the poor prognosis of COVID-19 patients.
Several hematologic anomalies were revealed in COVID-19 patients which were prevalent in lymphopenia and thrombocytopenia. NLR and PLR are available values that correlate and reflect indirectly the inflammation conditions. Their values can be easily obtained from blood routine counts in laboratory. The NLR is determined by dividing the absolute number of neutrophils into the absolute count of lymphocytes, while PLR is calculated by dividing a count of platelets by the absolute count of lymphocytes. They are inflammatory markers with low-cost and are replicable to measure, which can be assessed easily with a blood sample. Generally, NLR and PLR are quantitative and convenient suitable factors to assess the susceptibility of death through COVID patients.

Immunological research has shown that the severity characteristic in extreme COVID-19 cases is due to elevated levels of proinflammatory cytokines known as a cytokine storm. This intense elevation of cytokines leads to a major proinflammatory reaction occurring in MODS and ARDS, resulting in mortality. From this point of view inflammatory markers can help to evaluate the severity and mortality cases of COVID-19 patients.

Few studies assessed the role of NLR and PLR in severe and non-severe COVID-19 patients. The present study was carried out to determine the association of NLR and PLR to COVID-19 severity and mortality.

**Material and Methods**

Our retrospective study on one hundred twenty-four positive COVID-19 cases in patients hospitalized at King Abdulaziz hospital. All patients were confirmed using reverse transcription-polymerase chain reaction (RT-PCR Abbott company, USA). The study was approved by Unit of Biomedical Ethics Research Committee (Reference NO. 301-20). Demographic parameters and laboratory assessments of the complete blood count of the patients were obtained exclusively from the Laboratory Information System (phoneix) in the hospital. Patients with complete data, including 95 survivors and 29 non-survivors’ cases, were randomly selected. Whole venous blood samples have been collected on BD EDTA tube and CBC profile analysis has been performed on Sysmex xn-10 and Sysmex xn-20 analyzer (JAPAN).

**Statistical Analysis**

The statistical analysis and graphical representation were done using SPSS Statistics 25 (IBM SPSS) and R version 4.0.4. The categorical variables were described as the number/total number (%), and continuous variables were described using the mean, median and interquartile range (IQR) values. The Kolmogorov–Smirnov test was used to test the variables for the normality distribution. Otherwise, the Mann–Whitney test was used to compare the medians for continuous variables. The categorical data were compared by $\chi^2$ test or Fisher’s exact test. The receiver operating characteristic (ROC) curve was used to determine the sensitivity and specificity of NLR and PLR for all-cause mortality and severity. The logistic regression model was used to explore the risk factors associated with mortality and severity. A p-value $< (\alpha =0.05)$ was considered statistically significant, where $\alpha$ is a significant to test level.

**Results**

The current study enrolled 124 diagnosed patients of COVID-19. The number of male patients was 77(62.1%) while 47(37.9%) were female. The mortality rate was 23.4% as 29 deaths had occurred. The mortality is more likely to present with older age ($\geq 60$) with 62.1%, and more common in male than female (75.8%). At admission time all positive patients underwent blood routine examinations, most of them had peripheral blood system anomalies. Moreover, non-survivors’ patients obtained low value of MCH and hemoglobin levels, 75.9% and 100% respectively.
regarding the reference range. The increase of neutrophils counts percentage was obvious within 89.6% of non-survivors’ patients. More importantly, we found that 96.5% among non-survivor’s patients showed significant lymphopenia. Furthermore, 66.1% of all patients exhibited eosinopenia, while this percentage was 75.9.1% of non-survivors’ patients. The results revealed normal platelet counts with 98 (79%) among COVID-19 patients. While amongst survivors’ patients 26 (27.4%) were in intensive care unit ICU, whereas 28 (96.6%) were in ICU of non-survivors.

A null hypothesis Ho has no significant affiliation between mortality and the categorical variables. It was tested using Chi-squared test. For the continuous variables, the significance differences of median value between the two groups were tested using Man Whitney Test. Results show that the null hypothesis is rejected for all variables except gender, Hb, MCH, platelet, and eosinophils. This indicates a statistically significant difference between the two groups in the median values for age, WBCs, lymphocyte, neutrophils, and monocyte variables.

Table 1 shows the results in comparisons of the values of NLR and PLR between survivors and non-survivors’ patients using Man Whitney Test. The results show a significant difference for NLR and PLR variables between the two groups. Moreover, this indicates a statistically significant difference for NLR and PLR variables between ICU and Non-ICU patients. Among the non-survivors’ group, NLR and PLR were significantly elevated compared to survivors’ group. Moreover, PLR and PLR were considerably raised in severe cases, when compared to non-severe group.

Table 2 shows the logistic regression model analysis. The univariate analysis revealed that several clinical factors were statistically significant risks associated with COVID-19 mortality, which included age, WBCs, monocyte, eosinophils, NLR, and PLR. The forward stepwise logistic regression regression is used to explore the most important risk factors associated with mortality. According to forward stepwise logistic regression results. The multivariate analysis shows that NLR and monocyte are risk factors associated with mortality with OR 1.081(95%CI :1.013-1.154) and 0.657(95%CI :0.503-0.857), respectively. The results reveled that NLR a valuable biomarker in relation to the mortality in COVID-19. However, PLR was not found to be an independent risk factor for death in multivariate analysis.

According to multivariate analysis, Table 3 shows that NLR and Hb are significantly correlated with severity with OR 1.480 (95%CI:1.266-1.731) and 0.652 (95%CI: 0.520-0.817), respectively. However, PLR was not found to be an independent risk factor for severity in multivariate analysis.

From Fig. 1 and Table 4 based on the ROC curve analysis, NLR, PLR, Monocyte, and the combined effect (NLR and Monocyte together) had diagnostic values for COVID-19 mortality (P-value<0.05), and the AUC from highest to lowest was (combined>NLR>PLR> Monocyte) respectively. The Empirical optimal cut point of NLR correlated with mortality is 4.647, with a sensitivity of 93% and a specificity of 61% (area under the curve (AUC): 0.847, 95% CI 0.774-0.921; P = 0.001). Moreover, the optimal cut point of PLR associated mortality is 17.358, with a sensitivity of 90% and a specificity of 64% (area under the curve (AUC): 0.791, 95% CI 0.703-0.878; P = 0.005). Moreover, Fig.2 showed that NLR, PLR, Hb, and the combined effect (NLR and Hb together) had diagnostic values for COVID-19 severity (P-value<0.05), and the AUC from highest to lowest was (combined >PLR>NLR> Hb) respectively.
Table 1: The value of NLR and PLR for COVID-19.

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survivors</td>
</tr>
<tr>
<td>NLR</td>
<td>3.86(7.27)</td>
</tr>
<tr>
<td>PLR</td>
<td>10.31(20.34)</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range).

Symbol (*) denotes to that the difference between the medians is statistically significance at level of significance $\alpha=0.05\%$. Otherwise, are not significant using Man Whitney test.

Table 2: The Univariate and Multivariate analysis for risk factors associated with death in patients with COVID-19.

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Gender</td>
<td>2.286(0.890-5.869)</td>
<td>0.086</td>
</tr>
<tr>
<td>Age</td>
<td>1.039(1.011-1.069)</td>
<td>0.006*</td>
</tr>
<tr>
<td>WBCs</td>
<td>1.171(1.073-1.278)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Hb</td>
<td>0.858(0.731-1.005)</td>
<td>0.058</td>
</tr>
<tr>
<td>MCH</td>
<td>1.052(0.887-1.247)</td>
<td>0.560</td>
</tr>
<tr>
<td>Monocyte</td>
<td>0.579(0.452-0.743)</td>
<td>0.000*</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.557(0.322-0.966)</td>
<td>0.037*</td>
</tr>
<tr>
<td>NLR</td>
<td>1.151(1.079-1.228)</td>
<td>0.000*</td>
</tr>
<tr>
<td>PLR</td>
<td>1.029(1.013-1.045)</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

(*) denotes to the statistically significance at level of significance $\alpha=0.05\%$. 
OR denotes to odds ratio.

Table 3: The univariate and multivariate analysis for risk factors associated with severity in patients with COVID-19.

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>OR</td>
<td>P-value</td>
</tr>
<tr>
<td>Variables not in equation</td>
<td></td>
<td></td>
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<tr>
<td>Variables in equation</td>
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<tr>
<td>Gender</td>
<td>2.548(1.182-5.496)</td>
<td>0.017*</td>
</tr>
<tr>
<td></td>
<td>1.011</td>
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<tr>
<td>Age</td>
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<td>0.001*</td>
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<tr>
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<td>1.344</td>
<td>0.246</td>
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<tr>
<td>WBCs</td>
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<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>2.850</td>
<td>0.091</td>
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<tr>
<td>Hb</td>
<td>0.679(0.570-0.809)</td>
<td>0.000*</td>
</tr>
<tr>
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<td>0.652(0.520-0.817)</td>
<td>0.016*</td>
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<tr>
<td>MCH</td>
<td>1.021(0.886-1.177)</td>
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<tr>
<td></td>
<td>0.050</td>
<td>0.824</td>
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<tr>
<td>Monocyte</td>
<td>0.759(0.662-0.871)</td>
<td>0.000*</td>
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<tr>
<td></td>
<td>0.347</td>
<td>0.556</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.880(0.635-1.218)</td>
<td>0.440</td>
</tr>
<tr>
<td></td>
<td>2.171</td>
<td>0.141</td>
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<tr>
<td>NLR</td>
<td>1.416(1.247-1.607)</td>
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</tr>
<tr>
<td></td>
<td>1.480(1.266-1.731)</td>
<td>0.000*</td>
</tr>
<tr>
<td>PLR</td>
<td>1.119(1.073-1.167)</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>3.295</td>
<td>0.069</td>
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</table>

(*) denotes to the statistically significance at level of significance α=0.05%.

OR denotes to odds ratio.

Table 4: ROC analysis for COVID-19 mortality and severity.

<table>
<thead>
<tr>
<th></th>
<th>Mortality</th>
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<th>Severity</th>
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<td>95% CI</td>
<td>P-value</td>
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<tr>
<td>NLR</td>
<td>0.847</td>
<td>0.774-0.921</td>
<td>0.001</td>
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<td>0.894</td>
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<tr>
<td>Monocyte</td>
<td>0.172</td>
<td>0.090-0.255</td>
<td>0.063</td>
<td>Hb</td>
<td>0.218</td>
<td>0.135-0.302</td>
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<tr>
<td>Combined*</td>
<td>0.861</td>
<td>0.788-0.933</td>
<td>0.000</td>
<td>Combined**</td>
<td>0.928</td>
<td>0.886-0.969</td>
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</tbody>
</table>

AUC area under the curve, 95% CI confidence interval.
* Combined effect (NLR and Monocyte together).

** Combined effect (NLR and Hb together).

Fig. 1: ROC analysis of the NLR, PLR, monocyte, and combined effect (NLR and Monocyte together) for prediction of COVID-19 mortality.

Fig. 2: ROC analysis of the NLR, PLR, Hb and combined effect (NLR and Hb together) for prediction of COVID-19 severity.
Discussion

Several recent reports have described the correlation of age with survivors and non-survivors COVID-19 patients. Our finding is compatible with the recent study that the older patients had worse health results and higher mortality rate than the young age group. Thus, it can be said that COVID-19 is most found with older age males.

Moreover, the current study found an increase in the neutrophil counts percentage of non-survivors’ patients compared with the survivor’s patients. Furthermore, we found that 96.5% of non-survivors’ patients showed a significant substantial reduction in the number of lymphocyte counts. Additionally, 7.5% among non-survivors’ patients showed eosinopenia. The findings of our study were aligned with the results of other previous studies.

The immune response is highly lymphocytic-based, and activation of the immune system is mainly due to neutrophils. Moreover, the systemic inflammation will destruct the CD4+ T lymphocytes and induce the suppressor CD8+ T lymphocytes which leads to an increase of the neutrophil-lymphocyte ratio (NLR). Moreover, platelets play an essential role in innate immunity and inflammatory response, and their count reflects the inflammation and infection condition. COVID-19 can cause pulmonary endothelial damage, which leads to pulmonary endothelial injury, activation, accumulation, and preservation of platelets in lung, followed by platelet depletion. The cytokine storm induces the inflammation in SARS-CoV-2 patients to worsen, and the platelet-to-lymphocyte (PLR) ratio reflects the level of cytokine inflammation that can be used for evaluation purposes.

The evaluation of inflammatory diseases progression and their changes may be a crucial factor that scientists must take in consideration. In COVID-19 patients, inflammatory biomarkers can be used as potential prognosis predictor factor. Most studies show that inflammation plays a central role in the pathogenesis of the disease. More concerning, the severity and mortality characteristic of COVID-19 was identified by several inflammatory factors.

SARS-CoV-2 can lead to various infection conditions, particularly respiratory tract infection diseases, which can develop into serious complicated conditions exhibiting pneumonia, pulmonary edema, acute or multiple organ failures leading to patient death. So, it is highly demanded to discover biological markers that can be used to identify COVID 19 at an early stage, helping to save people’s life. The early detection will aid to decrease the progression of severity, decrease the mortality, and support the decline of financial expenses of the ministry of health all over the world. Therefore, any parameter that can help for the early diagnosis is crucial.

Recent studies considered NLRs and PLRs as a precursor element for COVID-19 admission condition in the intensive care unit, and they consider it as a useful marker to evaluate the severity and mortality. NLR and PLR are easily calculating from a complete blood count with a differential count.

In this study, we found a higher NLR and PLR values in non-survivors COVID-19 patients compared to survivors’ patients. However, univariate analysis identified high NLR and PLR as an independent prognostic factor for survival. After changing the other confounding variables in multivariate Cox analysis, the NLR remained as a risk factor of survival in patients with COVID-19. Our findings were consistent with earlier studies. Whereas other studies reported that PLR not correlated with COVID-19 prognosis at all.

Moreover, monocytes are pro-inflammatory cells that can induce inflammation by producing many
cytokines\textsuperscript{12,33}. A recent study detected significant decreased in monocytes count within critical COVID-19 patients\textsuperscript{34}. Our results demonstrated that monocyte has risk factors associated with mortality with OR 0.657 (95\%CI :0.503-0.857).

ROC analysis of our study showed that patients with non-serious symptoms compared to patients with severe COVID-19 signs had higher NLR and PLR values, and they have a diagnostic value for COVID-19 severity and mortality. It was also demonstrated that the empirical optimal cut point of NLR correlated with mortality is 4.647 with a sensitivity of 93\% and a specificity of 61\%. Moreover, the optimal cut point of PLR associated mortality is 17.358, with a sensitivity of 90\%.

Moreover, our results shown that NLR and PLR had diagnostic values for COVID-19 mortality (P-value<0.05). The measurement of their values is a reliable and low-cost test, which can be easily applied in a daily routine blood tests in the lab and may be act as an independent prognostic factor for severity and mortality of COVID-19 patients.

Finally, no universal cutoff value has been found to date for NLR and PLR, especially in patients with COVID-19. Future research should focus on the optimum cutoff value to use it as an important prognostic indicator before medicinal application and patient hospital admission.

Conclusions

NLR and PLR had diagnostic values for COVID-19 severity and mortality. The daily CBC routine blood test can be used to identify COVID-19 patients at early stage of the infection. This type of detection well saves people life and reducing mortality rates by avoid going through complication stages. Additionally, this is an economical diagnosis tool easily available in cases where logistics and financing are limited.

Limitation

Our retrospective study was done in a single center and as a result the sample size was limited due to logistic problems and administrative acceptance of many patients that need more processing time, so a multiple centers prospective research is recommended. Also, since this study was conducted on blood laboratory parameters, patients were not constantly tracked for all clinical manifestations.

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Conflict of Interest: Non.

Source of Funding: Self.

Ethical Clearance: Taken from Unit of Biomedical Ethics Research Committee (Reference NO. 301-20).

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Effects of Nutrition and Physical Activity in Prevention of Neurological and Cognitive Disorders- A Review Study

Shweta Patel¹, Diwakar Chauhan², Ranjana Patnaik³

¹PhD Scholar, Department of Bio-Sciences, ²Professor and Head Department of Basic Sciences, ³Professor, Department of Bio-Sciences, School of Basic and Applied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India

Abstract

Background: As neurodegenerative and psychiatric disorders are rising rapidly, unexpected demand for cost-effective and practical treatment is also growing. The present study reveals the synergistic role and mechanism of nutrition and physical activities in the transition of cognitive functions and mental wellbeing.

Objectives: To unravel the effect of nutrition and exercise in maintaining cognitive function and mental health. In this study some of these points were reviewed such as what is the influence of nutrition and exercise over mental health. How well the combined effects can help to combat neurological and behavioural disabilities. What mechanism is involved in promoting mental health.

Methods: A non-systematic approach was used in the present review study. This study summarizes the synergistic role and mechanism of nutrients and physical exercise in the transition of cognitive function and mental balance.

Results: The most important outcome is nutritional therapy in combination with regular physical exercise is a reliable, effective and economical alternative that can intensify the effects of standard drug therapy.

Key Words: Physical Activity, Exercise, Nutrition, Mental Health, Cognition, Memory

Introduction

As per World Health Organization, central and peripheral nervous system is centre of neurological disorders. Such diseases include epileptic syndrome, Alzheimer’s disease, dementia, stroke, migraine, multiple sclerosis, Parkinson’s disease, neuronal motor dysfunction, brain tumours, head injuries because of trauma and neurological problems due to poor nutrition.¹

It is widely accepted that the lifestyle pattern, eating habits and environmental changes have a strong influence on the mental and physical wellbeing of individuals, particularly in our modern societies where the lack of physical activities and low nutritious diet are the major risk factors for several
neurodegenerative and psychiatric disorders.2

Impact of physical activity on the brain functions influences the fundamental aspects of brain plasticity. Exercise increases the level of neurotropic factors (Brain Derived Neurotropic Factor) which is originated from the brain in hippocampus an important region for cognition and reminiscence.3-7 Conversely, high fats diet, sugar and a calorie-rich diet are found to be harmful for neuronal functions because they elevate oxidative stress and thereby decreasing the synaptic plasticity as well as cognitive functions.8,9 Adequate levels of physical exercise and a nutritious diet can be an alternative non-invasive, highly efficacious treatment approach to the standard drug therapy of neurological disorders.10 Accordingly, under this review we will examine the process by which nutrition and physical activity regulate brain health and synaptic plasticity along with circumstances under which potential benefits can be derived from their combined application.

MICRONUTRIENTS AND NERVOUS SYSTEM

Some microelements such as vitamins, minerals, trace elements, carotenoids and essential fatty acids impart role in the flourishing and maintaining nervous system at the initial stages of life and during aging.11 Different nutrients are involved in sustaining the regular functioning of brain; they take part in producing cellular energy, creating myelin, preserving and restoring cells and synthesizing neurotransmitters.12,13

Vitamin B₆ is essential for the breakdown and consumption of amino acid, and consequently for the synthesis of neurotransmitter such as dopamine, GABA particularly serotonin neurotransmitters.12 In one study, scientists suggested to give folic acid to the people suffering with Parkinson’s disease for reducing the level of homocysteine due to standard medication known as levodopa.14 As reported in multiple cases, haematological and gastrointestinal symptoms along with vitamin B₁₂ deficiency (150pmol/L) are sign of increased risk of neuropathy, demyelination, cognition impairment and dementia.15-17 In a recent research, experts found that sufficient supplies of Docosahexaenoic Acid (DHA) with long-chain omega-3 fatty acid would avert dementia.18 There is another components that is minerals that can attenuate the transmission of nerve stimuli by magnesium and calcium for proper functioning of the nervous system.

Nutrients as antioxidant

Antioxidant can arrest, suppress as well as restore any damages to cells what can happen because of oxidative stress (Figure 1).19,20 The minerals are non-enzymatic antioxidants, they works by promoting the activity of different catalytic enzymes or by repairing the damage, reducing hydro-peroxide, ion sequencing as well as free-radicals scavenging along with the suppression of ROS formation.21 Enzymes that protect from reactive oxygen species damage need amino acids and dietary minerals such as copper, zinc, manganese as cofactors for their synthesis. Fat soluble vitamins such as vitamin A, C and E are active dietary antioxidants present in fruit and vegetables can prevent the cytotoxic effect of free radicals, serve as ROS scavenger and enhance antioxidant activity of enzymes.19

Research Bridging Antioxidants and Mental Health

Some studies reported the convergent proof on the role of different antioxidants in protecting cell membrane from oxidative stress, alteration of mood as well maintenance of mental health. Some of the antioxidants are Vitamin A, C and E, carotenoids, polyphenols, zinc and selenium.20 A recent study showed the link of low carotenoids and depression.21,22 Another randomized controlled trial indicated that
ascorbic acid modulates catecholaminergic activity, releases prolactin and oxytocin which reduces anxiety and stress and is also associated with the sexual behaviour thereby increases intercourse frequency and improves mood.23

**Nutrition and cognition**

Non-invasive pictorial techniques shown that food thought alone can alter neural activity in some specific region of brain, known for taking part in appetite control and contributing to physiologic reactions such as involvement of saliva, gastric acid and insulin secretion.24,25 Polyphenols are abundant antioxidant-derived micronutrients. The most important source of polyphenols is various drinks and fruits such as coffee, tea, cocoa and red wine. They have been proved to shield neurons from neurotoxin-induced injuries, to suppress neuroinflammation, improve memory, learning and cognitive function using neuroprotective activities.26

Flavonoids are the diverse group of polyphenols. The dietary polyphenols have numerous protective effects in the brain which include neuroprotective effect against neurotoxin-induced injury, suppressive effect on neuro-inflammation; it also promotes learning and cognition.27 Long Term potentiation is commonly recognized by the activation of neuronal signal pathway as one of the key mechanisms that play role in memory development, strengthening and storage.28 Some of these pathways consist of protein kinase C, calcium – calmodulin kinase, protein kinase A, phosphatidylinositol-3 kinase /Akt, and protein kinase pathways activated by mitogen. Brain Derived Neutrotropic Factor is one of the neurotrophins that is known to control the growth of synapse, increases synaptic receptor and dendrites spinal density.29 (Figure 2).

Docosahexaenoic Acid (DHA) is a type of omega-3 polyunsaturated fatty acids essentially required for the brain development and neuroplasticity.30,31 Docosahexaenoic Acid exerts its effect by enhancing the function and fluidity of synaptic membrane, mediating cell signalling, regulating gene expression and enhancing long term potentiation.7

**Research Linking Nutrition and Cognitive Functions**

Research on the age related degenerative disorders has shown an association between mitochondrial aging and DNA disruption due to deficiency of micronutrients resulted into higher risk of cognitive impairment and stroke.29,32 The neural signalling pathway of flavonoids and its rich fruits is well known to attune, especially with extracellular receptors, synaptic plasticity. The neuronal signaling pathway, in particular with the extracellular receptor kinases and protein kinase B and Akt pathways, have been well-reported for modulating neuronal signalling pathways that is crucial for inducing synaptic plasticity.33,34 In children with Attention Deficit Hyperactivity Disorder, a randomized controlled analysis of Pycnogenol (containing phenolic acid, catechin, taxifolin and procyanidines) reported improved symptoms along with higher level of glutathione and overall antioxidant effects.35,36 In a past research, schizophrenia patient were given dose of vitamins C and E along with n-3 Polysaturated Fatty Acids, Eicosapentaenoic Acid and Docosahexaenoic Acid for four months has decreased psychopathology in different indicators.37

**EXERCISE, MEMORY AND COGNITION**

Exercise has demonstrated positive effects on cognitive efficiency in healthy individuals. A 12-week longitudinal study found that participants in exercise group had improved memory and cognitive skills compared to control group.38 Research has shown that the regular strength training has a positive effect on cognitive efficiency, acts by increasing synaptic
plasticity in the hippocampus. It has also been reported that in healthy brain, strength training is associated with structural changes in grey matter, white matter and putamen volume.\textsuperscript{39,40} Thus, a combination of moderate aerobic and resistance exercise would provide an ideal benefit to overall cognitive function.

**Benefits of physical activity in neurological condition**

Physical exercise can be beneficial in many ways, such as by maintaining cardiovascular health, by increasing blood supply to the brain, by minimizing inflammation, by reducing stress hormone levels. All of these factors have significant role in cognitive function.

- The Review study indicated that physical activity serve as a prototypic preconditioning stimulus that provides protection to the brain in patients with acute or chronic strokes.\textsuperscript{41,42}

- Exercise significantly improves the emotional and physical health of patients with Alzheimer’s disease.\textsuperscript{43}

- Patients with neurodegenerative conditions indicated that physical activity can improve posture, balance, tremor, mobility, handgrip as well as motor coordination in patients with Parkinson’s disease.\textsuperscript{44,45}

**POTENTIAL MECHANISM OF IMPROVED COGNITION WITH EXERCISE**

Important source of energy for neurons is brain glycogen which is mainly present in astrocytes.\textsuperscript{34} Extensive exercises with hypoglycemia contribute in reduction of brain glycogen. However some studies shown that loss of astrocytes glycogen are overcompensated by brain.\textsuperscript{35} Another research supported the hypothesis that the ability of brain to increase energy metabolism during activation is limited by the glycogen depletion.\textsuperscript{36} It means that the daily exercise would increases the stock of astrocyte glycogen, providing the brain protection from future potential hypoglycemic bouts and thereby enhancing cognition. Exercise is linked to the enhancement of neurotransmitter levels, neurotrophic factors, growth factors and to improve functional connectivity in temporal lobes.\textsuperscript{39} One of these factors Brain Derived Neurotropic Factor is indicated to modify brain mitochondrial respiratory efficiency; but this role tends to be inhibited by the presence of inflammatory cytokines.\textsuperscript{46,47} Brain Derived Neurotropic Factor controls energy homeostasis by regulating eating habits, physical activity and by modulating the peripheral metabolism of glucose.\textsuperscript{48}

Parkinson’s disease is a neurodegenerative disease where person with disease losses dopaminergic neurons and eventually, reduced mitochondrial activity. Exercise helps in elevate the dopamine level of that has many benefits such as increase antioxidant activity, Brain Derived Neurotropic Factor, IGF-1 level (Figure 3).\textsuperscript{48}

PGC-1\(\alpha\) plays important role in promoting beneficial effects of exercise on mental health. It has been reported that PGC-1\(\alpha\) takes part in the process through which exercise induces hippocampal Brain Derived Neurotropic Factor expression.\textsuperscript{50,51} Myokines help in maintaining biological homeostasis along with the metabolism of energy, angiogenesis and myogenesis.\textsuperscript{52,43} Myokines, Interleukin (IL-6), (IL-10), and (IL-1ra) are produced during exercise and attribute to their beneficial effects, studies reported that IL-6 would also have anti-inflammatory effects. Exercise induced IL-6 has been found to suppress the synthesis as well as activity of interleukin -1\(\beta\) and tumor necrosis factor-\(\alpha\).\textsuperscript{44} Animal study showed that the hippocampal TNF-\(\alpha\) (Tumour Necrosis Factor) level was considerably lower and IL-6, IL-1ra, and IL-12 were found to be higher in hippocampus among the exercise group when compared with control group.\textsuperscript{53}
SYNERGISTIC ROLE OF DIET AND EXERCISE

The diet and exercise became a part of the continuum with which the environment played a crucial role for millions of years in the development of human brain. Studies demonstrated that exercise acts as a supplement to a DHA-rich diet to affect cognitive function-based molecular systems. Exercise exerts its positive effects by repairing of the membrane homeostasis after brain injury, which is essentially needed to promote neuroplasticity and cognitive functions. The cumulative effect of a food rich in polyphenols along with exercise encourages the development of genes which usually benefit synaptic plasticity as well as mental wellbeing, at the same time reduces the growth of genes that are involved in adverse processes such as inflammation and cell mortality. (Figure 4)

![Image of healthy diet and brain benefits](image1)

Figure I Effect of healthy food on brain: Nutrients rich diet helps the brain to increase brain agility and vitality, enhance energy metabolism and neuronal survival. This reduces the oxidative stress, neuroinflammation and neurodegeneration in brain. (19)

![Image of Flavanoids mechanisms](image2)

Figure II Mechanism of action of Flavanoids: Flavanoids activates and inhibits several signalling pathways in brain. Flavanoids improves the oxygen delivery, increases synaptic plasticity, neuronal communication and memory through the activation of extracellular receptor kinase (ERK), Akt and protein kinase A (PKA). They inhibit the JNK, ASK and p38 involved signalling pathways. This reduces the neuroinflammation, apoptosis, neurodegeneration and brain ageing. (28)
Figure III Exercise balances the hormonal level in body: alteration of hormones in different regions of brain is linked to Parkinson disease, Alzhiemer disease. Dopamine (happy hormone) helps in alertness, motivation, clarity, pleasure and working memory. Serotonin hormone helps in relaxation, contentment, positivity. Norepinephrine promotes vigilance, concentration, energy level.\(^{(48)}\)

Figure IV Impact of Exercise on Brain function: Exercise increases motor control and improves cognitive and performance and learning power.

Conclusion

The present study summarizes multidimensional evidences indicating the role and functions of various nutrients in the healthy functioning of brain and cognition. Modification of the lifestyle with regular exercise and nutritious diet appears to advance brain functioning by activating specific cortical areas, stimulating the release of neurotransmitters, and promoting neural activity that is crucial for cognitive and emotional well-being, thereby stopping the progression of neurocognitive disorders. The causes behind the neurological disorders are described by different elements and cannot be related to the one source thus combined effects of nutrition and exercise may be used as a therapeutic approach for targeting
cognitive and neurological disorders. This means that nutritional therapy in combination with regular physical exercise can be a reliable, effective and economical alternative that can intensify the effects of standard drug therapy. Even though various studies have reported the beneficial effects of nutrients alone or exercise alone only very few researches exploring there synergistic role and mechanism. There is need for more human studies for in depth understanding of synergistic properties of different nutrients and physical activities at biochemical, physiological or psychological levels.

Conflicts of Interest

All the authors declare that no personal interest was involved in the course of study/there is no conflict of interest

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Ethical Clearance: Department of Bio-Sciences, School of Basic and Applied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India.

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The Flipped Teaching of Middle School Students from the Viewpoint of PE Teachers

Hussein Farhan Sheikh Ali

Associate Professor Misan Education Directorate Department, Ministry of Education, Iraq

Abstract

The research aims to the extent of the possibility of using inverted (flipped) teaching in the physical education lesson in the manner of video or via e-mail. Learning platforms after the process of reducing the official time for middle school students from the point of view of physical education teachers. Scientific research was limited to physical education teachers in the Directorate of Public Education in Misan Governorate for the academic year 20-20-20 21, the research sample consisted of a college (100) A teacher in secondary education, the researcher adopted an initial exploratory questionnaire to determine the extent of inversion of the teaching of T. And the evaluation, according to its stability by the method of re-testing, as it reached a degree of stability (0.83) on the basic sample consisting of (60) teachers and schools, and after processing the data statistically, the results of the research indicated that there are a number of reasons leading to the possibility of using (reverse education) in education Sports. The lesson and in light of the results, the researcher presented a set of conclusions and recommendations.

Keywords- Flipped Teaching, Middle School Students, PE Teachers

Introduction and Importance of Research

According to Beda Jogia’s educational competencies the state of the educational curricula cannot be preserved for years without changing or modernizing the educational process in Iraq and the attempts of educational movements by experts and thinkers in the field of education by providing insights about the possibility of high accuracy of Iraqi education. Even his transition from education focuses on the culture of memorization and repetition and a return to digital (electronic) education in line with developments in the educational field that explode human and creative energies, freedom and including flipped education and its teaching. Integration into our curricula, including the preparatory school curriculum in physical education, in line with this change with the current situation the world is going through in terms of difficulties, disasters and diseases, by introducing and spreading a new culture equipped with educational technologies outside the school walls. The importance of the research is evident: by presenting an identity represented in the method of reverse teaching, training teachers to use it, encouraging them to open up to different cultures, and the extent to which these technologies are used in schools and used as a tool. For intermediate studies by supporting freedom of thought and creativity and activating the culture of citizenship that works to achieve national and social cohesion 1.

Research Problem

Unfortunately, we do not have any developmental plans to change the student’s path towards using his creative powers to build life and embrace the future. Our educational and educational institutions are still keen on the culture of silence, memory and exclusivity without qualitative transformation and raising the student’s awareness and maturity at the
level of thought, behavior and vision of life, and there is no doubt that knowing the causes of weakness and weakness. Lack of curricula or study materials is the way to suggest solutions. When we say: The prevailing paper-based curricula are incompatible with our current era, the era of information flow, how can students absorb this vast amount of knowledge resources? The research problem is illustrated by the introduction of (reverse) electronic teaching in the physical education curricula for the preparatory stage through electronic platforms and submitting it to middle schools. You will witness a digital shift in our curricula and move away from rigid ones that are not up to date\(^2\). The researcher formulated the study problem and defined it with the following question: Is it possible to introduce the flipped teaching into the physical education curricula for the middle stage from the point of view of physical education teachers in the center of Maysan governorate?

**Research aims**

1. Preparing a questionnaire form for physical education teachers about the introduction of flipped teaching in the physical education curricula for the middle stage.

2. Knowing the reverse teaching method for middle school students from the teachers’ point of view

**Limit search**

Through this research and physical education, the separation of teachers in preparatory schools at the General Administration of Education in Misan has been determined for the academic year for the period from 11/29/2020 1/7/2021 instead of the specialist’s supervision.

**Defining terms**

- Teaching is upside down: Defined it as a digital educational model that aims to use modern technologies and the Internet in a way that allows the teacher to prepare the lesson through videos or platforms\(^3\).

- Intermediate stage: well-known\(^4\).

- This stage, which aims to help students, acquire concepts and form relationships with others, takes the age stage for students to stay in or to be deported.

- Procedural definition of the intermediate stage: It is the second stage after the primary stage at the age of (13) years, we start with the first intermediate grade and end with the third intermediate grade, after which the student moves to the preparatory stage.

**Research procedures**

To achieve the research objectives, the researcher undertook the following procedures

**First: Defining the research community**

Including teachers of the research community assigned to teach physical education at the middle stage in the schools of the General Directorate of Education in Misan Governorate 20 years 20 - 2021 School.

**Second: The research sample**

The sample is that part of the community chosen according to scientific rules and methods, which is a research that represents the community within O and because of the difficulty of conducting research on all elements of the original community. The research tends to choose a molecular group that represents the best representation of the elements of society, so that it is able to generalize its results to the study population\(^5\).

Where this sample was described as follows

- The Exploratory Sample: This sample was
chosen randomly from the original community of (40) teachers and schools, and directed to them for an open questionnaire that includes the following question:

- Is it possible, in your opinion, to include flipped online teaching instead of traditional teaching in the middle school physical education lesson? «

The original sample: the number of individuals reached (60) teachers and schools that were presented to them.

**Third: Research Tool (Questionnaire)**

Questionnaire: one of the information gathering tools through which the opinions of teachers and parents are determined.

**Fourth: The validity of the tool**

It is that the scale, questionnaire, or test measures what has been confirmed. If the tool measures any behavior or trait other than the one it was prepared to measure, then it is in this case a dishonest tool.

The researcher relied on the opinions of a group of experts in the field of teaching methods in physical education, measurement and evaluation to explore their views on the validity of the paragraphs of the closed questionnaire in measuring the content that was prepared. In light of the experts’ observations and suggestions, the researcher modified the wording of some paragraphs without deleting any paragraphs. If all paragraphs obtain approval of (85%) from expert opinions.

**Fifth: the stability of the tool**

Stability means the consistency of the tool product with itself, if it is applied again or multiple times to the same individuals in this way (retest - test (one of the best methods used to find the stability of the instrument (accuracy) in this type of research is to find the correlation coefficient) that you use to recognize perseverance.

The closed questionnaire was distributed to a sample from outside the main research sample of (30) teachers, and after (15) days is the best period of time to use this method, as shown where the questionnaire was distributed again to the same sample, and after performing the statistical operations, it was found that the stability factor of the resolution was (0.83), which is a good stability factor for the tool.

**Sixth: Application of the tool**

The researcher applied the final questionnaire (0 closed) to the basic sample on 3/1/2/2020. The researcher explained how to answer the regular paragraphs, a tool for them, and after completing a closed application. Questionnaire: The researcher examined the questionnaires and then completed the answers of the sample members in special forms prepared for this purpose and on which statistical operations were performed.

**Seventh: Statistical Means**

- correlation coefficient (Pearson)
- Percentage: used to convert the frequencies in each paragraph of the questionnaire
- Weighted position
- The researcher extracted the hypothesis with which the weighted average is compared through the questionnaire scale so that it is \( (1 + 2 + 3) \div 3 = 2 \)

**Presentation and discussion of the results**

Presentation of Results: This chapter includes the results of the researcher in light of the objectives of the research by revealing the extent to which reverse teaching is employed in the physical education lesson for the preparatory stage. The repeated answers of physical education teachers were counted in the closed paragraphs of the questionnaire. Then calculate the weighted average for each paragraph and the percentage of its weight, then the paragraphs are
arranged in descending order from the highest severity to the least serious, and in the following the results are presented.

Table 1: Yes, employment in the middle stage of the physical education lesson is reversed from the teachers’ point of view, in descending order, in terms of percentage and percentage weighted

<table>
<thead>
<tr>
<th>No</th>
<th>Paragraph</th>
<th>Paragraphs by rank</th>
<th>the middle Likely</th>
<th>The ratio Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>The flipped teaching is in line with developments in the educational field</td>
<td>91/2</td>
<td>97%</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>The teaching of flipped (electronic) differs from the traditional teaching in the mathematical education curriculum</td>
<td>87/2</td>
<td>66/95%</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>The flipped teaching covers many aspects of the intermediate stage satisfactory education curriculum</td>
<td>86/2</td>
<td>33/95%</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>Teaching flipped contributes to the investment of time and effort in the lesson of mathematical education</td>
<td>84/2</td>
<td>66/94%</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>The shift from traditional teaching to electronic teaching according to the inverse method helps in developing the mathematical education curriculum for the intermediate stage</td>
<td>81/2</td>
<td>66/93%</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>Losing evidence about reverse teaching faces great difficulties in bringing out the mathematical education lesson</td>
<td>80/2</td>
<td>33/93%</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>The teacher of sports education, according to the reverse teaching, can present new evaluation methods</td>
<td>79/2</td>
<td>93%</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>The flipped teaching in a sports education lesson helps the sports education teacher look to broad prospects in this field</td>
<td>75/2</td>
<td>66/91%</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>The flipped teaching allows the teacher of mathematics education to have sufficient familiarity with electronic techniques and skills</td>
<td>71/2</td>
<td>33/90%</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>There is an interest in setting educational and behavioral goals during the teaching flipped in the mathematics education lesson</td>
<td>70/2</td>
<td>90%</td>
</tr>
</tbody>
</table>
Discussing the Results

1. Table No. (1) includes a group of (30) paragraphs that represent the inverted teaching of middle school students from the viewpoint of physical education teachers. The severity of the vertebrae (weighted average) ranged between (2.91 - 1.69) and weight between (97% - 55.33%).

2. The results showed the sequence of Paragraph (3) (that the Ministry of Education can provide Internet system services to all schools) It ranked first among the negative vertebrae with a weighted average (1.66) and a weight ratio (55.33%). We also find that the state has withdrawn from driving the wheel of development in society due to unstable conditions, including the educational field. There is an absence of a vision and a political project that adopts education issues in Iraq. Perhaps the budget allocated to education in Iraq reveals the state’s lack of seriousness in providing Internet services and electronic devices to all schools, the problem of traditional culture that calls for preservation, hadiths and imitation, and the absence of a culture of criticism, creativity, dialogue and tolerance.

3. The sequence of paragraph (23) (increasing interaction between students while taking physical education lessons according to the teaching inverse) with a central weighted rate of (1.71) and weight ratio (57%) ranked second in the negative paragraphs, with the aim of starting. If the interaction prevails in the discussion room and the exchange of views between the teacher and the student and among the students themselves, we find here in the lesson outcomes through reverse teaching, the interaction between them will decrease.

4. The sequence of paragraph (5) (most physical education teachers have visions and experiences in teaching upside down) with a weighted average (1.82) (60.66%) came in third place of the negative paragraphs that do not match their responses. Physical education teachers. Where we see: Physical education teachers still have a blurry vision about inverted teaching, where the problem of stagnating the entire educational system in terms of educational content and teaching methods in Middle Eastern schools. In addition, there is teacher’s Physical education. They need skills and experience in educational technology, including flipped teaching, especially those who did not touch on online programs.

5. The sequence of paragraph (19) (there are workshops and training courses to confront teaching inverted in physical education curricula) with a weighted average (1.85) and relative weight (61.66) came fourth in the negative paragraphs. Since we do not find any kind of courses offered by the Preparation and Training Department, they are trained to face reverse electronic teaching in the physical education curricula and go that what any society goes through and social transformations require a change. The educational institution is just a cup of these shifts and changes in readiness to educate of electronic politics.

6. The paragraph came in its sequence (12) (Teaching flipped in the physical education lesson achieves creativity in educational curricula) with a weighted average (1.87) and a relative weight (62.33%) within the negative paragraphs that are not. Consistent with the responses of physical education teachers. As says: Education, in its current state in third world societies in general, allows generations of less skill, experience and knowledge, and contributes to the formation of traditional non-creative minds that are distinguished through a molecular point of view and they lack a holistic vision and thus do not possess awareness of the spirit Era and its requirements.

7. While the sequence of paragraph (21) (dispensing with physical education courses in school yards and moving towards electronic teaching) came sixth in the negative paragraphs with a weighted
average (1.89) and a weight ratio (63%) we see that the physical education lesson is a skill and an educational lesson, so it is necessary to teach directly in the boxes because it is a lesson that deals with students and corrects the wrong conditions and touches the body of the learner about how to perform the right and the right conditions, because he needs to correct the body from the wrong habits.\(^{16}\)

### Conclusions and Recommendations

#### Conclusions

The following can be concluded

- Teaching flipped (electronic) needs attention from specialists in teaching it, as well as providing supplies and internet networks for all schools.

- There are some physical education teachers who are averse to teaching flipped through electronic platforms due to their lack of experience in this field.

- There are several reasons that affect the introduction of flipped teaching in the physical education curriculum.

#### Recommendations

- Through the findings of this research, the researcher recommends the following

- The introduction of educational techniques, including the flipped teaching in the teaching of physical education.

- The diversity between online platforms and official teaching in physical education curricula.

- Physical education teachers include developmental courses in electronic technologies; in addition to that, the teacher must be an artist in communicating the course material to students.

### Ethical Clearance

People identified as potential research participants because of their status as relatives or carers of patient’s research participants by virtue of their professional role in the university and departments.

**Source of Funding:** Self-Funding

**Conflict of Interests:** The authors declare there is no conflict interests.

### References


Building and Standardizing the Location of Perceived Control of Young Footballers

Mohammed Fadhil Anber¹, Mustafa Abdulzahra Abbood², Ali Mutair Humedi³

¹Researcher, ²Lecturer, ³Prof. College of Physical Education and Sports Sciences, Misan University, Iraq

Abstract

The research aims to build and legalize the site of perceived control of young football players. The areas of research included the human field that represents football players’ clubs in Misan Youth Governorate, and defining the time zone for a period of (1/12/2020) until (10/5/2021), while the spatial domain was in the halls of the studied clubs.

The researchers used God C in a descriptive survey method, and the community research is (239) players from Misan Governorate youth football clubs, and the (SPSS) system was used to obtain the results of the research, the researchers concluded the following:

1- A tool was developed for measuring the position of perceived control in its three dimensions for young soccer players.

2- The perceptual control site scale that the researcher built and codified through the applied scientific procedures demonstrated the ability of this scale to recognize the individual differences between the players in the perceptual control site scale and its three dimensions.

3- Young players in Misan governorate have an average level in the perceptual control position variable.

The researchers recommended the following:

ü Adopting the scale as a tool to measure the psychological variable (location of perceived control in its three dimensions) for young footballers.

ü The perceptual control position measure can be used by coaches and the need to use it on players periodically during the sports season, which allows identifying this important psychological variable in competition as well as identifying the positive things that support it and the negatives to overcome and develop them with the players.

ü Conducting other similar studies according to the variables (gender, age, different sporting events).

Keywords: Young Footballers, Psychological Variable, Perceived Control Site

Introduction

The development witnessed in the world of scientific prospects and a huge information revolution that pushed people to search for new ideas, as the success of any work must depend on a set of appropriate rules and principles that depend on correct and accurate foundations and standards in the sports field in a manner commensurate with the type of sports activity. Good sports results are the result of the harmony of physical, skill, planning and psychological abilities, and achievement can only be achieved if all of the above are developed.

It is a football game that captures the interest of the people of the world at different ages, including
young people, youth and advanced, in an exciting and enjoyable way that is characterized by the games, and the world is competing with each other to determine even tournaments at various continental and international levels because football is the popular game. The first in the world, so it is receiving great interest from governments, agencies and institutions, and researchers are racing to present literature and research on this game. Everyone knows the importance of sports psychology in the process of preparation and access to achievement, which has become the mainstay in changing the results from negative to positive; Among those things that a coach should look at is the player’s control and control over the situations he is going through, and his close association with many positive outcomes such as mental health, optimism, achievement, self-esteem and adaptation.

Hence the importance of research in identifying the levels of players in this important psychological variable because it distinguishes the players among them, in addition to that it reflects the personal side of the players towards the team as a whole, which contributes to raising the level of the players. The team, because football is a team sport that needs solidarity and proper control in order for one team to reach the desired goals, as well as to be a scientific guide for the players and coaches to benefit.

Research Problem

Through the researchers’ review of many messages and theses, it was found that there is a lack of studies and research that dealt with this important psychological variable, which is the (perceived control site). The Perceived Control Site the researchers decided to study this problem by constructing and standardizing the scale and identifying the level of players in this important psychological variable; As well as to be a scientific guide to supplement the libraries and show the situation reached by the players skillfully and psychologically by extracting grades and standard levels and comparing his degree with his peers from the players and knowing his level in the future.

Research Objectives

1. Building the Perceptual Control Site Scale for Young Footballers in Misan Governorate.
2. Creating benchmark scores and levels for the Perceptual Control Position Scale for Young Footballers.
3. Recognizing the level of players in the scale of the perceived control position and its three dimensions for young football players.

Research areas

1. The human field: a player and clubs in Misan governorate in youth football
2. Spatial field: the halls of the clubs discussed
3. Field Temporal: from 1/12/2020 to (10/05/2021).

Research Methodology

The researchers used the descriptive approach in the survey approach, due to its relevance to the nature of the problem.

Community and Sample Research: All individuals, objects, or persons are the subject of the research problem, and all the elements related to the study problem are the ones that researchers seek to generalize the results of the study on (1).

The research community consisted of (239) players distributed from (12) clubs registered in the lists of the sub-federation of Misan football governorate. As for the research sample, it amounted to (200) players and they were published as follows:
A survey experience sample (15) players is higher by (6.28%), and the building of the sample included 130 players and a percentage (54.39%) of the research community, and the application of the sample included 70 players and by (29.29%) from the research community, (24) Players were eliminated with (10.04%) 2.

Means, equipment and tools used: Arab and foreign references and sources, personal interviews with experts and specialists, a personal computer (laptop) of the type (Compaq 6101), a portable electronic calculator, ballpoint pens.

Field research procedures:

Basic steps for building the scale (1):

The steps that can be followed when building a test or questionnaire are subject to many scientific steps, the most important of which are:

The purpose of building the scale: The first step to building the scale is to clearly define what purpose it is built and what it is needed for, and one of the goals of building the scale is to define the level of perceived control location of young footballers.

Determine the phenomenon to be studied: The phenomenon to be measured must be defined and its concept and limits be completely clear, and the phenomenon that the research aims to determine the location of control in the perceived range of young football players The concept of researchers for the location of perceived control of some of its dimensions (behavioral control, cognitive control, decision-making control decision) 3.

Determine the dimensions of the perceived control site scale:

See through the literature and resources on sports psychology knowledge and resources related to the specific research problem, researchers are controlling the behavioral personality theory that includes three dimensions of which he proposed (Skinner 1996), a (behavioral control, cognitive control, control over decision making) 4.

Scale dimensions validity: The dimensions of the perceived balance control site on persons with experience and competence within the competence of knowledge of sports psychology, testing and measurement and its dimensions Statement of validity, modification or suggestion of other dimensions and making any observations, all experts agree on the validity of the dimensions and with a percentage (100%) 5.

Determine the style and principles of drafting paragraphs.

The researchers adopted Likert’s method (Likert in correction of paragraphs and this method is one of the best methods of predicting behavior or phenomenon, and by reviewing the literature on the nature of the scale construction and how to formulate paragraphs and benefit from interviews with experts and specialists. Since (64) paragraphs were formulated distributed into (3). The dimensions of my devices (behavioral control (24 items), cognitive control (21 items), decision-making control (19 items) 6.

Scale Paragraphs Validity:

After preparing the scale in its initial form, which contained (64) items distributed in three dimensions, the researchers did the following:

The scale was presented in its initial form to a group of experienced and competent judges in the fields of mathematical and psychological sciences, testing and measurement. Next, the researchers analyzed the scale scores using percentage as a criterion for accepting or excluding scale elements 7. Agreed upon clauses are accepted (75%) or more of the arbitrators’ claim that they are correct and appropriate to the scale, and
(Bloom) notes, “Researchers must obtain agreement (75%) or more of the arbitrators’ opinions regarding this type of truthfulness (2), as well as Deleting the paragraphs in which the degree of (Ca₂) is calculated less than the order of the degree (Ca₂) arranged at the level (0.05) and at the temperature of freedom (1) equal to (3.84), and Table No. (1) Illustrates that ⁸.

Table (1) shows the percentage and score of Ca 2 experts’ responses in each scale paragraph

<table>
<thead>
<tr>
<th>Sig</th>
<th>Ca value²</th>
<th>The paragraphs</th>
<th>Inconsistent</th>
<th>agree</th>
<th>percentage</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>21</td>
<td>1,2,6,18</td>
<td>0</td>
<td>21</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>0.00</td>
<td>17.19</td>
<td>3,5,8,16</td>
<td>1</td>
<td>20</td>
<td>95.24%</td>
<td>2</td>
</tr>
<tr>
<td>0.00</td>
<td>13.76</td>
<td>13,14,15,20</td>
<td>2</td>
<td>19</td>
<td>90.48%</td>
<td>3</td>
</tr>
<tr>
<td>0.00</td>
<td>10.71</td>
<td>4,11,19,22</td>
<td>3</td>
<td>18</td>
<td>85.72%</td>
<td>4</td>
</tr>
<tr>
<td>0.00</td>
<td>8.04</td>
<td>7,10,23</td>
<td>4</td>
<td>17</td>
<td>81.00%</td>
<td>5</td>
</tr>
<tr>
<td>0.12</td>
<td>2.33</td>
<td>9,12,17,21,24</td>
<td>7</td>
<td>14</td>
<td>67.00%</td>
<td>6</td>
</tr>
</tbody>
</table>

Cognitive control

<table>
<thead>
<tr>
<th>Sig</th>
<th>Ca 2</th>
<th>The paragraphs</th>
<th>Inconsistent</th>
<th>agree</th>
<th>percentage</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>21</td>
<td>4,6</td>
<td>0</td>
<td>21</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>0.00</td>
<td>17.19</td>
<td>18,20</td>
<td>1</td>
<td>20</td>
<td>95.24%</td>
<td>2</td>
</tr>
<tr>
<td>0.00</td>
<td>13.76</td>
<td>1,3,7,14,16</td>
<td>2</td>
<td>19</td>
<td>90.48%</td>
<td>3</td>
</tr>
<tr>
<td>0.00</td>
<td>10.71</td>
<td>5,8,11,12,17,19,21</td>
<td>3</td>
<td>18</td>
<td>85.72%</td>
<td>4</td>
</tr>
<tr>
<td>0.00</td>
<td>8.04</td>
<td>9,10</td>
<td>4</td>
<td>17</td>
<td>81.00%</td>
<td>5</td>
</tr>
<tr>
<td>0.27</td>
<td>1.19</td>
<td>2,13,15</td>
<td>8</td>
<td>13</td>
<td>62%</td>
<td>6</td>
</tr>
</tbody>
</table>

Control of decision making

<table>
<thead>
<tr>
<th>sig</th>
<th>Ca²</th>
<th>The paragraphs</th>
<th>Inconsistent</th>
<th>Agreed and n</th>
<th>percentage</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>21</td>
<td>1,9</td>
<td>0</td>
<td>21</td>
<td>100%</td>
<td>1</td>
</tr>
<tr>
<td>0.00</td>
<td>17.19</td>
<td>15</td>
<td>1</td>
<td>20</td>
<td>95.24%</td>
<td>2</td>
</tr>
<tr>
<td>0.00</td>
<td>13.76</td>
<td>3,10,11,14,16,19</td>
<td>2</td>
<td>19</td>
<td>90.48%</td>
<td>3</td>
</tr>
<tr>
<td>0.00</td>
<td>10.71</td>
<td>2,4,13,17</td>
<td>3</td>
<td>18</td>
<td>85.72%</td>
<td>4</td>
</tr>
<tr>
<td>0.00</td>
<td>8.04</td>
<td>5,6,12,18</td>
<td>4</td>
<td>17</td>
<td>81.00%</td>
<td>5</td>
</tr>
<tr>
<td>0.01</td>
<td>5.76</td>
<td>7,8</td>
<td>5</td>
<td>16</td>
<td>77%</td>
<td>6</td>
</tr>
</tbody>
</table>

Significant at significance level ≤ 0.05
And after deleting (8) Paragraphs that were not agreed upon by experts and specialists, the number of paragraphs has become) 56 A paragraph distributed on the scale dimensions, as shown in Table (2) ⁹.

Table (2) It shows the number of paragraphs of the scale in its initial form before and after it was presented to experts and specialists, distributed over the dimensions

<table>
<thead>
<tr>
<th>Number of Scale Paragraphs After Deletion</th>
<th>Sequence of Deleted Paragraphs</th>
<th>The Number of Paragraphs Deleted</th>
<th>The Number of Paragraphs of The Scale In Its Initial Form</th>
<th>The Dimension</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 paragraphs</td>
<td>9,12,17,21,24</td>
<td>5</td>
<td>24 a paragraph</td>
<td>behavioral control</td>
<td>1</td>
</tr>
<tr>
<td>18 paragraphs</td>
<td>2,13,15</td>
<td>3</td>
<td>21 paragraphs</td>
<td>Cognitive control</td>
<td>2</td>
</tr>
<tr>
<td>19 paragraphs</td>
<td>Not found</td>
<td>0</td>
<td>19 paragraphs</td>
<td>Control of decision making</td>
<td>3</td>
</tr>
<tr>
<td>56 paragraphs</td>
<td>_____</td>
<td>8</td>
<td>64 paragraphs</td>
<td>Total</td>
<td></td>
</tr>
</tbody>
</table>

Choose the scale rating:

Appropriate grading of the scale, a group of arbitrators with experience and competence in the field of sports psychology, testing and measurement, was handed to a statement behind them in the grading scale. The arbitrators on the proposed scale agreed a rating of 100%.

Correction method for scale paragraphs:

For the purpose of obtaining the total score for each individual of the sample, appropriate weights are given to replace the scale paragraphs, and clarify the importance of the paragraphs in a gradual manner, and by collecting the respondent’s scores on the five-point classification, we obtain the total score for each individual ¹⁰, and since the scale paragraphs are formulated in the negative and positive direction. Paragraph weights were determined from (1-5) degrees for each positive paragraph and (1-5) for negative paragraphs, and Table No. (3) Shows this.

Table No. (3) Shows the method for correcting scale expressions

<table>
<thead>
<tr>
<th>Never apply</th>
<th>They rarely apply</th>
<th>Applies sometimes</th>
<th>Often applicable</th>
<th>It always applies</th>
<th>Phrase type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>positive</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Preparation counter instructions

After the completion of the readiness to apply the scale as the first instructions for the history of business and how to respond to its paragraphs, the instructions specified that the answer to the sample intended to be used for the purpose of scientific research only will not be seen by one of the researchers, and the sample members were asked to accurately answer all the paragraphs in order to reach objective and fruitful results.

Experimental experience:

After the scale was ready for application, the researchers conducted an exploratory experiment before the final application of the research at an appropriate time, by applying it to a sample of (15) players for the purpose of finding the reasons for success when applying the main test sample and making sure that you understood the sample paragraphs of the scale, in order to avoid any errors or difficulties when applying during the main research test, researchers have done several things, namely:

1. Clarify the answer method for the individuals of the sample.

2. Knowing the difficulties faced by researchers and the assisting work team.

3. It will be a clear picture for the researcher that the assistant work team for the answers to the paragraphs of the scale.

The researchers found appropriate paragraphs, and the average time spent on answering ranged between (18-25 minutes).

Key Experience:

In conducting the main experiment, the researchers aimed to build a measure of the perceived control position in its final form on the research community, and after collecting the samples, the results were statistically analyzed to find the discriminatory strength of each paragraph in order to exclude and delete the unmarked paragraphs and find the correlation coefficient for the paragraphs, and to achieve this, the researchers applied the scale on the building sample numbered (130) players.

Statistical Analysis of Paragraphs:

1. The use of appropriate statistical methods is largely determined by the method of research design and the type of data to be collected. So:

2. Discrimination Indications:

3. The two final groups (discriminatory power):

4. It is intended to test the ability to distinguish between individuals who have a degree of personality or trait, and between individuals who have a low degree of capacity or disassociation or trait (1).

The discriminatory power is revealed by knowing the total degree of the response of the sample members, and then the models are arranged in descending order, after which two circumferential groups are selected at 27% of the total sample that was measured, a high group represented by the individuals with the highest scores, and a lower group represented by the individuals. Those with the highest score Lowest score. Then the T-Test for two independent samples is applied to determine the statistical significance of the difference between the two means - Ali from the minimum standard items promised with the value (T) calculated and evaluated) indicating the validity of paragraph (3) where the number of requests with higher scores reached (35) forms and the minimum The number of approved forms becomes (70). The W-shape from the night results shows that all the scale paragraphs are distinguished by using the Statistical Bag for the Social Sciences (SPSS), and it was found that all the scale paragraphs are distinct.
Whereas, the level of statistical significance was less than (0.05), which means that there are statistically significant differences between the upper and lower groups, and therefore all these items were approved, thus the number of scale items became (56) items.

**Internal consistency of paragraphs:**

This validity is formed by preparing a test consisting of a number of dimensions to measure a phenomenon, and the sum of the scores of these dimensions is the total score of the test, and to calculate the internal consistency of this test, the Pearson correlation coefficient for this purpose, by finding the correlation between the scores of each paragraph. The overall score of the scale, based on the responses of the research sample consisting of (130) players.

Paragraph relationship from undergraduate degree to scale:

I use the (Pearson) Bo S. A Plan pouch Statistical (SPSS) correlation coefficient between the scale items and the scale’s overall score, and it was found that all scale readings have a high direct correlation.

**Stability**

Reliability means “the extent of the apparent accuracy of the test in measuring the subject matter (1)”. The test results are consistent in preparing and approving the results of the tests. There are several methods for extracting the stability parameter, and the researchers have chosen, including:

**Alpha Cronbach method:**

To extract stability in this way, the (Alpha Cronbach) equation was applied to the scores of the sample members and their number (130) players. The value of the scale stability coefficient was (0.980), which is an indication that the test reliability coefficient is very high, and the closer the test reliability coefficient is to one. Indicate the correct one through the strength and firmness of the test (2).

**Half-split method:**

The split in half method is one of the most widely used persistence methods in paper and pencil tests. In this method, it is possible to obtain two scores for each individual by dividing the test into two halves, so that the first half includes odd numbers, and the second half includes even numbers, and accordingly we obtain two scores for each individual and the relationship between these two grades (scores of the two halves of the test) is considered as internal consistency. For the half of the test only and not for the test as a whole (3), since the Pearson correlation coefficient was (0.822) and in order to obtain the value of the reliability coefficient for the entire scale, the researchers used the Spearman - Brown equation, using the statistical bag (SPSS), and its value reached (0.902), which is Indicator of the gauge’s stability.

**Objectivity:**

The researchers consider that clear instructions and a model for how to answer, as well as clarity of statements, ease of interpretation, multiple answer alternatives, and the exclusion of questionnaires in which the answer is repeated for the same paragraph or where the answer is not completed in all paragraphs, all this makes answering the paragraphs of the scale a goal.
The scale in its final form:

Sample scale completed by Perceptual Control site

<table>
<thead>
<tr>
<th>Start</th>
<th>Scarcely</th>
<th>Sometimes</th>
<th>frequently</th>
<th>Always</th>
<th>The paragraphs</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>I think the success of my team depends on the effort I put in</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Strength and will make me face difficult situations in the match</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>I can avoid the problems that occur during the match</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Close people don’t influence my personal decisions</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>When I fail to perform a certain duty, I am not disappointed</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>If I make good use of my capabilities, I will reach a high level of performance</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>I feel that I have an experience that distinguishes me from others</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Setting a schedule to complete my tasks makes me complete them to the fullest</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>I aspire to be the best player physically and skillfully</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hard work is what sets me apart from my peers</td>
<td>10</td>
</tr>
</tbody>
</table>

**Statistical means**

The researchers used the following statistical systems: (Microsoft Excel) system, which is to download and separate data and calculate the modified standard Z and T scores, and the ready-made statistical package (SPSS. Ver 21):

**Presentation, analysis and discussion of results:**

Present site-controlled metric results, analysis, and discussion:

After completing the construction and standardization of the perceptual control site scale for young soccer players, the researcher applied the scale on the application sample consisting of (70) players representing three clubs (Naft Misan, Al Ahrar and Tigris) and then emptied the scale. Data for the purpose of statistical treatment and produced. So the tables appear later.

**Table No. (6) Shows the default average, the arithmetic mean, the standard deviation, the value of (t Hinted T ratio) and the value (sig) of the perceived control scale.**

<table>
<thead>
<tr>
<th>sig</th>
<th>d1 calculated</th>
<th>standard deviation</th>
<th>Arithmetic mean</th>
<th>Hypoththesized mean</th>
<th>Number of scale paragraphs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>6.249</td>
<td>26.35</td>
<td>187.67</td>
<td>168</td>
<td>56</td>
</tr>
</tbody>
</table>
Table (7) raw degrees, levels, number and percentage of the application sample for the scale (site of perceived control)

<table>
<thead>
<tr>
<th>percentage</th>
<th>the number</th>
<th>Raw grade</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>0</td>
<td>235.24-280.04</td>
<td>Too high</td>
</tr>
<tr>
<td>58.58%</td>
<td>41</td>
<td>190.43-235.23</td>
<td>high</td>
</tr>
<tr>
<td>32.85%</td>
<td>23</td>
<td>145.62-190.42</td>
<td>Average</td>
</tr>
<tr>
<td>8.57%</td>
<td>6</td>
<td>100.81-145.61</td>
<td>Low</td>
</tr>
<tr>
<td>0%</td>
<td>0</td>
<td>56-100.8</td>
<td>Weak</td>
</tr>
</tbody>
</table>

**Discussion of the Site Perceptual Control Scale:**

It is clear to us from Table No. (6) that the arithmetic mean of the research sample reached (187.67), which is at the average level, and as shown in Table No. (7), which is greater than the default average of (168), while the value of (t) is calculated (6.249) and at the level of significance 0.00), which is less than the level of significance (0.05). This indicates the existence of statistically significant differences between the two arguments and the arithmetic introduction in favor of the arithmetic mean of the research sample.

The researcher attributes these differences to the youth players’ ability to control their behavioral and cognitive behavior and even decision-making, since the youth stage is an important stage of life, and they transform during periods of time from one category to another. This puts them up to greater challenges to grasp as they go along. With them time was more mature and conscious; Despite this, they need to think of new ways and methods of building their futures, which make them more knowledgeable and knowledgeable about their life. The perceived control in this stage (the stage of youth) is of great importance through the regulation of the situations and duties of daily life, and it is one of the characteristics that distinguish the owners of organized thinking, as well as their ability in how to shape their future. The goals and setting the appropriate means to achieve them that make them able to face the difficulties or tasks that need to be managed in a distinct and effective manner, how to keep up with that and take the appropriate decision, and draw a picture of the required duty according to their abilities and capabilities in order to determine adaptation to the current situation by changing themselves. Or they change the environment around them for that.

Young people at this stage are more aware and aware of their capabilities and ideas to achieve the goals they aspire to achieve, and they have the ability to control and perceive life and its conditions by developing their ideas, beliefs and abilities to achieve positive results in order to realize the multiple options available to them and evaluate their potential and their ability to draw a vision of what is possible and this He agrees with the study which asserts that “young people have the ability to control their actions and choose what is best for them and feel responsible for what they adds, however, that perceived control is a relatively accurate assessment of their ability the individual’s actual control over life conditions and response to life events.
Conclusions

1- It was found to build a tool for measuring perceived locus of control in its three dimensions for young footballers.

2- It was reached to extract scores and record levels for the three-dimensional perceived control position scale for young footballers as a scientific guide for coaches and players to take advantage of.

3- The perceptual control site scale, which the researcher built and standardized through the followed scientific procedures, indicates the ability of this scale to identify individual differences between the players in the perceptual control site scale and its three dimensions.

4- Young players in Misan governorate have an average level in the perceptual control position variable.

Recommendations

1- Adoption of the perceptual control locus scale for young football players.

2- Coaches can use the perceptive control attitude measure and the need to use it on players periodically during the sports season, which allows identifying this important psychological variable in competition, as well as identifying positive and negative things to overcome in personal areas. Players’ preference and development.

3- Conducting other similar studies according to the variables (gender, age, different sporting events).

Ethical Clearance: People identified as potential research participants because of their status as relatives or carers of patient’s research participants by virtue of their professional role in the university and departments.

Source of Funding: Self-Funding

Conflict of Interests: The authors declare there is no conflict interests

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The Effect of Sudden Weight Loss During Competitions For Young Weightlifters in (RMR) and the Concentration of (Sodium-Potassium) Salts in the Blood

Natiq Abdulrahman Werytha Allami¹, Waritha Al-Lami²

¹Associate Professor, ²Researcher, College of Physical Education and Sports Sciences, Misan University, Iraq

Abstract

The study aimed to identify the level of the weight difference between young weightlifters when a sudden drop occurred during the competition period, to identify the level of (RMRF) difference in the concentration of salts (sodium - potassium) in their blood during this period, and to identify the effect, relationship and contribution of sudden weight loss during this period. The period of competition in (RMR) and the concentration of (sodium potassium-) salts in their blood, and they adopted the descriptive method of relational relations on weightlifting youth (18) - 20 years under the weight category 69 kg were chosen randomly and (20) weight lifter, as That the number of those who lost weight unapproved is (12) weightlifters make up the percentage (22.917%) from the origin of their community. Fitmate pro) at rest time after weight loss, at rest time after weight loss, and withdrawing (5 cc) of blood every before and after intentional weight loss to measure sodium and potassium cytokine by laboratory examination, and the researcher verified that the data were processed by the cyst system Statistical Abstract (SPSS) for conclusions and extrapolations Bikat that the process of sudden weight loss is associated with a negative relationship in the level of (RMR) and the concentration of salts (sodium - potassium) in the blood, and affects and contributes to reducing the levels of young Al Rafi ‘i for weightlifting during the competition period, and it is necessary to nurture the coaches to support the culture of the ideal weight and the true athlete of the youth of Al Rafei to lift Weights, as can be the coaches who influence them a lot. To prevent sudden weight loss disorder, they should reduce this phenomenon, which can easily cause eating disorders, as can those in charge of men’s tournaments. Agement at the local weightlifting association and its training, providing advice to the youth eye class on the unhealthy DONC weight loss, if there are no other professionals who can intervene (physiologists, doctors ... and others), it is necessary for the basic trainers to have the information. Correct information about the risks of eating disorders in sport and to be fully aware of the symptoms and consequences, to be able to act and build a level of preventive discoveries for the relevant predictions and to take the necessary steps when these problems arise, and whoever learns adequately about nutrition supervises the diet and gives Lempid tips to reduce Weight healthily for the prevention and treatment of eating disorders and counseling (for example, when an athlete needs to lose a kilo or more of body weight).

Keywords: Weight Loss, Young Weightlifters, Treatment, Concentration

The Research Problem and Its Importance

Most of the youth’s ideas revolve around in general practice, about to be, and a little less weight, especially the weightlifters for them to compete, which merged this reality with the diet of modern day pressures that civilization has created today, and those around them to victims of beauty. The socially imposed models, and that many of them are dissatisfied with the shape and weight of their bodies, although this fact does not expect weight disorders in the future according to what weight loss regimes bring, but it effectively contributes to the emergence of dietary
disorder factors in sport, this fact calls for Reconsidering the education of sports nutrition for those who exercise to be an essential element in evaluating food habits and means of losing weight, especially sudden before the race, as the term disorders refer to the sudden weight that some individuals suffer Eating disorders by eating too little quantities of foods forcefully to the point that it puts them at risk to their health or life, and other neglect. O eat frequently, can take risky actions; To get rid of the calories consumed as studies and scientific research have proven that health and proper nutrition are not only responsible for the formation and building of the body but they play an important role in guiding society and morals. And the intellectual behavior of the individual, and direct evidence of the quality of life, and indirect evidence of the extent of social and economic development of the importance of proper nutrition. In normal growth and optimal mental development in the health and health of the patient, and the maintenance of good health throughout a person’s life, where weight-loss disorders are defined by the presence of distorted attitudes and behaviors rooted in eating, weight and obesity, or as a compulsive eating misuse with the aim of achieving a desired type of psychology. It is also known as disorders represented in the presence of wrong behaviors towards eating in an unusual way in an attempt to reach a certain shape of the body or to participate in competitive programs that depend on a certain weight, and some mental illnesses appear as well as some organic diseases that lead to death. The development of today’s world is always affected by the advancement of new technologies, as a clear spread scenario has recently started, which is an ideal scenario for preventing sudden weight loss disorders in websites that cover these matters depending on the results of the benefit, and can be used to inform athletes about the dangers. Associated with poor nutrition or diet, which is the result of anxiety about weight and physical appearance, as well as in the case of dissatisfaction with weight and body shape, although this fact does not expect eating disorders in the future according to what has been done of weight loss regimens, but it contributes in a way. Effective in the emergence of disorders of weight-loss factors in sports, and this fact calls for a review of sports nutrition education. For those who participate in training and providers to compete to be a necessary component of assessing dietary habits. As is the case in this particular era, the report of young athletes begins to continue their sporting activity in a more serious way, and to continue training and competing in dangerous environments, and this fact is in contrast to what some studies have produced results, and this call to direct coaches and parents to Encouraging healthy eating and different habits because they most influence the prevention of such a disorder. Which will definitely lead to a decrease in the level of training and competition as well, it is imperative for coaches to recognize the fact that they have the correct information about the risks of disruptions to losing weight in sport and to be fully aware of the symptoms. The results to be able to act and build a preventive level discovers the relevant predictions, and applies the necessary steps when these issues show weakness in PDM including the thoughtful and non-specialist lifting of views, and is characterized by the jumping and jumping weightlifters that they are the most athletes who have the ability to provide a stock of phosphorus creative from other athletes. (Theodore and Michael), as is the process of weight loss, it is the reduction of weight lifter weights from the normal average in a deliberate and intentional manner in order to make all players ready to compete in the higher weight, and this process often takes place in sports with a single bout, where the weight plays The body plays a major role in achieving results like weight lifting. In the context of sports and in this particular era, young athletes begin to report that they continue their sporting activity in a more serious way,
and to continue training and competing in these truly dangerous environments, in contrast to what came with the results of some studies, as participation in the activity. The normal is associated with a high degree of approval of a person's body shape and then comparing it with non-athletes, in order to maintain a high degree of control in this case, the achievements and level of the athlete must be taken into account, and in an interesting investigation with adolescents, we concluded that the messages from parents predicting the strength of both strategies for losing weight. Among the children more than the media, and the best of friends, as it is known that the source of my energy, the human being is the food intake, which consists of the head of carbon, hydrogen and oxygen as well as nitrogen in the case of Protina T, and it is known in the number of food that these bonds are weak, and therefore they only provide (Molecular bonds) as well as to bind the limited molecular thread when broken down, and thus the energy stored in food is chemically dissolved inside the cells of the body and stored on the body an energy-rich compound called adenosine triphosphate (ATP), which turns into (ADP) in the metabolism processes, then ended up transformed into (ADP), a charge recycled again to become a high-energy ATP, that food is important for athletes in sports that emphasized the importance of lightening body shape or weight and reducing eating. As far as possible within levels of exposure to disorders and in these cases prevention of sudden weight loss that can actually appear in the athletic field, it is necessary to understand the significance of these types of weight and their number related to each other. The researcher believes that it is certain that any sudden case of body mass for a weightlifter will cast its evidence on the cell physiology, composition and effectiveness of the elements and minerals in which there is a kind of disorder that calls for a balance and avoids the risks of blocking the body to cause the type of compatibility between the internal environment and the requirements of the external environment are from The duties of this body in this new situation in which the weight is unbalanced and the imposition of chemical changes on the state of the body, and the means of ridding the body of excess weight is in fact the process is not in the amount of fat because burning it in a short or limited period of time is a difficult process, so the loss is in relation to For fluids that come out of the body through urination and intense sweating, or in abnormal physiological conditions, where sodium, potassium and chlorine are linked to each other by you will see the interconnection relationship between their functions in the body, and each depends on the other to become complementary functions of utmost importance in general and for athletes who they lift it not particularly foul, becoming human sodium chloride and potassium chloride. The body needs (8-15 grams of sodium chloride per day, (3-4) turbid potassium chloride, and this amount increases, when practicing training. The importance of the availability of potassium and sodium in it is responsible for the absorption of E. The absorption of sugars in the
The intestine, which is responsible for the contraction of muscles, supports the amount of water inside the cells of the body, and regulates the acids in the intestine blood and various body fluids. The role of sodium helps in maintaining blood pressure at normal levels and supports neuromuscular work and regulates fluid balance in the body, and the normal level of sodium ranges. Blood between (135-145) milliliters is equivalent to a liter of plate D, hyponatremia occurs when the level of sodium in the blood falls below (135) milliequivalent/liter (RAA - Renin Angiotensin Aldosteron System). Sodium consumption and potassium excretion by the kidneys. Therefore, the increased secretion of one of these substances (renin, angiotensin, and aldosterone) leads to the excretion of excessive amounts of potassium from the kidneys, which in turn leads to a lack of potassium in the blood, which causes weakness and muscle pain. Muscle weakness. In more difficult cases, failure of the face may occur. Respiratory bladder due to weakened breathing muscles, and abnormalities in the ventricular rhythm of the heart, where there is a living substance in the form of positively charged ions and it actively contributes to the catabolism and synthesis of proteins and carbohydrates, and the regulation of fluid movement in the body, the regulation of blood pressure and body fluids. Al-Baalouji or the graduation muscle with the effectiveness of the carpenter’s hand to produce the required strength for competition in the sport of weightlifting. The required proportions must be of the availability of mineral elements, salt and metabolic efficiency, and the sudden weight loss process during the period of the lifter competition for youth weightlifting effects that must be studied academically to avoid risks that may occur. On this cat of athletes, through the work of an academic researcher in the physiology of sports training and as a specialized trainer for the youth category, he noted that weightlifters had to resort to this process that requires delving into some of the accompanying effects that may occur to them in the short term. The research aims to determine the level of the weight difference between young weightlifters when a sudden drop occurs during the competition period. And to know the level of difference (RMR) and the concentration of salts (potassium-sodium-). In the blood of young claimants in weightlifting in a surprising and irreducible duration, and to identify the effect of the relationship and the contribution to sudden weight loss. During the RMRF competition period, the concentration of salts (sodium-potassium) in the blood of young weightlifters.

**Research Methodology**

In this type of research, the ethics of scientific research dictates that a person should not suffer some kind of health harm by the experience. For this reason, I adopt the descriptive approach in studying the problem as it is to clarify the related relationships, by adopting relational studies from this descriptive approach, which is defined as the research that seeks to try to define the relationship between two or more.

**Research and Designated Community**

The limits of the research community for weightlifting represented the youth (18-20) years of age between (69-77-85 kg) with a total of (48) lifters because they represent a phenomenon observed by the community in the research problem of themselves, as they compete in championships. Clubs and institutions set up by the Iraqi Central Weightlifting Federation at Tameem Hall in Baghdad late (2020) officially registered within the youth category. A sample of (69) weight class was purchased randomly (20) weight lifter, and the number of those who lost unaccredited weight is (11) weight lifter. (22.917%) of their original community, and this is a phenomenon that requires a number of field study and important calls.
Measurement tests and research procedures

The specificity of the study required knowing the weight difference (mass) for each weight lifter because before the deliberate reduction of its weight and after to measure the values of this quantitative difference are influenced by an electronic unit balance of the kilogram and its weight. The components, according to the RMR measurement) by the Italian Fitmate pro system (COSMED) made of a respirator with a chest strap with a (Bluetooth) pulse device at rest time before weight loss, and at rest time after weight loss to extract quantitative values of the RMR difference), and (5 cubic centimeters) of blood from each lifter was withdrawn before and after the intentional weight loss, and each of them was placed in a tube (1.5) mL with added (1000 μl) of RBC solution by means of tests. Lab for both sodium and potassium to extract quantitative values for this difference as well. These differences are matched by each of the tests for examining each lifter for measurements of the difference in weight (mass) resulting from the sudden decrease in diuretics that these young men take from the lifters and with that, and after collecting data for each verification of the weightlifter the researcher can completely process the data Statistical Package System (SPSS) version (V 26) Elijah extracted each of the values of percentage, central arithmetic mean, standard deviation, single-eye (t-test) test, linear (regression) coefficient (linear correlation coefficient), contribution ratio, error criterion evaluation, and test (F) Corresponding quality, tilt test (influence) (T) your mind of linear regression.

Results and Discussion

Table No. (1) Illustrates the statistical parameters of the variables

<table>
<thead>
<tr>
<th>Skewness</th>
<th>standard deviation</th>
<th>Arithmetic mean</th>
<th>N</th>
<th>measuring unit</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.346</td>
<td>70.582</td>
<td>1232.73</td>
<td>11</td>
<td>Cloud</td>
<td>Weight loss difference</td>
</tr>
<tr>
<td>-0.072</td>
<td>2.212</td>
<td>16.09</td>
<td>11</td>
<td>Milli Mall</td>
<td>Sodium concentration difference</td>
</tr>
<tr>
<td>-0.167</td>
<td>0.176</td>
<td>1.509</td>
<td>11</td>
<td>Milli Mall</td>
<td>Potassium concentration difference</td>
</tr>
<tr>
<td>0.138</td>
<td>40.113</td>
<td>239.09</td>
<td>11</td>
<td>Calorie</td>
<td>Metabolic rate difference</td>
</tr>
</tbody>
</table>

Table2. J between correlation and regression and contribute

<table>
<thead>
<tr>
<th>Standard error of estimation</th>
<th>Contribution rate</th>
<th>Coefficient of the regression of the linear 2 (R) Coefficient of determination ( )</th>
<th>Simple Correlation Coefficient ( )R</th>
<th>Affected</th>
<th>Influencer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.835</td>
<td>0.857</td>
<td>0.872</td>
<td>0.934</td>
<td>Sodium concentration difference</td>
<td>Weight loss difference</td>
</tr>
<tr>
<td>0.071</td>
<td>0.839</td>
<td>0.855</td>
<td>0.925</td>
<td>Potassium concentration difference</td>
<td>Weight loss difference</td>
</tr>
<tr>
<td>20.861</td>
<td>0.73</td>
<td>0.757</td>
<td>0.87</td>
<td>Metabolic rate difference</td>
<td>Weight loss difference</td>
</tr>
</tbody>
</table>
Table 3. Test shows) F) To examine the quality of Tawfiq E model of a linear regression

<table>
<thead>
<tr>
<th>Affected Variables</th>
<th>Regression</th>
<th>Sodium concentration difference</th>
<th>Weight loss difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight loss difference</td>
<td>42.628</td>
<td>1</td>
<td>42.628</td>
</tr>
<tr>
<td>Sodium concentration difference</td>
<td>0.698</td>
<td>9</td>
<td>6.281</td>
</tr>
<tr>
<td>Weight loss difference</td>
<td>0.264</td>
<td>1</td>
<td>0.264</td>
</tr>
<tr>
<td>Potassium concentration difference</td>
<td>0.005</td>
<td>9</td>
<td>0.045</td>
</tr>
<tr>
<td>Weight loss difference</td>
<td>12174.321</td>
<td>1</td>
<td>12174.321</td>
</tr>
<tr>
<td>Metabolic rate difference</td>
<td>435.176</td>
<td>9</td>
<td>39.588</td>
</tr>
</tbody>
</table>

M * significance level (0.05) N = 11 values of (F) a function if the score value (Sig)> (0.05)

Table No. (4) Shows the values of the constant range and estimates of the slope (effect).

<table>
<thead>
<tr>
<th>The moral</th>
<th>(Sig)</th>
<th>( t )</th>
<th>Standard error</th>
<th>Beta b</th>
<th>Variables</th>
<th>Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moral</td>
<td>0.002</td>
<td>4.321</td>
<td>4.621</td>
<td>-19.969</td>
<td>Fixed limit</td>
<td>Sodium concentration difference</td>
</tr>
<tr>
<td>Moral</td>
<td>0.000</td>
<td>7.815</td>
<td>0.004</td>
<td>0.029</td>
<td>Weight loss difference</td>
<td></td>
</tr>
<tr>
<td>Moral</td>
<td>0.008</td>
<td>3.404</td>
<td>0.391</td>
<td>-1.33</td>
<td>Fixed limit</td>
<td></td>
</tr>
<tr>
<td>Moral</td>
<td>0.000</td>
<td>7.279</td>
<td>0.000</td>
<td>0.002</td>
<td>Weight loss difference</td>
<td></td>
</tr>
<tr>
<td>Moral</td>
<td>0.011</td>
<td>3.209</td>
<td>115.386</td>
<td>-370.299</td>
<td>Fixed limit</td>
<td></td>
</tr>
<tr>
<td>Moral</td>
<td>0.001</td>
<td>5.289</td>
<td>0.093</td>
<td>0.494</td>
<td>Weight loss difference</td>
<td></td>
</tr>
</tbody>
</table>

Signal level (0.05) N = 11 significant (t) values if (Sig)> (0.05)

The results of the negative contribution decrease and the Thayer difference sudden weight loss for the rest of the variables surveyed were valid, as the greater the weight difference decreases during the competition period, the greater the difference caused by both sodium, potassium and average. From cellular metabolism at rest, and this result is a decrease in the stress of the events of the weightlifter, which is supposed to support its performance in producing the energy required for you in terms of energy, and the negative impact caused by the nervous overreach required for that. Muscular action also abolishes this imbalance that leads to harm to the contraction process if these results are scrutinized. The first effects of such a mechanism are according to its impossible interpretations, especially the mechanical
interpretation, which requires its availability. The energy required for protein sliding chains to shrink muscles, leading to increased weight of the lifter, the negative effect on wasting muscle strength gain by stressing the mechanism that contracts the muscles responsible for lifting, and it is also assumed that standard training will have a positive effect in creating positive and healthy adaptations for the weightlifter to prepare To participate in competitions while he is in a state of physiological adaptation. Positive, not vice versa, when you lose weight suddenly, this is like a loss of coaches’ efforts and a decrease in the level of effectiveness rather than progress, which leads to a decline in the professional level that it is hoped that they will be able to do so. The international move to reach the tournaments of the Olympics and in this only entrenched case they have false beliefs about sudden weight loss that they will resort to other quick ways and means to reach the lead regardless of the cost of the matter in terms of damage. On their bodies, and this goes against the Olympic Ideology Charter in competitive sports that place health and safety as a priority.

Since changes in attitudes and behavior require reduced risk in terms of sports according to the prevalence of eating disorders, these changes about athletes should not be directed only but rather extend to a social context, they serve in the opposite culture and an environment surrounding a thousand answers, as young people lack the skills The mentalities to overcome these changes, and some of them within the social nature do not have enough experience to know how to help them do so during this transitional time. Judy, 2007) and (Driskell) Mineral salts help in preserving the internal environment and in the formation of many tissues and to show the need for them because they give strength and vitality and help many vital functions during the motor work of the body ... and keeping muscle contraction during motor performance is crucial with minimal stress. Stress on the joint during movement and increase muscle contraction with a negative performance, and this serves the arena of players more than others and in the field and a faster rate of work. In muscles, they must be equipped with (ATP), and a biochemist believes that fatigue occurs due to the ineffectiveness of energy processing for (ATP) and it is fast in the use of muscles 16, as potassium, sodium and magnesium work to regulate the acid-base balance within the muscle cell and require compensation, to maintain high physical activity, which results in chemical reactions that increase as well as its role in regulating the pressure of insects 17. Potassium is also an important mineral for the heart muscles and has a direct role in lowering blood pressure, and the action of potassium is closely related to the action of magnesium in the body, as it works. To relax muscles, which helps to regulate the cellular regulatory mechanism through this action, the lost sodium level from the blood must also be maintained. At the normal level, because the increase and decrease in its concentration in the blood is inversely proportional to the blood and vice versa, as is its role in regulating the permeability of perforation of the cell membrane 18. The importance of sodium availability and avoiding its deficiency or impairment in neurotransmission causes the release of the peripheral plate that drives the neurotransmitter Acetyl Kolayn, which is the specialized connecting nerve between the nerve end and the muscle fibers (neuromuscular forum) and binds to the receptor for the my fiber sheath acetylcholine , Which is the reason for the opening of the sodium channels, it leads to the influx of sodium into the muscle fibers, which leads to the depolarization of the membrane, so the effect of the instruction applies to the length of the sarcoplasmic muscle fibers in both directions and below the membranous joints and leads to a complete contraction of the muscle fibers, sending the instructions to the sites where the links are located. The membrane adjacent to the sarcoplasmic
reticulum (FR) the latter causes the release of calcium (temporarily the calcium ion) and the sarcoplasm releases a calcium concentration that rises above 10a (μM), this is the lowest level that begins to form cross bridges as previously described. Then the active calcium pump continuously returns the calcium to the grid. Sarcoplasmic (usually in the range of 30 °C) ms and when the calcium concentration in the sarcoplasm becomes too low, it inhibits terbomycin again, and this chain is repeated when another motor neuron stimulus reaches the plate from the moving limb, and at a high burst frequency, the calcium ions persist in Escape from the sarcoplasmic reticulum, and the calcium concentration in the sarcoplasm that surrounds capillaries greatly increases. In this condition, the muscle fibers do not completely rest between the stimulation and the successive contraction and will be stronger and more stable (somewhat) until the stimulation of the nerve stops. This deficiency in sodium and potassium concentrations will lead to a loss (acclimatization) in the individual, and an adaptation to physiological compensation that lasts for days or weeks as a result of an external factor change or internal environmental factors. To damage endogenous homeostasis responses (local equilibrium responses), which are another set of reflections of responses other than actions, when a change occurs in the internal environment or the external environment, this change is in an alert state and this stimulus causes a change in cellular activity town as a result of the final control of Stimulation, so that this E. responds like all electronic responses that start and end with a response, but differs from other responses (reflexive action) in that they only occur in the stimulus region. Special adaptations must occur in the biochemical muscle in training the specialist with the difficulty experienced by the athlete. Proper nutrition and weight loss programs should go hand in hand with athletic training goals, which during high exertions naturally increase RMRs during exertion and this, is reflected in the increase in the rest of the time and this requires muscles Able to maintain chemical content balance. It is the duty of coaches to notice sudden changes in young people who play sports that require muscle strength and should quickly view it as a kind of continuous evaluation of their planning for their future. The lack of water and fluids from inside the body leads to decreased plasma volume, which leads to a decrease or decrease (stroke volume, cardiac impulse, and hypotension). A study also showed that low serum sodium intake indicates access to this salt and in such cases, a lack of serum sodium leads to a loss of water, which is the clearest, and a rapid body weight loss is evident. What is known is that when the mind is stored from carbohydrates during a violent muscular effort, the body begins to represent the stored fatty substances, and this leads to the oxidation of large amounts of fatty acids, which increases the formation of other (ketones). Acids that increase the acidity of the blood, so the abuse of alkaline salts such as citrate has a benefit in removing this acidity to a large extent, and among the natural substances that contain abundant substances, citrate is citrus and lemon in particular. Where weight disorders lead to eating disorders are extreme attitudes and behaviors related to eating and weight, and in the form of anorexia or bulimia nervosa, which reflects psychological and physiological problems that can have devastating effects, and severe consequences on the lives of those who suffer.

Conclusions and applications

1. The process of sudden weight loss is associated with a negative relationship in the level of (RMRF) salt concentration (sodium-potassium) in the blood, which affects and contributes to reducing the levels of young weightlifters during the period of competition.

2. The need to pay attention to coaches to support the culture of the true ideal athlete who has
the leverage for weightlifting youth, as coaches can influence them a lot to prevent sudden weight loss disorder in them to reduce this. Which can easily cause nutritional disruptions, and tournament administrators at the local weightlifting federation and their training can advise young eyes that Wayne should go to lose weight. But DONC is unhealthy. If there are no other specialists who can intervene (physiologists, doctors ... and others), it is necessary and basic trainers to have the correct information about the risks of eating disorders in sport and for Dr. Preventive detects relevant predictions and applies confidential appearance steps when these problems arise, and who learns adequate nutrition to be a nutritional supervisor and provides advice on healthy weight loss methods to prevent and treat eating disorders and a counselor for example (when an athlete needs to lose a kilo then of His body weight).

**Ethical Clearance:** People identified as potential research participants because of their status as relatives or carers of patient’s research participants by virtue of their professional role in the university and departments.

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**Conflict of Interests:** The authors declare there is no conflict interest

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The Effect of Human Resource Management Practice on Sharing Tacit Knowledge of Managers an Applied Study on Misan Center for Cardiac Diseases and Surgery

Soulaf Abdalqadir Hameed Alsady

Research Scholar Department of Nursing, College of Nursing, Misan University, Iraq

Abstract
The aim of the research is to identify the impact of human resource management practices used on the exchange of tacit knowledge, which were represented in each of (the practice of recruitment, selection and appointment, training practice, and the practice of motivation). The management of human resources is an effectiveness on which the future strategy of the organization depends, since previous decades, strong companies were those companies that possessed machines, technology and materials where the human element was insignificant and was treated as machines without taking into account its potential, feelings, development and capabilities, witnessing the economic market An accelerated daily development, and through this, the important role played by the human resources department in raising the performance of employees is evident, as it provides the specialized and efficient human element that affects the organization and its performance. The main objective of this research is to indicate the effect of human resource management practices on the sharing of implicit knowledge of managers, as well as to explain the importance of implicit knowledge in employee formation in reaching executive positions in a business organization. The topic of knowledge management has received great attention all over the world by academics, including writers, researchers, and executives from managers and officials, due to the economic and competitive advantages this practice achieves, bearing in mind that the most important goal of this research revolves around the business organization. It is one of the most important keys to the success of the organization, and thus it is a source of competitive advantage and its importance is widely accepted at the present time. Therefore, many individuals in executive positions acknowledge that knowledge creation has an important role in their companies. The trend towards knowledge management began in the 1990s, when companies are now being urged to consider knowledge creation as a source of success, because the tremendous benefit that knowledge management promises is innovation.

Keywords: Human Resource, Management Practice, Knowledge, Business Organization

Introduction
We live today in an era in which crises abound as a result of changes and openness to the world and thus the employees and managers of institutions in the contemporary environment have no choice but to think and act in a strategic way ¹. Numerous at the global and Arab level, and the practice of human resource management is one of the most important management concepts that have gained attention and spread in recent years. Knowledge management processes are of great importance in business organizations and the health sector by providing competitive advantages, but most organizations face challenges in managing and developing tacit knowledge and converting it into explicit, tacit knowledge refers to knowledge stored in the human mind, such as good things, skills and ideas, and represents an urgent necessity to ensure the success of organizations and individuals in general and tacit knowledge represents one of the types of knowledge in addition to explicit knowledge ².
**Research problem**

The health sector - like other aspects of human activity - is witnessing an unprecedented stage of change, as it has become difficult to predict the future, and human resource management practices have become an effective means of honesty and maintaining movement in the right direction. It is an ongoing process. Based on the above, it becomes clear that the impact of the human resource management practice on the exchange of tacit knowledge, in order to achieve the desired development, and to face the successive societal changes such as globalization, communications revolution, technological revolution, and for this reason the research problem can be crystallized in the following questions:

1- What is the concept of human resource management practices, and what is the terminology associated with it?

2- Implicit knowledge and its dimensions?

3- Dimensions of human resource management practices?

4- The concept of tacit knowledge?

**Research objectives:**

The research seeks to achieve the following objectives:

1- Understand the concept of human resource management practices, and what terminology is associated with it?

2- Clarify the reality of applying human resource management practices in the health sector.

3- Standing on the dimensions of implicit knowledge.

4- Determine the importance of human resources.

5- Determine the justification for the introduction of human resources in the health sector.

6- Applying a questionnaire on the impact of human resource management practices on the sharing of implicit knowledge of managers.

**Research hypothesis**

The first main hypothesis: This states that there is a moral correlation between the practice of human resource management and the tacit knowledge.

\[ H: \text{There is no significant correlation between HRM practice and tacit knowledge.} \]

\[ H_1: \text{There is a significant correlation between the human resource management practice and the tacit knowledge.} \]

The second main hypothesis: which states that there is a significant moral effect between the practice of human resource management and the tacit knowledge?

\[ H_0: \text{There is no effect between HRM practice and tacit knowledge.} \]

\[ H_1: \text{There is an effect between the practice of human resource management and the tacit knowledge.} \]

**The importance of research**

The importance of this research stems from the importance of human resources, which is the main and important resource capable of achieving the goals of the organization, especially in light of the intensification of competition and the rapid environmental changes that are imposed on organizations to keep pace with them constantly. The importance of this research is evident in identifying the impact of the practice of human resource management on the exchange of knowledge implicit in managers. Nor does it show the role of tacit knowledge present in the minds of experts and its role in developing procedures for
securing facilities, and that it represents an important source and reference for building future plans, and a key factor in developing procedures for securing the health sector, and this is based on feedback and using it in filling gaps and addressing errors that were exploited in previous operations.

**The importance of the current research is crystallized in:**

Ø The current research may open new vistas in the field of human resource management; As it is considered an extension of previous studies in the field of human resource management practices in the health sector, and at the same time it is a prelude to other new studies.

Ø The current research may benefit both the competent authorities in the health sector and its institutions, and those responsible for its administration; as it contributes to crystallizing the concept of human resource and its importance ... etc.

**Research community and sample**

The research sample is represented by the Misan Center for Cardiac Diseases and Surgery, and since the aim of the current research is to know the relationship and effect of the practice of human resources management on the implicit knowledge of managers, so the research is directed to the community being represented by everyone who occupies administrative positions (general manager, assistant director, head of department Division director, unit official, lounge official, ward official, counseling officials, pharmacy officials) who have the authority to make decisions, as the study population reached (177).

**Research variables**

The search variables are as follows:

1. The independent variable: the human resource management practice that includes the dimensions (recruitment and selection, training and development, incentives, compensation).

2. Dependent variable: Tacit knowledge

**The theoretical model for research**

The research is based on a theoretical framework indicating the nature of the objective of the current research, which is to determine the effect of the human resource management practice on the exchange of implicit knowledge of managers at Misan Center for Cardiac Diseases and Surgery, and to test the influential relationships between the practice of human resources as an independent variable on the implicit knowledge as a dependent variable, as illustrated by it. The following figure:
Methodology

Human Resources Management Practices

It is a group of activities that would put human resources strategies into practice and are directed towards improving performance. Human resource management practice is the process of attracting, motivating and retaining employees to ensure the survival of the organization. Human resource management practices are designed and implemented in a way in which employee performance plays an important role in achieving the goals of the organization, and the proper use of human resource management practices positively affects the level of commitment of the employer and the employee. Human resource management practices such as training, development and performance evaluation encourage employees to work better in order to achieve organizational performance. The practices selected for this research depend on the impact of human resource management practices on the sharing of implicit knowledge of managers. Human resource management (HRM) is a managerial function concerned with recruiting, motivating and maintaining people in an organization, focusing on people in organizations, policies and practices involved in implementing “people” or human aspects of resources for a management position, including recruitment, screening, training, and reward from the Human Resources Department. In short, human resource management (HRM) can be defined as the art of purchasing, developing and maintaining an efficient workforce to achieve the goals of the organization in an effective manner. The Human Resources Department works to direct all activities and events in the organization to achieve optimal use of the human resources in the organization and contribute to achieving the organization’s goals such as attracting, selecting, appointing, wages and incentives to meet its needs of human resources in terms of quantity and quality.

The importance of practicing human resource management

Behind the success of every successful organization stands a successful human resource management in its programs, whether these programs are within the framework of selecting individuals, training them, evaluating their performance, maintaining and retaining them, or through good programs for rewards and incentives, so there is a strong correlation between the success of the organization and its programs human resources.

Dimensions of the practice of human resources management

Recruitment and selection

The recruitment process is one of the most important jobs that the human resources department in the organization must accomplish. Success in that determines the course of career activities in the organization. The essence of the recruitment process is related to attracting the largest possible number of qualified individuals and encouraging them to submit job applications in companies to allow the company to choose and appoint the most efficient individuals. Polarization can be defined as the discovery of potential applicants to fill actual or anticipated organizational vacancies, and the sources of polarization have been identified with two main sources (internal polarization and external polarization).

Training and Development

Training is defined as that organized and continuous process that aims to provide and acquire the individual with new knowledge, capabilities and skills, and training is an investment in the human assets of the organization, so training is a useful means with the changes that are sponsored by technological innovations, as training and development constitute three main activities. Training, Education, and
Development. Training and development in general refers to the organized efforts that contribute to teaching job-related knowledge, skills and employee behavior, and it is also a knowledge system for human resources in the organization and developing their current skills.

**Incentives**

The existence of an effective incentive system applied in the organization is considered one of the most important components of a successful and effective work environment, and the importance of an effective incentive system is highlighted in that it mainly contributes to raising the morale of workers, developing their loyalty to the facility and making them feel stable and secure, and incentives were defined as the process of activating the reality of working individuals in ways positive or negative, with the aim of increasing production rates and improving performance, and satisfying their material and moral needs.

**Compensation**

Compensation relates to compensation of the employee with all kinds of wages or returns that he obtains in connection with working a certain job, and these compensation include two basic elements, namely, direct financial payments, which may take the form of wages, salaries, incentives, and indirect financial payments, which take the form of financial benefits such as insurances and paid leave (2007). As the information resulting from the job analysis process allows estimating the size and quality of compensation that is commensurate with the characteristics of each job and helps in assessing the relative importance of this compensation for all jobs in the organization.

**Tacit knowledge**

It is knowledge acquired through the accumulation of previous experiences and it is present in the minds of individuals, and it is often of a personal nature, which is difficult to obtain despite its great value because it is stored within the mind of the owner of knowledge. (Coakes) believes that tacit knowledge contains important cognitive dimensions such as (mental models, beliefs, and intuition), and therefore this type of knowledge is generated through the use of past experience in new contexts.

The knowledge implicit in consists of:

1- Hard facts, data, and mental patterns.
2- Views, shapes, images and concepts.
3- Judgments, expectations, general assumptions and beliefs.
4- Thinking strategies, and theological approaches.

Tacit knowledge is subjective and circumstantial and its implementation requires overlap and coordination, and thus the more implicit knowledge is, the more difficult it is to transfer and share and knowledge possesses an important implicit dimension, which makes the processes of studying about it, obtaining, transferring, storing and exchanging it difficult and sometimes impossible to achieve. There are controversial relations between the two types of explicit and implicit knowledge, as much of the implicit knowledge is affected by what is available to its owners of explicit knowledge, and the latter is due in its origins to the tacit knowledge that its owners decided to release and declare, as it is also a partial product of that knowledge. The first (implicit), when declared, contributes to forming the second (explicit). Tell can we than more know we” This phrase sheds light on the fact that many human knowledge and skills remain implicit and unregistered until the owner takes them out, so they do not appear and cannot be employed or defined except through dialogue and discussion, and this is what is considered the greatest
challenges in Knowledge management applications for modern organizations that strive towards excellence.

**Dimensions of tacit knowledge**

The dimensions of tacit knowledge are represented by experience, thinking and skill, in a way that gives a clear meaning to the tacit knowledge that relates to people and their intellectual perceptions rather than acquiring the knowledge they need.

**Expertise** - is the accumulated knowledge over time and experience with all facts, rules and procedures in a specific field of work. The experience is due to the technical dimension of knowledge, which is knowing how, which is the knowledge accumulated over the course of a person’s life that he has acquired through his exposure to many situations and his learning from them. There are two approaches to interpreting the experience. During his work in a specific field, the second is seen as a feature of individuals and is a result of the human capacity for widespread adaptation in the physical and social environment.

**Thinking** - Thinking is defined as the ability to perceive various alternatives to deal with the problem. It is also a series of mental processes that the brain performs when it is exposed to a stimulus that is received through one of the five senses.

**Skill** - Skill is the level of personal merit in accomplishing tasks from the point of view, while sees it as the tacit knowledge that is embodied in multiple forms. If the organization embraces this skill, it will generate a logical feeling for decision-making.

Attributes or characteristics of implicit knowledge (Al-Otaibi is a source previously mentioned)

1- Composition and accumulation complex.

2- It cannot be shared, shared or stored.

3- Self-configuring.

4- You can direct the behavior individually only.

**Nonaka and Takushi SECI model**

The cycle of knowledge developed by Nonaka and his colleagues, known for short as the (SECI) cycle, and this model assumes that individuals create their knowledge through the interaction between explicit knowledge and their tacit knowledge.

Knowledge passes through the process of its quantitative and qualitative expansion through four stages, namely:

1- The stage of socialization - the process that takes place through the creation of tacit knowledge through the exchange of experiences, ideas and skills among individuals.

2- The externalization stage - meaning the transformation of tacit knowledge into explicit knowledge, in a form that is easy to share with others.

3- The combination phase - which is the process of converting explicit knowledge into a more complex form.

4- The internalization phase - is the stage that individuals give to knowledge.

The explicit and transforming it into tacit knowledge through practice or learning by doing, and it is done through self-education.

**The role of learning in developing tacit knowledge of human resources**

Education, both formal and informal, contributes to the development of tacit knowledge of human resources by enriching all components of its dimensions. However, informal education has some peculiarities that are available in it and are not available in formal education (training), as follows:

1- In the field of friction with competencies:
Individuals interact with each other according to team spirit, and they learn from each other some necessary knowledge, skills and behaviors. Therefore, the institution’s management is responsible for providing the appropriate climate for its members in order to guarantee them learning (direct or indirect) through Good selection of team members and taking into account the balance between them in knowledge levels.

2- The field of ability to transfer implicit knowledge: Nonaka and Takeshi presented a model for knowledge transfer within the organization, and the various processes related to tacit knowledge are manifested in:

Input: transferring explicit to implicit knowledge, by means of learning by doing.

Output: transferring knowledge from implicit to explicit by modeling or codifying that knowledge.

Sublimation: transferring knowledge from implicit to implicit through social interaction and discussions between individuals.

3- In the area of skill: The skill is known by some as synonymous with tacit knowledge, as the contact of individuals with varying levels of skills would contribute to the transfer of skills from an efficient person to an incompetent person.

4- In the field of knowledge application: The culture that encourages human resources, which is embodied in the evaluation of what the individual has learned inside and outside the work of implicit knowledge related to work.

A practical framework for research

First: Description of the study sample

Study sample:

The sample of the study included an applied study on the Misan Center for Cardiology and Cardiac Surgery, where (180) questionnaires were distributed to an employee, and (177) questionnaires were retrieved.

Sample collection method:

The researcher used the simple random sample method in distributing the questionnaire forms to employees on the assumption that the community is homogeneous. Therefore, the researcher used the simple random sample, and then the appropriate sample was drawn, as the sample size reached (177) employees, which represented the study population.

Statistical indicators:

In the statistical analysis, the researcher relied on the data and information obtained from the research according to the Likert five-point scale, and the researcher used the most important statistical indicators to fit the research hypotheses and questions related to it, which are as follows:

1- Frequencies and percentages: to find out the number and percentage of respondents within the research sample.

2- The weighted arithmetic mean: It is used to find out the degree of agreement of the questioned sample to the questions.

3- Standard deviation: It is used to find out the extent of dispersion of the answers of the researched sample from the degree of approval.

4- Person correlation coefficient: It is used to measure the extent to which the research variables are related to each other and to determine the type of relationship whether it is positive (positive) or opposite (negative).

5- F test: It is used to find out the presence of an effect of the independent variables in the search on the dependent variable.
6- Regression equation: It is used to find out the effect of the independent variable on the dependent variable.

**Notice**

The significance value (sig.) was adopted in testing the research hypotheses instead of the tabular values for the accuracy of the results calculated from the significant value (sig.). It should be noted here that all these indicators were calculated by the Statistical Package for Social Sciences (SPSS V26) program 16.

Second: The demographic variables of the research: It shows a description of the demographic variables of the individuals of the research sample

<table>
<thead>
<tr>
<th>Table No. (1) Frequencies and percentages of demographic variables for the research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of job experience on the job</td>
</tr>
<tr>
<td>Valid</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>education level</td>
</tr>
<tr>
<td>Valid</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Employment</td>
</tr>
<tr>
<td>Valid</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

We note in Table (1) the following:
With regard to the period of job experience in research, where the frequency of the category (3-6) years was the highest, reaching (64) with a rate of (36%). With regard to the level of education in the research, the highest frequency of university qualification was (51) by (28%). With regard to the field of work in research, the frequency of the highest was in the field of director, reaching (56) by (31%) 17.

Third: Description and diagnosis of research variables

This paragraph includes a description of the main study variables and their diagnosis represented by the practice of human resources management as an independent axis and tacit knowledge as an approved focus in the research. To achieve this, appropriate statistical analyzes were used 18.

Arithmetic means and standard deviations of the sample responses for human resource management practice

Table No. (2) Below describes the research variables for the practice of human resources management

<table>
<thead>
<tr>
<th>No</th>
<th>Human resource management practice</th>
<th>Standard</th>
<th>Arithmetic likely</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Interviews and testing are used upon appointment</td>
<td>1.07381</td>
<td>4.1000</td>
<td>4.07381</td>
</tr>
<tr>
<td>2</td>
<td>Human resource offices are used to fill vacancies</td>
<td>0.96890</td>
<td>4.0000</td>
<td>0.96890</td>
</tr>
<tr>
<td>3</td>
<td>Selection of employees based on criteria commensurate with the work</td>
<td>0.90351</td>
<td>4.0000</td>
<td>0.90351</td>
</tr>
<tr>
<td>4</td>
<td>There is a variety of training types</td>
<td>0.30534</td>
<td>4.4500</td>
<td>0.30534</td>
</tr>
<tr>
<td>5</td>
<td>A budget is allocated for training and development</td>
<td>1.17038</td>
<td>4.2400</td>
<td>1.17038</td>
</tr>
<tr>
<td>6</td>
<td>There are programs for training and development</td>
<td>0.69985</td>
<td>4.2000</td>
<td>0.69985</td>
</tr>
<tr>
<td>7</td>
<td>There are many positive incentives</td>
<td>1.08496</td>
<td>4.0800</td>
<td>1.08496</td>
</tr>
<tr>
<td>8</td>
<td>The incentive system in place contributes to improving employee performance</td>
<td>0.81541</td>
<td>4.2200</td>
<td>0.81541</td>
</tr>
<tr>
<td>9</td>
<td>Emphasis is placed on the material incentives of the employees</td>
<td>0.78895</td>
<td>4.1000</td>
<td>0.78895</td>
</tr>
<tr>
<td>10</td>
<td>The educational qualification is taken into consideration in determining the remuneration</td>
<td>0.98582</td>
<td>4.2600</td>
<td>0.98582</td>
</tr>
<tr>
<td>11</td>
<td>I feel satisfied with the monthly salary compared to the career ladder</td>
<td>0.74751</td>
<td>4.1800</td>
<td>0.74751</td>
</tr>
<tr>
<td>12</td>
<td>Remuneration is granted on the basis of time regardless of the amount of completion</td>
<td>0.92390</td>
<td>4.0000</td>
<td>0.92390</td>
</tr>
<tr>
<td>13</td>
<td>The overall average</td>
<td>0.872362</td>
<td>4.1525</td>
<td>0.872362</td>
</tr>
</tbody>
</table>
The results of Table No. (2) indicate that the practice of human resources management has obtained a general arithmetic mean of (4.1525) and a standard deviation (0.872362), and this indicates the homogeneity of the sample answers about the value of the arithmetic mean. This axis, which is (there is a diversity in the types of training), is the most homogeneous, as it obtained an average (4.4500) and a standard deviation of (0.30534) and that the direction of this paragraph is (agree), and the general trend in the practice of human resources management is (agree) 19.

The arithmetic mean and standard deviations of the sample responses for implicit knowledge

**Table No. (3) Below describes the variables of the special research implicit knowledge**

<table>
<thead>
<tr>
<th>Tacit knowledge</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiate the transmission of available tacit knowledge to individuals</td>
<td>1</td>
</tr>
<tr>
<td>The worker can devise solutions around the problems</td>
<td>2</td>
</tr>
<tr>
<td>The ability to identify problems</td>
<td>3</td>
</tr>
<tr>
<td>Enhancing the supportive environment for the exchange of knowledge in ideas among all employees</td>
<td>4</td>
</tr>
<tr>
<td>Providing systems and technologies that allow workers to share their knowledge</td>
<td>5</td>
</tr>
<tr>
<td>Easy access for employees to the available knowledge bases owned by the bank</td>
<td>6</td>
</tr>
<tr>
<td>Providing information about those with expertise in the bank</td>
<td>7</td>
</tr>
<tr>
<td>Evaluating the performance level of employees according to knowledge sharing systems</td>
<td>8</td>
</tr>
<tr>
<td>The overall average</td>
<td></td>
</tr>
</tbody>
</table>

The results of Table No. (2) indicate that the implicit knowledge has obtained a general arithmetic mean of (3.8359) and a standard deviation (1.160973), and this indicates the homogeneity of the sample answers about the value of the arithmetic mean, and as for the paragraphs level, paragraph No. (5) Came in this dimension. Which is (the provision of systems and technologies that allow workers to share what knowledge they possess) is the most homogeneous, as it obtained an average (4.3200) and a standard deviation of (1.07457), and the direction of this paragraph is (agree), and the general trend of implicit knowledge is (agree) 20.

**Third: Testing the hypotheses**

**The first main hypothesis test:** which states that there is a significant correlation between the practice of human resource management and the tacit knowledge?

H0: There is no significant correlation between
HRM practice and tacit knowledge.

H1: There is a significant correlation between the human resource management practice and the tacit knowledge.

Table (4) represents the correlation relationship

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Tacit knowledge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human resource management practice</td>
<td>Pearson Correlation</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).

Table No. (4) Indicates the existence of a significant correlation between the practice of human resources management and the knowledge implicit in the research, where the value of the Pearson correlation coefficient was significant and equal to (0.720), because the significant value is sig. It is equal to (0.001) and it is less than 0.01 with 99% confidence. This means that the first main hypothesis is fulfilled, meaning there is a significant correlation between the human resource management practice and the implicit knowledge.

The second main hypothesis test: This states that there is a significant effect between the practice of human resources management and the tacit knowledge.

H0: There is no effect between HRM practice and tacit knowledge.

H1: There is an effect between human resource management practice and tacit knowledge ²¹.

Table No. (5) Represents a schedule for analysis of variance

<table>
<thead>
<tr>
<th>ANOVA b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

a. Predictors: (Constant), Human resource management practice

b. Dependent Variable: Tacit knowledge
The results of the F test indicate the presence of the impact of the human resource management practice and the tacit knowledge shown in Table (4) that the calculated F value reached (37,622) at a significant level (0.05) where the P-value was equal to (0.000), which is less than 0.05 this means rejecting the null hypothesis and accepting the alternative hypothesis, and this means that there is an impact of human resource management practice and tacit knowledge.

Table No. (5) Represents the impact of human resource management practice and implicit knowledge

<table>
<thead>
<tr>
<th>Model</th>
<th>Coefficients a</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
</tr>
<tr>
<td>(Constant)</td>
<td>0.996</td>
<td>0.577</td>
<td>1.728</td>
</tr>
<tr>
<td>Human resource management practice</td>
<td>0.890</td>
<td>0.225</td>
<td>0.670</td>
</tr>
</tbody>
</table>

Table No. (5) Indicates that the value of R² is equal to (0.437). This means that the practice of human resources management has explained (43.7%) of the changes that occur in the implicit knowledge, and the values of B = (0.670) that is, the increase in the variable of the resource management practice Humanity one unit of standard deviations will increase the implicit knowledge by 67% of the standard deviation unit.

Conclusions and Recommendations

Conclusions:

1- There is a lot of confusion between information and implicit knowledge management, which causes confusion.

2- In order to raise the efficiency of workers in the management of human resources, it is necessary to create what helps them in the completion of their work.

3- Job experience is the highest in the research community, and the level of education comes second after it.

Recommendations

1- Urging the spread and learning of tacit knowledge, its empowerment and action.

2- Creating activities in Misan Center for Cardiac Surgery and Diseases related to public relations in order to coordinate work and ideas to enable knowledge.

3- The need for health institutions to realize the importance of the role that human resources management plays and to work on developing it.
through developing the capabilities and capabilities of workers.

4. Motivation of human resources, taking into account the difference in individual skills and abilities, as material stimulation will be an important incentive for better performance.

5. There is a close correlation between the practice of human resources management in all its dimensions as a practice of human resources strategy and the implicit knowledge.

6. Conducting a study on human resource management practices and their role in achieving institutional excellence.

**Ethical Clearance**: People identified as potential research participants because of their status as relatives or carers of patient’s research participants by virtue of their professional role in the university and departments.

**Source of Funding**: Self-Funding

**Conflict of Interests**: The authors declare there is no conflict interest

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The Effect of Mental Maps on Creative Abilities in Learning the Two Skills of Correction from High Jumping and Shooting Accuracy with Hand Ball

Yasser Mohsen Aliwi¹, Wissam Qais Khazal²

¹Lecturer, ²Teaching Assistant Misan Education Directorate Department, Ministry of Education, Iraq

Abstract

Mind maps are one of the modern means of expressing ideas through symbols and shapes, and it is one of the techniques used in the art of teaching and education through the use of mental skills in the transfer of external stimuli that are formulated to the inner world. The brain (nervous system) and thus stimulating the processes of remembering, creativity and thinking, it is a successful method for the teacher and the student, as the teacher uses it to convey the idea and the student uses it for memory and memorization. It is also a great model for increasing the common senses in the learning process. Hence the researchers formulated the problem with the following question: Do the exercises prepared by the researchers using the mind mapping method develop my creativity and learn my corrective skills from high jumping and accurate handball? One of the aims of the research is to prepare exercises according to mental maps to teach high jump correction skills and accuracy of shooting with handball to school students. The research also assumes that there are differences between the tests and measurements of pre and dimensions in the creative abilities and learning the skills of correction of high jump and accuracy in manual shooting for the two experimental and control in favor of the meta-tests. The researchers used an experimental Lee approach on a sample of Yeh fringe students. The most important finding of the researchers is that prepared exercises, formulation and application contribute to the development of creativity among school students. They added that the mind mapping strategy is better than the traditional method through research results in the creative abilities and basic skills under study.

Keywords: Hand Ball, High Jumping, Shooting Accuracy, Witnessing

Introduction

The world is now witnessing a tremendous development beyond its imagination, and this development is the result of great efforts in which everything new has been used at the level of educational technologies and the possibility of using the human mind in dealing with it in all differences. Including the educational field, which has now become one of the fields that give an impression on the progress of countries and their progress, so it was necessary to conduct a lot of studies and research in order to achieve progress in the use of modern educational methods and through solving the problems facing this progress, as it is noticed that countries the applicant spends huge sums in order to access everything new and developed to achieve the best ways to use modern technologies in the educational process and that this development did not stop at a specific category, but it included all academic groups from the primary level to the university level, which are maps that represent the symbols and shapes that the basic pillars of the art of teaching and education are formed by transferring those external stimuli that have been formulated to the inner world, the brain (nervous system), which stimulates the processes of memory, creativity and thinking, as it is a method of memorization.
information in memory in a manner consistent with the memory pattern the man (190: 2) The physical and skill levels of handball players and mental abilities all affect performance and winning in matches, and that the skill and physical and mental familiarity and how to employ them to reach the top in performance has become an important element for winning and its development through training and training, which made the trend in the study of capabilities Mental control to control it in other abilities and determine its results, hence the importance of searching for numbers for a set of exercises based on the method of mental maps, raising creative abilities and teaching the two skills of correction for high jumping and accuracy of shooting with the hand ball.

**Research Problem**

The researchers pointed out that the process of learning skills in the game of handball, especially correction, requires repeated training and condemnation, and it is not an easy skill, especially among students, and therefore requires diversity in education and training techniques and finding modern alternatives as well as attention to the factor of individual differences, in accordance with their abilities and capabilities. Physically and mentally, which drives them to seriously participate in the educational process, as well as search for all new methods and means that develop and develop the learner’s ability to use his mind and thinking in order to learn new skills in the best way. The researchers also indicated that there is a weakness in the use of modern methods such as mind maps, which are considered one of the strategies that rely on drawing everything you want on one sheet in an organized way, where words are replaced by drawings. Refer to it in a concise, beautiful and easy to remember, and it helps to develop ideas about a topic in an easy, organized and artistic way that simulates the work of the brain, develops thinking skills and stimulates creativity and the learner. The desire to learn the educational material because it delivers the joy of learning the researchers also believe that the exercises that are formulated should take me into consideration considering how they contribute to the development of creative abilities in addition to discovering learners and their future readiness in any type of activities, as well as tests in creativity abilities that did not occupy enough space, and from here the researchers formulated the problem with the question Next (Do exercises prepared by researchers using the mind mapping method develop my creativity and learn my skills in correction from high jumping and accurate handball shooting).

**Research Objectives**

1. Preparation exercises in accordance with the mental maps to teach the skills of the correction of the high jump and precision correction roller hand for middle school students marginal.

2. Identify the differences between pre and dimensional tests and measurements in creative abilities and learn the two correction skills of high jumping and the accuracy of shooting with hand ball for the experimental and control groups.

3. Identify the differences in tests and dimensional measurements in creative abilities and learn some basic handball skills between the experimental and control groups.

**Research hypotheses**

1. There are differences between the pre- and post-tests and measurements in the creative abilities and learning some basic hand reel skills for the experimental and control groups and for the benefit of the post tests.

2. There are differences in the tests and dimensional measurements in the creative abilities and learning of some basic handball skills between
the experimental and control groups and in favor of the experimental 9.

Research areas

- Field of human: a sample of medium students’ marginal b for boys in the province of Misan.
- Spatial area: Hall Square and middle school marginal b.

Research Methodology

The experimental approach was chosen by designing the equivalent groups for its suitability to solve the research problem and its objectives

Research community and sample

A research community for middle and marginal students was identified in Yahya Governorate, Maysan Governorate, for the academic year 2019. 135 students were selected. Two sections were selected from each section (15). Any sample that was taken was (30) and the sample was divided by (22.22%) into two groups, where each section represents one group, which is the experimental group and the second the control group. The researchers did not need to perform the homogenization process, as the research sample was not from the specialist players, but rather a raw sample of the same age and school stage, so the conditions for homogeneity were provided 10.

Age - Weight

<table>
<thead>
<tr>
<th>Values )T( Calculated</th>
<th>Experimental</th>
<th>Control</th>
<th>Variables</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard deviations</td>
<td>Arithmetic means</td>
<td>Standard deviations</td>
<td>Arithmetic means</td>
</tr>
<tr>
<td>1.33</td>
<td>4.12</td>
<td>158.73</td>
<td>3.76</td>
<td>155.43</td>
</tr>
<tr>
<td>1.79</td>
<td>1.73</td>
<td>59.83</td>
<td>2.85</td>
<td>59.75</td>
</tr>
<tr>
<td>1.19</td>
<td>2.04</td>
<td>14.23</td>
<td>1.65</td>
<td>14.78</td>
</tr>
</tbody>
</table>

The degree of freedom is (28) and the level of significance (5%) equals (2.05)

And when the results of the test (T (At the level of freedom (0.05), the values of the variables are less than the tabular value and therefore there are no differences between the two groups, which confirms the parity between them, as shown in Table No. (1)

Means, devices and tools used

Methods of gathering information
- Arab and foreign sources.
- International information network (the Internet)
- Scientific observation.
- Creative Capacity Scale
Tests and benchmarks

Devices and utilities

- Mid-Hadidiya Square
- Video camera (Sony - Digital (With accessories).
- Plasma TV size TH-49PV8M Type Sony.
- Computer Pentium 4 type HP with CDs) dvd (Type Sony.
- Sagging
- Maps (Flex boards on which mental maps were drawn, with dimensions of 3 x 3 m, horizontal shape designed by the researchers) were used during the educational units, number 3
- Handouts
- Terraces
- Hand balls

Defining tools for measuring research variables

Tests and measures used in the research

First: Creative Capabilities

After reviewing the sources and references, the researchers chose the Creative Abilities Scale translated by Sayed Khairallah, 1974 (5: 123)

Second: The tests used in the research

1- Test the technical performance of the shooting skill from high jump.

- Name of the test: Technical performance of the skill of shooting from high jump.

- The objective of the test: - Evaluating the technical performance of the shooting skill from jumping high: 175.7

- Tools used: Legal handball court (3) legal hand balls. A pre-prepared evaluation form.

- Performance specifications: The test student performs the correction skill by jumping high from a point located on a right angle with the middle of the goal line and 10 meters away from it. The laboratory is given three consecutive attempts to perform the skill.

- Registration: Each laboratory student is granted three consecutive attempts, the best assignment for each evaluator is calculated, and after that the arithmetic mean of the best three attempts of the three evaluators is calculated. Note, “The final score for the evaluation is (10) degrees, as shown in Figure

Figure (1) The technical performance evaluation test demonstrates the skill of shooting high through the handball
2- Accuracy test of high jump aiming skill.

- Name of the test: Test of the accuracy of the skill of shooting from high jump: (175,7)

- The objective of the test: To measure the accuracy of the correction from jumping high with the handball.

- Tools used: Legal handball court. Legal handballs, count (6) Pre-prepared evaluation form. - Squares accuracy (60 cm - 60 cm)

- Performance specifications: The test student performs the correction skill by jumping high at a point located at a right angle with the middle of the goal line and a distance of (10 m) and the correction is done by jumping high with taking three steps and from a distance of (10) meters where the correction is done on the square The right, then the left square

- Registration: Each laboratory student is awarded six consecutive attempts, counting the number of successful and failed attempts recorded by the laboratory student. Note that the maximum score for evaluation is (6) scores, as shown in Figure (2)

![Diagram of high jump aiming skill](image)

Figure 2: The precision evaluation test demonstrates «high jump scoring skill» with handball

Pre-exams

The researchers over two days on 13-12/ 15/2020, and in at ten Saba Ha Square and in the middle school marginal b tests tribal research sample group experimental and control, as follows: On the first day were tested and measured in the experimental group:

A measure of creative abilities

The two shooting skills of jumping high and the accuracy of shooting with hand ball. On the second day, the control group was tested in the same place, timing and verse used for the experimental group.

Educational curriculum (experimental variable)

After reviewing the sources and references, the researchers formulated the exercises in the content of the educational curriculum according to the mental maps, and arranged the mechanism of the
mental maps strategy in a way that includes first the educational section, which is the teacher explanation. The skill clearly and accurately for all its stages and its division in detail as well as the presentation of the model in the performance near the ideal and then the use of students who are able to implement the skill correctly, and includes the use of mental presentation. Maps prepared by researchers that were hung on the walls and yard of the school throughout the education period. In addition, students were assigned, starting with the third unit of each skill, to draw different mental maps of the acquired skills to familiarize students and teach them how to plan their minds as well as anchoring information in the student’s mind. Attempt to fix the information. Moreover, the researchers gave students an incomplete mind map of a certain part so that they would be asked to complete the missing part. The practical side, which lasted for 25 minutes, included the application of all that the student had learned in the educational aspect, in addition to the accompanying performance notes. The two researchers took several points in implementing its educational approach, namely:

1. The main research experiment was carried out on 11/11/2019 until 2/9/2020
2. The educational curriculum was implemented for a period of 12 weeks, at a rate of (2) educational units per week, and the total number is (24)
3. As (8) educational units were allocated for each skill.
4. The time of one educational unit is (45) minutes, in which the introduction and warm-up time is (10) minutes
5. The educational section time (10) minutes
6. The applied section time is 25 minutes

Note that the only variable between the control and experimental groups is only the applied section, as the experimental group implements the educational curriculum prepared by the researchers and the control group, it implements the educational curriculum of the (traditional) teacher, while the rest of the sections are similar to the two groups.

**Dimensional tests**

Researchers after the end of the educational variable over two days on 14-15/2/2020, and in at ten in the morning of and in the yard middle school marginal b tests tribal research sample group experimental and control, as follows: On the first day were tested and measured the experimental group in:

1. A measure of creative abilities
2. The two correction skills of high jumping and accurate shooting with hand ball.

In the second, the control group was tested with the same place, timing and verse followed for the experimental group.

The researchers also tried to simulate the conditions in the post-tests with the pre-tests.

**Statistical methods: (6: 335)**

The researchers used a set of statistical methods

)The arithmetic mean - a standard deviation - coefficient of variation -T For correlated and non-correlated samples(

**Chapter Three: Presentation, Analysis and Discussion of Results**

**Presentation and analysis of results**

Presenting the results of tests and measurements of pre and dimensional creative abilities, the two correction skills of high jumping and the accuracy of shooting with hand ball for the experimental and control groups:
Table No. (2) It shows the mean, standard deviations, mean difference, and value T Calculated and tabular between measurements and tests, before and after, for creative capabilities and skills of correction from high jumping and shooting accuracy of the experimental group.

<table>
<thead>
<tr>
<th>Moral</th>
<th>indication *</th>
<th>T Calculated</th>
<th>The error Standard</th>
<th>Average The differences</th>
<th>Post test</th>
<th>The pretest</th>
<th>Alone Measurement</th>
<th>Variables</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>moral</td>
<td>0.001</td>
<td>3.54</td>
<td>1.09</td>
<td>5.75</td>
<td>9.11</td>
<td>66.43</td>
<td>12.43</td>
<td>60.68</td>
<td>1</td>
</tr>
<tr>
<td>moral</td>
<td>0.003</td>
<td>3.12</td>
<td>1.21</td>
<td>2.06</td>
<td>1.43</td>
<td>5.74</td>
<td>1.12</td>
<td>3.68</td>
<td>2</td>
</tr>
<tr>
<td>moral</td>
<td>0.000</td>
<td>4.98</td>
<td>2.93</td>
<td>11.03</td>
<td>7.63</td>
<td>42.87</td>
<td>11.23</td>
<td>31.84</td>
<td>3</td>
</tr>
<tr>
<td>moral</td>
<td>0.001</td>
<td>3.57</td>
<td>1.89</td>
<td>5.25</td>
<td>1.32</td>
<td>8.42</td>
<td>0.69</td>
<td>3.17</td>
<td>4</td>
</tr>
<tr>
<td>moral</td>
<td>0.000</td>
<td>2.79</td>
<td>2.45</td>
<td>2.07</td>
<td>1.01</td>
<td>4.13</td>
<td>1.16</td>
<td>2.07</td>
<td>5</td>
</tr>
</tbody>
</table>

It is evident from the table that there are significant differences between the results of the pre and post tests and the validity of the post tests.

Table No. (3) It shows the mean, standard deviations, mean difference, and value T Calculated and tabular between measurements and tests, tribal and dimensional, of creative capabilities and the skill of correction from high jumping and shooting accuracy of the control group

<table>
<thead>
<tr>
<th>Moral</th>
<th>indication</th>
<th>T Calculated</th>
<th>The error Standard</th>
<th>Average The differences</th>
<th>Post test</th>
<th>The pretest</th>
<th>Alone Measurement</th>
<th>Variables</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not moral</td>
<td>0.02</td>
<td>1.63</td>
<td>1.97</td>
<td>1.68</td>
<td>10.01</td>
<td>61.36</td>
<td>11.74</td>
<td>59.68</td>
<td>1</td>
</tr>
<tr>
<td>Not moral</td>
<td>0.04</td>
<td>1.89</td>
<td>1.07</td>
<td>1.24</td>
<td>1.38</td>
<td>4.46</td>
<td>0.78</td>
<td>3.22</td>
<td>2</td>
</tr>
<tr>
<td>Not moral</td>
<td>0.03</td>
<td>1.53</td>
<td>1.87</td>
<td>3.59</td>
<td>7.14</td>
<td>35.63</td>
<td>13.65</td>
<td>32.04</td>
<td>3</td>
</tr>
<tr>
<td>moral</td>
<td>0.001</td>
<td>4.21</td>
<td>1.02</td>
<td>3.03</td>
<td>0.68</td>
<td>6.12</td>
<td>1.29</td>
<td>3.09</td>
<td>4</td>
</tr>
<tr>
<td>moral</td>
<td>0.000</td>
<td>2.40</td>
<td>1.67</td>
<td>1.2</td>
<td>0.82</td>
<td>2.60</td>
<td>0.86</td>
<td>1.40</td>
<td>5</td>
</tr>
</tbody>
</table>
It appeared from the table that the attached statistical significance is smaller than (0.05), which indicates the absence of significant differences in (fluency - kinematic originality - kinetic flexibility), while differences were made in (high-jump scoring and shooting accuracy) between the pre and post tests and the validity of the posttests.

**Table No. (4) Shows the arithmetic mean, standard deviations, and values of T( Calculated and tabular between measurements and dimensional tests of creative abilities and correction skill from high jumping and shooting accuracy with hand ball between the experimental and control groups**

<table>
<thead>
<tr>
<th>Moral</th>
<th>indication</th>
<th>T Calculated</th>
<th>Experimental group post-test</th>
<th>Control group Post test</th>
<th>Alone Measurement</th>
<th>Variables</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>deviation Standard</td>
<td>the middle Arithmetic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Standard</td>
<td>Arithmetic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>66,43</td>
<td>9,11</td>
<td>10,01</td>
<td>61,36</td>
<td>1</td>
</tr>
<tr>
<td>Moral</td>
<td>0.001</td>
<td>3.76</td>
<td>10,01</td>
<td>61,36</td>
<td>Degree</td>
<td>Fluency</td>
<td>1</td>
</tr>
<tr>
<td>Moral</td>
<td>0.002</td>
<td>3.86</td>
<td>1,38</td>
<td>4,46</td>
<td>Degree</td>
<td>Kinetic originality</td>
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</tr>
<tr>
<td>Moral</td>
<td>0.000</td>
<td>4,32</td>
<td>7,14</td>
<td>35,63</td>
<td>Degree</td>
<td>Kinetic flexibility</td>
<td>3</td>
</tr>
<tr>
<td>Moral</td>
<td>0.001</td>
<td>3,01</td>
<td>0,68</td>
<td>6,12</td>
<td>Degree</td>
<td>Shoot from jumping high</td>
<td>4</td>
</tr>
<tr>
<td>Moral</td>
<td>0.002</td>
<td>3,65</td>
<td>1,01</td>
<td>2,60</td>
<td>Degree</td>
<td>Aim accuracy</td>
<td>5</td>
</tr>
</tbody>
</table>

It appears from the table that there are significant differences between the post-tests and in favor of the experimental group.

Discussion and Analysis of the results of the tests and measurements of tribal and dimensionality of creative abilities and the skills of voting by jumping high and the accuracy of the correction experimental and control groups:

The researcher attributed the reason for the difference, disparity, progress and differences that occur in the experimental group in the creative abilities and skills of jumping shooting and the accuracy of correction in the dimensional tests to the educational method tests based on mental maps and exercises carried out in them. And the characteristics it contains that enable learners to understand and interact with the teacher quickly. Increased alerts about solution finding, engagement and exploration in learning the skills he receives from the teacher. It also helps them increase their cognitive abilities so that it will facilitate them to perform mental operations and apply them in the practical side (12: 167). The researchers also attributed the improvement and higher level of learning to the skills under study. The use of mental maps that increase the motivation and desire of the
learners due to the use of images, colors, shapes and written texts, which is a modern method that raises the individual learner to explore and track them to improve the state of learning and motivate students. The internal motivation plays a crucial role in the creativity process, because it stems from the inside of a specific goal. It shows the desire for research, knowledge, and a feeling of happiness in discovering reality and giving new ideas. Researchers consistent with what was indicated by Wissam Salah: “A study that increases the clarity of the skill and the method of learning it, in addition to increasing the ability to remember, in short and not branching, nor are you limited to presenting facts but rather than relationships. Between facts and this achieves meaningful learning” (14: 105). This is in agreement with a study confirming that the use of graphics and images in the mind map makes the learning process easier, as pictures and drawings represent a large part of memory, so it is easy for one to remember the mental map. The control that depends on the traditional method of the teacher showed some differences while other variables did not occur. The researchers attribute this to the nature of the traditional curriculum and the method of explanation in it for the educational department, and the method of the applied department is the other that allows and helps in progress, but within relative limits due to the old method and method. Reduces student interaction and not actually utilizing lesson time as in the experimental group. Therefore, the mind mapping strategy requires the student to be able to think about how the diagrams are formed and thus get a complete idea of the pain. Which helps to learn the skill better and this strategy is one of the new and unconventional strategies, which leads to an increase in learners’ desire and enthusiasm for learning while eliminating their boredom factor, as well as the presence of drawings and Pictures and colors that increase learners’ interest and thus help them learn. As this method of learning raises alerts to more than one sense among the learners, including the use of modern technologies such as the plasma device, which increases mental processes such as attention and focus and confirm that the use of more than one sense during learning has an effect. Effective in the learning process (8: 134). The researchers also attributed the reason for the difference and the disparity in the results of the two groups of research to the fact that the educational curriculum and the sequential exercises that they prepared from easy to difficult. Repetition and method of performance also contributed to increasing the effectiveness of learning basic skills. Emphasized: “It is necessary to focus on the implementation of the educational activity from the lesson using modern methods to repeat the task of the movement and its training, in addition to making the most of the implementation time in the lesson and achieving more effectiveness. (89: 8) Handball is a game. Their performance requires mastering all basic skills, whether physical, skill or planning, in order to succeed in the offensive plans in place and thus score more points that lead to winning matches, and these requirements depend on the existence of a sufficient number of physical numbers for the players. The close connection between physical attributes and skilled performance is what leads the team to optimal performance and achieving the best levels and under the most difficult circumstances, and we note that these physical characteristics are explosive power, speed, flexibility and accuracy. Sports training depend on raising the efficiency of the functional body organs through the internal load that will lead to the adaptation of the body systems and the acceptance of the load. Handball confirms interest in the principle of using pressure (training load) and rest properly, when given high pressure or high training load, adequate positive rest periods must be provided for the purpose of restoring the nervous system the ability to function through the sensory receptors as well as “to save energy.
The researchers agree with what pointed out that (determining the training load depends on knowledge of the basic power supply system) (1998, (75)). The sample has a clear difference in the explosive force variable of the arm and its connection to the good performance of the shooting skill, and the researchers also noted that some players had physical requirements for better performance than others, and there are some players who have good performance with high technology and thus lead to achieving points, which formed a clear picture for the researchers of the relationship between these requirements and the shape of the performance and the end of the movement is one of the important factors in achieving a high score in the test, which leads us to say that these two matters require emphasis and training. Equally and with different intensities to stabilize the performance form and access the performance mechanism, researchers agree with what he indicated. The basic motor skills of the game are the backbone of the training process at the present time, as it is difficult to correct errors later on, and the unskilled player cannot “control the ball E and its movements”.

**Conclusions**

1. The prepared exercises and their formulation and application contribute to the development of students’ creative abilities

2. The mind map strategy is better than the traditional method through the research results in the creativity and shooting skills of high jumping and shooting accuracy

3. The use of mental maps in the learning process reduced the wasted time and shortened time in the learning process in learning the skills of correction and accuracy.

**Recommendations**

1. Emphasis on the application of the mental maps method in learning correction and accuracy in handball.

2. It is necessary to emphasize in formulating exercises how to use them to develop creative capabilities.

3. You must choose the appropriate method and method with the mental, age and physical stage of the learners, to make use of time fruitfully.

4. The necessity of creating the educational environment with the capabilities and tools necessary to implement the mental maps strategy.

5. Conducting similar studies to develop mental processes, motor abilities and intelligence, using the mental mapping strategy.

**Ethical Clearance:** People identified as potential research participants because of their status as relatives or carers of patient’s research participants by virtue of their professional role in the university and departments.

**Source of Funding:** Self-Funding

**Conflict of Interests:** The authors declare there is no conflict interest

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Effectiveness of Antenatal Educational Guidelines on Maternity Nurse’s Performance

Samah Abd Elhaleim Said1, Amira Mohammed Salama1, Hemmat Mostafe Elbana1

1Assistant Professor of Obstetrics and Woman’s Health Nursing. Faculty of Nursing, Benha University, Egypt.

Abstract

Antenatal care is a detailed, systematic examination and follow-up of pregnant female that includes education, counseling, monitoring and therapy to address and treat minor issues and provide appropriate screening during pregnancy. Aim of the study: Evaluate Antenatal education guidelines’ effectiveness on maternity nurses’ performance (Knowledge and Practice). Design: A quasi-experimental design (pre-test-post-test). Setting: study was conducted in obstetric skill lab of faculty of nursing, Benha university. Sample: A purposive sample was used to recruit 140 maternity nurses from Maternal and child health centers at kalioubia governorate. Tools: two data collection tools: A self-administered questionnaire sheet and an observational practice checklist. Results: Highly statistically significant improvement in maternity nurse’s knowledge score, it changed from 51.4% to 85% at post-intervention. Maternity nurses’ practice mean score was satisfactory improved, as the mean score increased from 56.4% to 82.1% at the post-intervention phase. Highly positive correlation between total nurse’s knowledge and practice score regarding antenatal care was found. Conclusion: Provision of antenatal educational guidelines helped strengthen the awareness and practice of maternity nurses on the various aspects of the antenatal period. Recommendations: The care of pregnant women should be an integral part of the educational curriculum for nursing students.

Keywords: Antenatal care, Educational guidelines, maternity Nurses’ performance, pregnant women, Misconceptions.

Introduction

The antenatal period is considered one of the most stressful events in every woman’s life. It is often associated with some complications and diseases that may threaten the mother and baby’s life. Hence, antenatal care is very important to ensure a healthy baby’s delivery from a healthy mother and reduce maternal and fetal morbidity and mortality (1).

The key aim of antenatal care is to reduce child and maternal mortality rates. (ANC) is pregnant women’s support for safe pregnancy and healthy infants. ANC is essential to healthy motherhood, but its use varies widely. ANC is potentially one of the most successful health strategies to prevent maternal morbidity and mortality, especially where women’s overall health status is low (2). Education is an essential component of prenatal care, particularly in the 1st time pregnancy (3).

Regular interaction during pregnancy with nurses or midwives enables women to access services vital to women’s health. In order to minimize perinatal mortality and improve the experience of women in treatment, the World Health Organization (WHO) recommends a minimum of eight antenatal care contacts. However, important measures can be given at prescribed intervals over four visits, at least for
healthy women without any underlying health issues (4).

Preventing unwanted pregnancy outcomes depends on an organizational continuum of service before and during pregnancy, childbirth, and the postnatal period, with affordable, high-quality care. It also relies on the assistance available to help pregnant women access facilities, particularly when complications arise. The reliable ANC is an essential element in this continuum of treatment. The purpose of the ANC plan is to prepare for birth and parenthood and to avoid, recognize, mitigate or treat the three forms of pregnancy health problems that affect mothers and babies (5).

In the advancement of reproductive health, maternal health services have a potentially important role. The use of health care is related to availability, efficiency, cost of services, social structures, health values, and personal characteristics. Every year, over half a million women die from pregnancy or childbirth complications. Most maternal deaths occur during childbirth, and these numbers could be significantly decreased by the involvement of skilled medical professionals (6).

Behaviors related to the health of their patients, the awareness and practices of health professionals, especially maternity nurses, have an important role. During pregnancy, this effect can be much greater since there is regular interaction between women and health providers during periodic prenatal visits. These professionals will serve as health promoters when educated, assisted, and inspired (7).

Nurses play a major role in helping the pregnant woman to achieve the goal of optimal health through ongoing assessment and education so, Awareness and qualification of the practitioners participating in the care process must be raised, so that the requisite technical and scientific information is coupled with the dedication to a satisfactory health care outcome (8).

Significance of the study:

There is no more tragic situation as a mother loses her life when she gives life to her baby. Millions of women in developing countries are unable to receive antenatal care for a variety of factors. This results in increased maternal and newborn mortality and morbidity rate. The world health organization estimated that 4.3 million fetal deaths occur before or during delivery due to poorly managed pregnancies and delivery (9).

There is exceptionally high maternal mortality. In 2017, nearly 295,000 women died during and after pregnancy and childbirth. In low-resource environments, the vast majority of these deaths (94 percent) occurred, and most could have been avoided. Approximately 86 percent (254,000) of the reported global maternal deaths in 2017 were in Sub-Saharan Africa and Southern Asia. Sub-Saharan Africa alone accounted for about two-thirds (196,000) of maternal deaths, whereas approximately one-fifth (196,000) of maternal deaths were in Southern Asia (58,000) (9).

The maternal mortality rate in Upper Egypt is higher than in Lower Egypt (74-61 percent), according to the Egyptian Ministry of Health. In addition, post-partum hemorrhage (19.7 percent) is the most common cause of maternal mortality in Egypt, while cardiovascular disease (16 percent) is the most common indirect cause (1400 women and 50 percent of their newborns die each year in Egypt due to complications of pregnancy and childbirth). MMR is still high in Egypt according to global standards, so more efforts should be made (10).

The available Egyptian research that measures the satisfaction of women with prenatal care obtained from primary health care centers of various aspects of standard of care reported that sixty percent of mothers were very low or low satisfied with the data they
received during prenatal care (11).

Current study was carried out to evaluate maternity nurses’ knowledge and practices regarding antenatal care, design, implement and evaluate guidelines focusing on pregnant women’s health promotion.

**Aims of the Study**

The present study aimed to evaluate the effectiveness of antenatal education guidelines on maternity nurse’s performance (Knowledge and Attitude) through:

- Assessing nurses’ knowledge and practice about antenatal care.
- Designing and implementing an educational guideline for nurses to improve their knowledge and practice regarding antenatal care.
- Evaluating the effect of an educational guideline on maternity nurse’s knowledge and practice of antenatal nursing care.

**Research hypothesis:**

Maternity nurses who receive antenatal educational guidelines will exhibit an improvement in their knowledge and practice regarding antenatal care after intervention than before.

**Subjects and method:**

**Design:** A quasi-experimental design (pre & post-test) single group. The beginning was in January (2020) to the end of September (2020)

**Setting:** study was conducted in obstetric skill lab of faculty of nursing, benha university.

**Sample:** A purposive sample of 140 maternity nurses from 25% of the total 185 maternal and child health centers at kalioubia governorate. They were randomly selected. **Extracted from the research project.**

**Tools:** two data collection tools were used:

**Tool I: Self-administered questionnaire sheet:**

After reviewing current and related national and international literature, the researcher constructed this tool. It consisted of two parts:

**Part one:** Personal and socio-demographic data.

**Part two:** Assessment of maternity nurses’ knowledge regarding pregnant women’s antenatal care included three sections.

**Section (1):** Knowledge about nursing care during antenatal care included five items (the concept of antenatal care, antenatal care objectives, and the principle of antenatal care, first visit procedure, and follow-up appointments).

**Section (2):** Knowledge about pregnancy included nine items (symptoms of confirmation pregnancy, early signs of pregnancy, investigation required for pregnant women, vaccinations required during pregnancy, minor discomfort and danger signs during pregnancy, the group at risk during pregnancy, physiological and psychological changes during pregnancy).

**Section (3):** Knowledge about health education during antenatal care included 12 items (health education about the importance of nutrition, essential elements for pregnant women and fetus, exercise, reduce fatigue, sleep, clothes, occupation during pregnancy, sexual relationship, personal hygiene, taking medication, travel during pregnancy and preparation for breastfeeding.)

**Scoring:** When the answer was completely correct, each item was scored as (2), a score (1) was given when the answer was not completely correct, and a score (0) was given when the answer was incorrect. The overall knowledge score was categorized as follows:
-Inadequate knowledge: < 60% of total knowledge score.

Adequate knowledge: ≥60% of total knowledge score.

**Tool II: Practice Observational checklist:** The researcher designed this tool after reviewing related literature (12) to assess the practice of maternity nurses during antenatal care: it was included seven sections (history taking, physical, abdominal examination-uterine fundus, auscultation of fetal heart rate, urine analysis, and breast care).

**Scoring:** Each item was scored as (0) for not done and (1) for done. Then adding up the scores of the items in each procedure and the overall scores gave practice score. Total practice score was classified as the following: -

- Incompetent practice < 75% of total practice score.

- Competent practice ≥75% of total practice score.

**Supportive material :-( Arabic Leaflet booklet)**

The researcher designed the educational guideline in the Arabic language supported by figures after reviewing related literature. It included two theoretical and four practical parts. The theoretical part, the concept of antenatal care, antenatal care objectives, and the principle of antenatal care, first visit procedure and follow-up appointments, investigation required for pregnant women, discuss pregnancy’ signs and symptoms, and minor discomfort of pregnancy. Vaccinations required during pregnancy, minor discomfort and danger signs during pregnancy, group at risk during pregnancy, physiological and psychological changes during pregnancy the basic elements of the requirements of a pregnant woman and fetus while the practical part explained the procedure’s nurses need to take care of women during the antenatal period, such as history taking, abdominal examination, auscultation of fetal heart rate, urine analysis, and breast care and so on.

**Tools Validity**

By presenting the questionnaire to five maternity nursing experts, the tool validity was tested by face validity and asked them to read it and assess the content in terms of whether it represents the principles intended to test and to decide its readability and consistency in order to achieve a consensus on the best method to be implemented. The required adjustment was done in the form of some questions being added and omitted.

**Reliability:**

The test-retest was replicated on two occasions on the same sample of maternity nurses and then the findings were compared. The Cronbach alpha coefficient for the knowledge questionnaire was 0.7 and 0.82 for practice.

**Pilot study:**

After designing instruments, a pilot study was performed on 10 percent of the studied subjects (14) maternity nurses. It was aimed at determining the tools’ usability and clarity. It also helped to predict the time required for the forms to be filled in. As no change was done, participants of the pilot study was included in our sample.

**Fieldwork:** Maternity nurses were recruited and followed-up from the beginning of January (2020) to the end of September (2020), completing nine months. From 9 am to 2 pm, two days weekly, until the previous predetermined sample size, the researchers attended the pre-mentioned study setting. Written permission was obtained from the predetermined institutional authority of health centers prior to conducting the research. After that, the researchers introduced themselves to women and told them of the purpose of this study in order to obtain their
approval and cooperation. Data confidentiality was ensured to gain the trust and confidence of women. The researchers designed and created the various data collection instruments, developed the materials for antenatal educational guidelines, and updated them by maternity nursing and obstetric medicine experts. Data collection included two periods: pre-test and post-test periods; data from pregnant nurses was collected in the pre-test. At the same time, after applying educational guidelines, the researchers initiated the intervention and assessed them immediately. Data collection was carried out in four phases: the assessment phase, the planning phase, the implementation phase and the evaluation phase.

1- Pre-implementation phase (Initial assessment):
   A. Interviewing Phase:

   This phase encompassed interviewing to collect personal and socio-demographic data and maternity nurses’ knowledge regarding antenatal care of pregnant women. It was used two times (pre-post-test). The questionnaire (pre-test) self-administered to each maternity nurse individually. It was directed in simple Arabic language, and answers were recorded immediately. The researchers greeted the maternity nurse, introduced themselves to each maternity nurse included in the study, explained the study’s purpose, and explained how to fill the tools accurately after obtaining their acceptance to share in the study. The tools of data collection required approximately (15-25 minutes).

   A- Practice observational checklist: It was filled by the researchers using an observational checklist for pregnant women’s antenatal care to assess maternity nurses’ practice pre implementing educational guidelines. The checklist was used two times (pre-post-test). The maternity nurses were unaware that they were being observed. Each observation sheet was filled immediately while observing the maternity nurse when performing the procedure. The average time needed to complete each observational checklist took about (30 – 45) minutes.

   **Planning phase:**

   The researchers developed the educational guidelines based on the interview sheet results from the pilot study, assessment phase (pre-test) and review the related literature. Identified needs, requirements and weaknesses were translated into educational guidelines’ goals and objectives. The educational guidelines’ contents were selected based on identified needs about knowledge and practice regarding antenatal care. Different teaching strategies and methods were chosen to fit small group discussion. An objective of the educational guidelines was to improve knowledge and practices for maternity nurses regarding antenatal care.

   **Implementation phase:**

   The theoretical and practical parts of educational guidelines were discussed and demonstrated through group discussion sessions. The researchers conduct six sessions (two theoretical sessions, the duration of each session was ranged from 30-45 minutes, including periods of discussion according to nurses’ achievement and feedback followed by four practical sessions, and the duration of each session was ranged from 45-60 minutes included) used for each group. Nurses have been split into small groups (10-14 nurses/session). Each group perceived the content of the educational guidelines using the same teaching strategies and handouts. The total number of groups was (10 groups) and the total time for achieving the educational guideline was (6 hours) for each group under the study.

   - **First theoretical session:** concerned with introducing the theoretical part of the educational guidelines and provided nurses with general knowledge about pregnancy.
- **Second theoretical session**: started with feedback about the previous session and an introduction to the new session’s objective. The researcher then provided nurses with knowledge about nursing care during antenatal care and health education provided during antenatal care. At the end of the session, the researchers allowed nurses to ask questions and provided a discussion period.

- **Third practical session**: It implied the implementation of the practical part of the educational guidelines for all antenatal care cases and concerned with training nurses regarding history taking and physical examination.

- **Fourth practical session**: started with feedback and re-demonstration of the previous session and introducing the new session’s objectives. Then the researchers demonstrated the procedure abdominal examination.

- **Fifth practical session**: included re-demonstration of previous procedures and demonstration Auscultation of fetal heart rate.

- **Sixth practical session**: included re-demonstration of previous procedures and demonstrating auscultation of heart rate and urine analysis for albumin and sugar and breast care.

**Evaluation phase:**

The educational guideline’s effect was assessed using the same format of tools used before implementing the educational guideline tool I theoretical session and tool II practical parts (pre-test). An immediate evaluation was conducted after implementing the educational guidelines to evaluate nurses’ knowledge gain and practical skills.

**Data Analysis**

Data was summarized and analyzed using statistical package for social sciences (SPSS) software, version 20. Frequency distribution for categorical variables was used for their description. means with standard deviations was used for continuous variables description. To measure tool reliability, Cronbach’s alpha was used.

**Results**

**Table (1): Distribution of the studied nurses according to socio-demographic characteristics (n=140).**

<table>
<thead>
<tr>
<th>socio-demographic characteristics</th>
<th>no</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>7</td>
<td>5.0</td>
</tr>
<tr>
<td>25-&lt;30</td>
<td>42</td>
<td>30.0</td>
</tr>
<tr>
<td>30-&lt;35</td>
<td>30</td>
<td>21.4</td>
</tr>
<tr>
<td>35+</td>
<td>61</td>
<td>43.6</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>38.24±7.84</td>
<td></td>
</tr>
<tr>
<td><strong>Educational level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diploma</td>
<td>16</td>
<td>11.4</td>
</tr>
<tr>
<td>Associated degree</td>
<td>40</td>
<td>28.6</td>
</tr>
</tbody>
</table>
Continued... Table (1): Distribution of the studied nurses according to socio-demographic characteristics (n=140).

<table>
<thead>
<tr>
<th>Experience</th>
<th>Bachelor degree</th>
<th>Post graduate</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>5</td>
<td>3.6</td>
</tr>
<tr>
<td>5-&lt;10</td>
<td>43</td>
<td>30.7</td>
</tr>
<tr>
<td>10+</td>
<td>92</td>
<td>65.7</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>12.57±8.95</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Training program</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>119</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>85.0</td>
<td>15.0</td>
</tr>
</tbody>
</table>

Table (1) represented the studied nurses’ socio-demographic characteristics; the results revealed that the women’s mean age was 38.24±7.84. Moreover, 51.4% of the studied nurses had a Bachelor’s degree in nursing. Besides, 65.7% of the nurses had more than ten years of experience, and 15.0% had training programs.

Table (2): Mean score of the studied nurse’s knowledge regarding antenatal care pre and post-application of educational guidelines (n=140).

<table>
<thead>
<tr>
<th>Total knowledge</th>
<th>Maximum score</th>
<th>Pre Intervention</th>
<th>Post Intervention</th>
<th>Independent t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nursing care during antenatal visits</td>
<td>5</td>
<td>2.32±1.04</td>
<td>4.15±0.61</td>
<td>5.11</td>
<td>0.000**</td>
</tr>
<tr>
<td>Nursing information</td>
<td>9</td>
<td>5.01±2.05</td>
<td>7.42±1.13</td>
<td>8.02</td>
<td>0.000**</td>
</tr>
<tr>
<td>Health education regarding antenatal period</td>
<td>12</td>
<td>5.65±1.71</td>
<td>9.61±2.21</td>
<td>10.75</td>
<td>0.000**</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>13.68±4.20</td>
<td>21.31±3.24</td>
<td>15.67</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

Table (2) Mean differences score of total nurse’s knowledge regarding antenatal care pre and post-application of educational guidelines. The results showed that there was a significant improvement between pre, post percentage of nurses’ knowledge.
Table (3): Mean score of the studied nurse’s practices regarding antenatal care pre and post-application of educational guidelines (n=140).

<table>
<thead>
<tr>
<th>Total practices</th>
<th>Maximum score</th>
<th>Pre-intervention</th>
<th>Post-intervention</th>
<th>Independent test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>History taking</td>
<td>10</td>
<td>5.12 ±1.21</td>
<td>7.24 ±2.51</td>
<td>9.31</td>
<td>0.000**</td>
</tr>
<tr>
<td>Physical examination</td>
<td>38</td>
<td>15.51 ±3.15</td>
<td>27.89 ±7.23</td>
<td>28.12</td>
<td>0.000**</td>
</tr>
<tr>
<td>Abdominal examination</td>
<td>10</td>
<td>4.85 ±1.41</td>
<td>8.71 ±1.21</td>
<td>7.75</td>
<td>0.000**</td>
</tr>
<tr>
<td>Uterine funds</td>
<td>4</td>
<td>2.08 ±1.20</td>
<td>3.31 ±0.54</td>
<td>4.67</td>
<td>0.000**</td>
</tr>
<tr>
<td>Auscultation of fetal heart rate</td>
<td>12</td>
<td>5.47 ±1.54</td>
<td>7.21 ±2.13</td>
<td>5.54</td>
<td>0.000**</td>
</tr>
<tr>
<td>Urine analysis</td>
<td>5</td>
<td>0.28 ±0.24</td>
<td>2.35 ±0.98</td>
<td>2.15</td>
<td>0.042*</td>
</tr>
<tr>
<td>Breast care</td>
<td>16</td>
<td>7.36 ±2.51</td>
<td>10.54 ±3.25</td>
<td>10.72</td>
<td>0.000**</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>38.92 ±7.91</td>
<td>67.65 ±12.25</td>
<td>13.21</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

**Table (3)** Mean differences score of total nurse’s practice regarding antenatal care pre and post-application of educational guidelines; the results revealed a significant improvement between pre and post percentage of nurses’ practice.

**Table (4):** correlation between total knowledge and total practices among studied nurses pre and post-application of the educational guideline (n=140).

<table>
<thead>
<tr>
<th>Practices</th>
<th>Knowledge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-program</td>
</tr>
<tr>
<td></td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>0.71</td>
</tr>
</tbody>
</table>

**Table (4)** illustrated the correlation between total knowledge and total practices among studied nurses; the results showed a positive correlation between nurses’ knowledge and practice pre and post-application of educational guidelines.
Discussion

Pregnancy is a crucial time for promoting good habits and parenting abilities. Strong antenatal care (ANC) integrates the mother and the family of women with the formal health system, improves the likelihood of using a trained birth attendant and leads to good health across the life cycle. Insufficient support during pregnancy breaks a vital connection in the continuum of care and impacts women and infants alike (WHO)\(^{(9)}\).

The present study aimed to evaluate the effectiveness of antenatal educational guidelines on maternity nurses’ performance (knowledge & practice). The present study results significantly proved the research hypothesis that an educational guideline help improving maternity nurses’ knowledge and practice regarding antenatal care.

The current study’s findings were discussed in the following four sections: Section I: shows the socio-demographic characteristics. Section II: related to mean score of nurses’ knowledge regarding antenatal care of pregnant women. Section III: related to mean score of nurses’ practice related to antenatal care of pregnant women, and Section IV: represent the correlation between total knowledge and practice among studied maternity nurses pre and post-application of educational guidelines.

Regarding socio-demographic characteristics, the present study findings revealed that the mean age of study nurses was 38.24±7.84, and half of them had Bachelor’s degrees. More than two-thirds of them had years of experience, more than ten years. The minority of the studied nurses had training programs regarding antenatal care. These findings followed maria et al.,\(^{(7)}\) who found that the health professionals’ mean age was 39.4 years, with more than ten years of antenatal care experience. On the other hand, these results disagreed with Ruby et al.,\(^{(13)}\) who found that most nurses belonged to the age group 18-32 years. This may be because nurses are full of energy and hyperactivity at a young age, which is always required in such crucial departments.

Additionally, this result disagreed with Babulal \(^{(14)}\) who stated that most studied nurses had less than ten years of experience.

Concerning educational level, this result disagrees with Nasser et al.,\(^{(15)}\) who found that most of participants (two thirds) hold a diploma in midwifery and nursing secondary school graduates.

Additionally, regarding the training course, this result agrees with Lisette \(^{(16)}\) who reported that nurses were not always given sufficient training during education or after begun clinical work, which increases stress and makes them less efficient in the work.

Moreover, concerning studied nurses’ total knowledge about antenatal care of pregnant women at the pre-test, the study revealed that more than half of the studied nurses had inadequate knowledge regarding antenatal care, which improved their knowledge after implementing educational guidelines. This result may be due to the absence of training courses regarding antenatal care among study nurses, the absence of refreshing guidelines related to the concept and objective of antenatal care, and the limited number of nurses who attended the training courses.

Additionally, these results were supported by Mohamed & Salem,\(^{(17)}\) they suggested that near two-thirds of studied nurses had inadequate and low scores of knowledge related to antenatal care. This may be due to improper knowledge and lack of motivation to update their knowledge.

Moreover, the present study findings elaborated that there were highly statistically significant differences at pre & post-application of educational
guidelines (p<0.001) mean total scores of maternity nurses knowledge was (13.68 ± 4.20 & 21.31 ± 3.24) pre and post-intervention, respectively. These findings were congruent with Saber, (18) they reported that there was a statistically significant difference regarding all items of the knowledge before and after implementation of educational guidelines with increased knowledge of nurses after implementation regarding antenatal care during pregnancy (p=< 0.000**).

In addition to the studied nurses’ total knowledge about pregnant women’s nursing care at the pre-test, the study revealed that there were highly significant differences of the studied nurses in the nursing care of pregnant women (2.32±1.04 & 4.15± 0.61) pre and post-intervention, respectively. These results were in the same line with Mohamed et al., (19) who reported a significant improvement in the nurse’s knowledge score about nursing care during pregnancy.

Regarding nurses’ knowledge about health education given to women during antenatal care, the present study findings showed a highly significant difference in the mean scores of the studied nurses (5.65±1.71& 9.61±2.21) pre and post-intervention, respectively. Lee et al.(20) supported these findings, they found that the women had inadequate awareness of the nutritional guidelines and minimal knowledge obtained from their providers of service. Similarly, as well as time constraints, insufficient knowledge of nutrition and a lack of nutrition preparation have impaired the ability of clinicians to provide adequate nutrition education.

Regarding nurse’s total antenatal care practice, the present study findings reported that more than half of nurses had incompetent practice regarding antenatal care. This finding was in line with (Mohamed et l., (19) who stated that about half of the nurses studied had an unsatisfactory pre-program total practice score, which improved to a highly satisfactory post-program total practice. On the other hand, this result was in contrast with Gooda et al(21) who reported a significant improvement in nurses” practice regarding antenatal care at the pre-intervention phase.

Concerning the mean total practice score of nurses regarding pregnant women’s care, the present study revealed a highly statistically significant difference between pre & post-intervention (p<0.001). The mean total score of maternity nurses’ practice was(38.92 ± 7.91& 67.65±12.25) at pre-post intervention respectively. This result agrees with Mohamed et al., (19) who reported that most of the studied nurses had unsatisfactory total practice score pre-program, which improved to high satisfactory total practice post-program. This result was also consistent with Elmorsy(11) who reported unsatisfactory practice of most nurses regarding antenatal period pre-application of educational guidelines. Meanwhile, the majority of them had satisfactory practice regarding the antenatal period post-application of the educational guideline. This result could be explained by lack of nurses’ knowledge, which reflected in their practice.

Regarding the correlation between total scores of maternity knowledge and practices, the present study’s finding showed a statistically significant positive correlation between total knowledge and total practice scores pre and post-application of the educational guideline. This result may be because a good level of knowledge positively affects the level of practice.

This result is in the same line with Gooda et al. , (21) who reported a positive, statistically significant relationship between nurses’ knowledge and practice and their age and years of experience before and after application of the nursing protocol (p<0.001).

Conclusion

Based on our findings, the research hypothesis is supported, and nurses’ performance (knowledge &
practice) shows a significant improvement in post-implementation of educational guidelines regarding antenatal care for maternity nurses compared to pre-implementation. Furthermore, there was a positive correlation between total knowledge and total practice of studied nurses at pre-implementation phases, while there was no correlation between total knowledge and total practice of studied nurses at post-implementation phases.

**Recommendations**

- The care of pregnant women should be an integral part of the educational curriculum for nursing students.
- Implementing regular training programs for nurses to update their knowledge and practice towards antenatal care.
- Further research:
  - Applying health education for all maternity nurses about antenatal care schedules in all Maternal and Child Health Centres (MCH).
- Effect of simulation training on nurses’ performance regarding antenatal care.

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**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Ethics:**

Ethical approval was taken from Faculty of Nursing’s Ethical Committee, Benha University, this research was conducted. Written and verbal informed consent was taken from the participants before collecting data and participants’ privacy and confidentiality were maintained during data collection and publication.

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A Study to Assess the Effectiveness of Deep Breathing Exercise on Blood Pressure and Heart Rate Among Hypertensive Patients in Selected Area of Kheda District

Vahora Anika¹, Patel Charmi¹, Ninama Hetal¹, Thakor Prachi¹, Momin Sakina¹, Damor Sarjana¹, Sachi Christia²

¹Final year B.Sc. Nursing Student Dinsha Patel College of Nursing, Nadiad, Gujarat, India, ²Assistant Professor, Dinsha Patel College of Nursing, Nadiad, Gujarat, India

Abstract

Background of Study: Despite the significance of the problem with respect to overall health and its undesirable health consequences, high blood pressure still is not adequately controlled and far from being optimal. An estimated 1.13 billion people worldwide have hypertension (WHO may 2019). In India incidence rate of hypertension is 29.8% (WHO September 2018). In Gujarat incidence rate of hypertension is 27.1% (JNC-7). Quasi experimental research design (pre test post test research design), Quantitative research approach was used to assess the effectiveness of deep breathing exercise on blood pressure and heart rate among hypertensive patients in selected area of Kheda district. The collected data was tabulated and analyzed using descriptive and inferential statistics. Mean, and standard deviation was calculated by the obtained data in which mean score of blood pressure was 127.46 in pre test and 78.63 in post test and score of standard deviation was 5.44 in pre test and 4.88 in post test. The mean score of heart rate was 86.2 in pre test and 63.01 in post test and score of standard deviation was 3.86 in pre test and 2.59 in post test.

Keywords: - Deep breathing exercise, Blood pressure, Heart rate, Hypertensive patient.

Introduction

Hypertension is a widespread health problem and is called the “silent killer” because it often has no warning signs or symptoms, and many people don’t realize that they have it. Hypertension is another name for high blood pressure. It can lead to severe health complications and increase the risk of heart disease, stroke, and sometimes death. Many pharmacological and non-pharmacological interventions effective in treating essential hypertension, Pharmacological therapy depends on antihypertensive drugs, while non-pharmacological therapy involved five lifestyle modifications are recommended by JNC 7 for reducing blood pressure: reducing sodium intake, increasing exercise, limiting alcohol consumption, dietary changes, losing weight in addition to; other lifestyle interventions as smoking cessation, dietary supplements and meditation. The last one includes careful attention to the process of breathing, to achieve a state of inner calm, detachment, and focus (Mancia et al.,2013, McElroy, et al., 2012, a Deep breathing increases blood and oxygen flow to the brain to function in its optimal state. It improves circulation, which improves heart health, energy levels and helps the body eliminate toxins, as well as reduces

Corresponding author:-
Ms. Sachi Christian,
Assistant Professor, Dinsha Patel College of Nursing, Nadiad, Email Id: sachichristian@gmail.com
Address:- Dinsha Patel College of Nursing, Behind Hyundai Showroom, College road, Nadiad, District-Kheda, Gujarat-387001.
stress (Shakeshaft, 2012). Hence, practicing deep breathing exercise influence autonomic functions and has therapeutic benefit to hypertensive patients (Almahrezi, et al., 2008) and (Ribeiro, et al., 2011).

Need of the Study:

Hypertension is major health problem in India, contribute to significant morbidity as well as mortality among with the significant sociology economically losses. Study on the hypertensive patients either in community, inpatient, outpatient, or different hospital have shown that hypertension is the commonest medical health problems. Previously study has found that breathing exercise is very beneficial for hypertensive patients.

Deep breathing is an easy exercise, non-invasive and painless. It can be done at any place, less time consuming, and cost effective. Therefore, this study was conducted to examine the efficacy of deep breathing exercises on hypertension and heart rate as a non-pharmacological complementary approach to treat essential hypertensive patients; make these gentle practices a worthwhile activity to incorporate into a healthy lifestyle. Hopefully this study could add an important evidence based practice to the concerned patients and health care providers.

In that Deep Breathing exercise is one of the exercise and relaxation technique which helps to maintain the normal blood pressure. The goal of the study is to determine the effectiveness of deep breathing exercise on blood pressure among patient with hypertension.

Objectives of the Study

1. To assess the blood pressure level of the hypertensive patient.

2. To evaluate the effect of deep breathing exercise on blood pressure among the hypertensive patients.

3. To assess the pre test and post test level of blood pressure and heart rate among hypertensive patient between control and interventional group.

4. To find out the association of heart rate and blood pressure level with selected demographic variable among hypertensive patients.

Hypothesis

H1 There will be a statistical significant difference in blood pressure before and after deep breathing exercises intervention at the level of p<0.05.

H2 There will be statistical significant difference in heart rate before and after deep breathing exercise intervention at the level of p<0.05.

Material and Methods

Research Approach: Quantitative research approach is used for this present study.

Research Design: Quasi experimental research design (pre test post test research design).

Population:

Target population of study was all hypertensive patients in selected area of Kheda district.

Sample Technique:

Purposive non probability sampling technique used to select sample

Sample Size:

The sample size comprised of 60 hypertensive patient (30 experimental group and 30 control group)

Description Tool:

Part A: Demographic data

Part B: modified deep breathing exercise
Inventory was used.

**Results and Finding of the Study**

**Table No. 1 Demographic variables**

<table>
<thead>
<tr>
<th>Demographic variable</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) 41-50</td>
<td>17</td>
<td>28.33</td>
</tr>
<tr>
<td>b) 51-60</td>
<td>19</td>
<td>31.66</td>
</tr>
<tr>
<td>c) 61-70</td>
<td>14</td>
<td>23.33</td>
</tr>
<tr>
<td>d) 71-80</td>
<td>10</td>
<td>16.66</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Male</td>
<td>27</td>
<td>45</td>
</tr>
<tr>
<td>b) Female</td>
<td>33</td>
<td>55</td>
</tr>
<tr>
<td>c) Transgender</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Illiterate</td>
<td>1</td>
<td>1.66</td>
</tr>
<tr>
<td>b) Primary</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>c) Secondary</td>
<td>19</td>
<td>31.66</td>
</tr>
<tr>
<td>d) Higher secondary</td>
<td>17</td>
<td>28.33</td>
</tr>
<tr>
<td>e) Graduation</td>
<td>10</td>
<td>16.66</td>
</tr>
<tr>
<td>f) Post graduation</td>
<td>1</td>
<td>1.66</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Unemployment</td>
<td>34</td>
<td>56.66</td>
</tr>
<tr>
<td>b) Self employment</td>
<td>4</td>
<td>6.66</td>
</tr>
<tr>
<td>c) Private employment</td>
<td>5</td>
<td>8.33</td>
</tr>
<tr>
<td>d) Govt employment</td>
<td>8</td>
<td>13.33</td>
</tr>
<tr>
<td>E) Business</td>
<td>1</td>
<td>1.66</td>
</tr>
<tr>
<td>f) Retired</td>
<td>8</td>
<td>13.33</td>
</tr>
<tr>
<td><strong>Year of suffering from HTN</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) &lt;5yr</td>
<td>23</td>
<td>38.33</td>
</tr>
<tr>
<td>b) 5-10yr</td>
<td>27</td>
<td>45</td>
</tr>
<tr>
<td>c) 11-15yr</td>
<td>10</td>
<td>16.66</td>
</tr>
<tr>
<td>d) &gt;15yr</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Year of taking medicate on of HTN</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) &lt;3yr</td>
<td>22</td>
<td>36.66</td>
</tr>
</tbody>
</table>
Reveals with regard to age, the majority of the patients 19 (31.66%) belonged to 51-60 years, 14 (23.33%) patient belong to 61-70 years, 17 (28.33%) patients belong to 41-50 years and only 10 (16.66%) belonged to 71-80 years in group of hypertensive patients.
Regarding gender, 27 (45%) were males and 33 (55%) were females in selected group. Regarding duration of hypertension, majority 27 (45%) were suffering from hypertension for 5-10 years duration, only 0 (0%) sample in group was suffering from hypertension below 15 years.

Regarding the number of years one is taking medication majority of 26 (43.33%) patients belongs to 8-12 years of age.

Assessment of dietary pattern revealed that regarding 37 (61.66%) of the male were vegetarian.

### Table No. 2 Details of Samples Blood pressure level

<table>
<thead>
<tr>
<th>Categories of blood Pressure</th>
<th>Systolic</th>
<th>Diastolic (mmHg)</th>
<th>*Mean Arterial pressure (MAP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal blood Pressure</td>
<td>120-139</td>
<td>80-90</td>
<td>93-103</td>
</tr>
<tr>
<td>Mild Hypertension</td>
<td>140-159</td>
<td>90-99</td>
<td>107-119</td>
</tr>
<tr>
<td>Moderate Hypertension</td>
<td>160-179</td>
<td>100-109</td>
<td>120-132</td>
</tr>
<tr>
<td>Severe Hypertension</td>
<td>&gt;180 mmHg</td>
<td>&gt;110 mmHg</td>
<td>133.3</td>
</tr>
</tbody>
</table>

### Table No. 3 Blood pressure outcome among hypertensive patient in experimental group

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Pre-test</th>
<th>Post-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Outcome</td>
<td>127.46</td>
<td>5.44</td>
</tr>
</tbody>
</table>

### Table No. 4 Heart rate outcome among hypertensive patient in control group.

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Pre-test</th>
<th>Post-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Outcome</td>
<td>86.02</td>
<td>3.86</td>
</tr>
</tbody>
</table>
Table No. 5 Blood pressure outcome among hypertensive patient in control group.

<table>
<thead>
<tr>
<th>variable</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure outcome</td>
<td>128.30</td>
<td>7.713</td>
</tr>
</tbody>
</table>

Table No. 6 Heart Rates outcome among hypertensive patient in control group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate outcome</td>
<td>73.10</td>
<td>2.894</td>
</tr>
</tbody>
</table>

MAJOR FINDINGS OF THE STUDY:-

Findings related to demographic variables of samples:

1. According to demographic variable of age, the majority of the patients 19(31.66%) belonged to 51-60 years, 14(23.33%) patient belong to 61-70 years, 17(28.33%) patients belong to 41-50 years and only 10(16.66%) belonged to 71-80 years in group of hypertensive patients.

2. Regarding gender, 27 (45%) were males and 33(55%) were females in selected group.

3. Regarding duration of hypertension, majority 27(45%) were suffering from hypertension for 5-10 years duration, only 0(0%) sample in group was suffering from hypertension below 15 years.

4. Regarding the number of years one is taking medication majority of 26 (43.33%) patients belongs to 8-12 years of age.

5. Assessment of dietary pattern revealed that regarding 37 (61.66%) of them were vegetarian

6. According to blood pressure outcome among hypertensive patient, in experimental group, the mean pre-test score is 127.46 with standard deviation 5.44 and the post test mean is 78.63 with standard deviation 4.88. The obtained paired t test value is 45.73.

7. According to heart rate outcome among hypertensive patient, in experimental group, the mean pre-test score is 86.02 with standard deviation 3.86 and the post test mean is 63.010 with standard deviation 2.59. The obtained paired t test value is 28.0031.

8. According to variable of blood pressure outcome, mean is 128.30 with standard deviation of 7.713.

9. According to variable of heart rate outcome, mean is 73.10 with standard deviation of 2.894.

**Conclusion**

The main conclusion drawn from the present study was that most of the hypertensive patients had high level of Blood Pressure. After receiving breathing exercises there was a significant reduction in the level of Blood Pressure. Samples became familiar and found themselves comfortable and also expressed satisfaction. It is concluded that the breathing exercises is effective and a simple strategy to maintain the blood pressure and heart rate.

**Conflict of Interest:** Nil

**Source of Funding:** Self Funding
**Ethical Clearance:** The study was approved by the research committee, IEC –DPCN/ 2019 and a formal written permission was gathered from the authority of hospital for study setting.

**Statement of Informed consent:** Informed consent was acquired from the participants

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6. Anderson, D., McNeely, J. & Windham, B. Regular slow-breathing exercise effects on blood pressure and breathing patterns at rest. J Hum Hypertens 24, 807–813 (2010). [https://doi.org/10.1038/jhh.2010.18](https://doi.org/10.1038/jhh.2010.18)


A Theoretical Study for Determination of Capoten by Analytical Methods

Rana.S.AL-Shemary¹, Enass Najem Oubaid²

¹Babylon University, College of Pharmacy, Pharmaceutical Chemistry Dept., Hilla, Iraq,
²Babylon University, College of Pharmacy, Pharmacology and Toxicology Dept., Hilla, Iraq

Abstract

The present analysis article points out numerous analytical methods for the quantitative identification of ACE inhibitor (ACE Inhibitor) by one in all the spectroscopical techniques (UV-spectrophotometry) and separation techniques, like superior liquid natural action (HPLC). additionally, we tend to examine the contribution of optical biosensors to the clinical and pharmaceutical study of ACE inhibitor, which has applicable analytical procedures for internal control, pharmaceutical drug formulations and human.

In this report, a comprehensive sample of scientific papers published in different journals relating to medicinal, clinical and analytical chemistry was collected. Captopril was chosen because it is a popular medication in the pharmacy, a first-line antihypertensive agent, with a fast release, limited course of operation.

Key words : - Captopril, ACE inhibitor, HPLC, UV, biosensor

Introduction

Captopril is the first oral angiotensin converting enzyme inhibitor to become available. It was used mainly for hypertension. In mild to severe hypertension, captopril is nearly as effective as normal doses of hydrochlorothiazide or propranolol, nearly one-half of these patients need the use of a diuretic to gain satisfactory regulation of blood pressure. (¹)

In extreme hypertension captopril plus a diuretic (and in some cases a beta-blocker) blood pressure has typically lowered considerably better than could be done with ‘normal combination treatment’ in cases whom have not responded sufficiently to this protocol, and this also resulted in an increased sense of well-being in highly hypertensive patients that have previously undergone comprehensive multiple drug therapy. Indeed, at the present stage of drug production, patients who do not respond to or accommodate ‘ordinary’ antihypertensive treatment are the most effective candidates for captopril therapy (²).

Ultimately, the final role of captopril in the treatment of hypertension may depend on further confirmation of its profile of adverse effects. Captopril has shown promising changes in a limited number of patients with serious congestive heart failure resistant to traditional treatment, in addition to research on hypertension (³).

Captopril must be considered an interesting addition to the clinical armamentarium; it will continue to create a lot of excitement as its final role in therapy is best described by additional well-designed experiments to evaluate captopril, such as HPLC, UV-visible spectroscopy, GLC, optical biosensor, etc. (⁴). Captopril tablets are shown in Fig.1.
Discovery and synthesis of captopril

One of the primary medications marketed for lowering blood pressure. Three researchers at the U.S. drug maker Squibb (now Bristol-Myers Squibb) made-up angiotensin converting enzyme inhibitor in 1975: Miguel Ondetti, Claude Bernard Rubin, and David Cushman. In February 1976, Squibb applied for U.S. patent rights for the drug and the U.S. In September 1977, a patent was issued (5). Captopril’s development was one of the early achievements of the groundbreaking idea of ligand-based drug design.

In the mid-20th century, the renin-angiotensin -aldosterone pathway was thoroughly researched and this mechanism provided many important goals in the production of new therapies for hypertension. The first 2 goals that were tried were proteinase associate degree an angiotensin converting enzyme inhibitor. ACE inhibitor was the results of {attempts|makes associate degree attempt|tries} by Squibb’s labs to supply an angiotensin converting enzyme inhibitor. Ondetti, Cushman, and colleagues distended on work undertaken among the 19 Sixties by a team of researchers semiconductor unit by John Vane at the Royal college of Surgeons of European nation. (6). Kevin K.F. created the first breakthrough. In 1967, the transfer of angiotensin I to angiotensin II was discovered to take place in the pulmonary circulation rather than in the plasma. Sergio Ferreira, on the other hand, noticed that bradykinin dissolved after its transit through the pulmonary circulation. It was believed that angiotensin I to angiotensin II conversion and bradykinin inactivation were regulated by the same enzyme (7).

In 1970, the transfer of angiotensin I to angiotonin was blocked throughout its passage through the circulation mistreatment bradykinin potentiating factor (BPF) equipped by Sergio Ferreira metric Ng and Vane. Later, BPF was found to be a peptide in the lancehead viper venom (Bothrops jararaca),
which was the transforming enzyme’s “collected-product inhibitor.” A sting from this snake triggers an immediate decrease in its prey’s blood pressure, rendering it difficult to run. After it absolutely was found by QSAR-based modification that the terminal sulphydryl moiety of the amide created a high efficiency of ACE inhibition, Captopril was formed from this peptide.

A non-peptide with a proline residue at the C-terminal is found in the venom. An effective regulator of the so-called angiotensin-converting enzyme (ACE) was found to be this non-peptide. It is a zinc-containing enzyme that catalyzes the synthesis of the angiotensin I peptide into the hormone angiotensin II active peptide that raises blood pressure. While the venom peptide’s very quick action can be useful for a starving snake, it is not helpful for humans (8).

Medicinal chemists have produced a sequence of proline-containing zinc-binding compounds using the catalytic mechanism of similar zinc-containing enzymes as the starting point. Succinyl-(S)-proline was the first ACE inhibitor to be synthesized. Medicinal chemists were able to improve the production of the ACE inhibitor more than 1000-fold by replacing the succinyl group with a structurally similar sulfhydryl group. In 1980, Captopril entered the market as the first inhibitor of ACE (9), as seen in Fig.2.

![Figure 2: ACE-inhibitor activity.](image)

It was proprietary in 1976 and approved in 1980 for medicative use. Attributable to its mechanism of action and conjointly attributable to the event method, it absolutely was the primary angiotensin converting enzyme inhibitor developed and was thought-about a breakthrough. At E, Captopril was found and grown. Centered on ideas pioneered by Nobel laureate Sir John Vane, R. Squibb & Sons Pharmaceuticals is now sold by Bristol-Myers Squibb.

On April 6, 1981, Captopril received FDA approval. In Feb 1996, once the business exclusivity maintained by Bristol-Myers Squibb for Capoten all
over, the medication became a generic medication within the U.S. The production of Capoten was claimed as an indication of ‘biopiracy’ (marketing standard medicines), as no benefits flowed back to the associate estral Brazilian community WHO 1st used viper venom as an point poison (10).

Indication

1. Hypertension

Adult / Initially twelve.5-25 mg doubly daily, then augmented to a hundred and fifty mg daily in a pair of separated doses if necessary, doses to be augmented at periods of a minimum of a pair of weeks, once daily dose are often decent if any concomitant medicine medications square measure measure taken.

Initially 6.25 mg doubly daily, then augmented to a hundred and fifty mg daily if needed in a pair of split doses, doses to be augmented at intervals of a minimum of a pair of weeks, once daily dose are often decent if any concomitant medicine medicines square measure taken.

2- Prophylaxis of symptomatic internal organ failure in clinically healthy patients with symptomless left chamber malfunction (beginning 3-16 days when infarction) following infarction (under close to surgical supervision)

Adult / at first 6.25 mg daily, then hyperbolic to twelve.5 mg three times daily for two days, then hyperbolic to twenty five mg three times daily if tolerated, then hyperbolic to 75-150 mg daily if tolerated in 2-3 separated doses, rising steady to doses larger than seventy five mg daily.

3-Diabetic nephropathy in type 1 DM

Adult/75-100 mg in split doses daily.

4-Heart failure

Adult/(under shut medical supervision) at first 6.25-12.5 mg 2-3 times every day, then hyperbolic more and more at periods of a minimum of a pair of weeks, if tolerated up to a hundred and fifty mg daily in divided doses.

5-Short-term treatment of clinically competent patients with myocardial infarction within 24 hours of onset.

Captopril analytical measurement

Several assay strategies, like coulometric (13), conductometric, and colorimetrical, are developed for the quantitative determination of Capoten (14). Infrared spectroscopy (15), mass spectroscopy and nuclear magnetic resonance spectroscopy (16) are used to determine Captopril. Captopril’s UV spectrum with one band at two hundred nm was obtained, whereas the CD spectrum consists of one peak at 210 nm (17).

Captopril by spectrophotometric method was calculated by Alberto (18) and iron and copper complexation with ACE inhibitor was additionally assayed (19) by ultraviolet illumination photometer. the amount of chromatographical strategies for the determination of ACE inhibitor has been defined as gas chromatography-mass spectrometry (20) Ahmed et al. (21) also published HPLC stability-indicating methods for its determination.

This approach is employed for the assessment of angiotensin converting enzyme inhibitor in pure type within the presence of the disulphide chemical compound and in pharmaceutical preparations, an answer containing 0.025 C and w / w Pd(II) chloride was used as a mobile step in a very mixture of acetonitrilemethanol-water comprising ten millimetre Britton-Robinson [BRb] pH scale 4.00 and 0.25 M KCl solution [1:4:5 v / v / v].

Another HPLC methodology determined by Stulzer et al. angiotensin converting enzyme inhibitor in controlled unleash tablets and analyzes
were performed at temperature at the inverted part Phenomenex Luna column C18 (250 millimetre × 4.6 mm), mobile part water: methyl alcohol (45:55; v / v) and pH scale 2.5 at 1.0 mL.min⁻¹, and also the response was linear at zero.3–1.5 mg.mL⁻¹ (r² = 0.9983) (22).

Ivanovic et al. have revealed a valid RP-HPLC method for analyzing hydrochlorothiazide and Capoten in tablets. Jankowski et al., calculated by HPLC in blood with captopril (23). The captopril adduct recovery achieved 93.1% and the detection limit was 15 ng.mL⁻¹, while the conceptual limit was 30 ng.mL⁻¹. Inter and intra-assay RSD were below 9%, but precision was found to be below 8%. Captopril was calculated by Saleem et al. and Amini et al. (24) in plasma.

For the quantitative determination of angiotensin converting enzyme inhibitor by the quantity of scientists victimisation HPLC (25), variety of assay strategies are developed. a range of examinations are recorded victimisation HPLC for the determination of angiotensin converting enzyme inhibitor in bulk drug substances and their formulations (26). Direct determination of the four ACE-inhibitors Zestril, Enalapril, angiotensin converting enzyme inhibitor and Fosinopril in prescription drugs and blood serum by HPLC analyst separation was accomplished by RP-HPLC gradient with a mobile step consisting of acetonitrile: water (60:40 v / v) with ortho oxyacid changed to pH 3.0 (26), Fig.3 demonstrates captopril’s chemical composition.

Figure 3: captopril’s chemical structure.
Monitoring of in vitro trials of ACE inhibitor association with LC-UV symptom agents and parallel LC determination of rosuvastatin, lisinopril, captopril, and angiotensin converting enzyme inhibitor in API, medicament formulations, and human blood serum (27).

Another Facile and Manifest Liquid chromatographical method for coinciding Determination of ACE inhibitor and NSAIDs in API and Pharmaceutical Formulations has been according and CAP has been isolated from NSAIDs by means that of a column of Purospher STAR C18 (250.4.6 mm, five μm) and a mobile section consisting of alcohol, water (80:20, v/v) (28).

Therefore, ACE inhibitor with water pill HydroDIURIL and diuretic in active pharmaceutical ingredients, medicative indefinite quantity formulations and human body fluid area unit used as combination indefinite quantity forms for alternative distinctive forms of drug substances like metal channel block medicinal drug, diuretics, etc. (29).

Other hypoglycaemic Capoten, medicinal drug and H2 receptor antagonist ways ar recorded in bulk, formulations, and human humor by RP-HPLC (30-32).

Biosensors have become important bioanalytical instruments in the last few years for environmental testing, biotechnology, pharmaceuticals, food safety and other consumer industries. Due to their high sensitivity, high precision, low expense, compact size and simple activity, the use of biosensors to test chemical species is an exciting opportunity.

High sensitivity and fast detection are key criteria for a sensor for detecting biomaterials. There has been a great deal of study over many decades to develop quick and responsive biosensors for various applications. In particular, owing to the probability of fast and direct (unlabelled) detection, optical methods have been reported to have a high potential.

A variety of biosensor instruments, including several interferometers, surface plasmon resonance sensors and micro ring resonator sensors, have been used to detect various biomaterials. While all these sensors offer sensitive and quick detection, they are only suitable for items up to 100 nm in size (33).

A light detector resistance (LDR) is AN optical device mounted in an exceedingly black PTFE cell and paired to a cheap multimeter (Ohmmeter). The chemical analysis is based on the reduction of ammonium ion molybdate by Capoten, producing a green-yellow compound in the presence of sulphuric acid (λmax 407 nm). By plotting the electrical resistance of the LDR against the CPT concentration, the standardization curves were obtained within the vary of 4.60 x 10^-4 to 1.84 x 10^-3 mol.L^-1 with an affordable constant of determination (r^2 = 0.9962) (34).

Moreover, as a result of their uncommon optical properties and catalytic ability, Molybdenum compound nanomaterials have recently attracted widespread interest. There is, however, no literature up to now on the utilization of photoluminescent nanomaterials of Molybdenum compound in biological and pharmaceutical sensing.

Via Associate in Nursing easy method, photoluminescent Molybdenum oxide compound quantum dots (MoOx QDs) were synthesized and so the synthesized MoOx QDs were more additional as a replacement sort of photoluminescent probe to create a replacement off-on angiotensin converting enzyme inhibitor (Cap) detector supported the idea that the quenched photoluminescence of MoOx QDs by Cu^{2+} was fixed up to Cap by precise interaction between thiol cluster of Cap and Cu^{2+}.

The rebuilt photoluminescence strength showed a powerful linear relationship with the Cap material, variable from 1.0 to 150.0 μM, with a 0.51 μM (3σ / k) detection most, beneath ideal conditions. Additionally,
with the recently designed off-on device, the content of Cap was with success known in pharmaceutical samples, and therefore the recoveries were 99.4-101.7 percent, indicating that the newest off-on device includes a high accuracy.

Replicate (n = 10) measurements were created on an equivalent answer comprising the equivalent of \(1.15 \times 10^{-3}\) mol.L\(^{-1}\) of CPT to check the repeatability and preciseness of the measure methodology. Determinations (n = 10) were created with an equivalent answer containing CPT adequate to \(1.15 \times 10^{-3}\) mol. L\(^{-1}\).

This resolution was tested in conjunction with the prescribed approach for measurement the CPT content of pharmaceutical formulations. In terms of share relative variance (% RSD)\(^{(36)}\), the accuracy was measured.

**The Biosensors Specifications**

The essential specifications for biosensors are the following:

Ø Ability to provide real-time data during a project at each development stage or at multiple time points. This would help in improved tracking and regulation of blood sugar levels, for example.

Ø It is feasible to customise several biosensor technologies to permit continuous flow analysis that’s terribly helpful in food process, observance of installation, and air quality.

Ø Through miniaturization, biosensors can reduce usage costs and can be incorporated into efficient, highly capable lab-on-a-chip tools.

Ø Biosensors is used for point-of-care or on-the-spot analysis wherever progressive molecular analysis is conducted while not the requirement for a progressive laboratory\(^{(37)}\).

**Biosensor system**

In general, a biosensor uses a facet of biological recognition that detects the presence of an analyte (the species to be detected) and produces a physical or chemical change that’s remodeled into a symptom by an electrical device (detector). The general block diagram of the framework of biosensors is defined in Fig. 4. Specific system for biosensing. An analyte is inserted into the detector by the sampling machine. The identification issue attaches or responds to a specific analyte, giving a biodetection specificity. Enzymes, antibodies, proteins, DNA or additionally cells like yeast or bacterium are used as parts in biorecognition\(^{(38)}\). In general, stimulus may be generated by optical, electrical, or other kinds of force fields that, as a result of biorecognition, extract a reaction. The transduction mechanism converts the physical or chemical process of biorecognition into an optical, electrical or another variety of signal within the presence of external stimuli that’s then detected by the detector device. For identification of the analyte, the detection device may provide pattern recognition.

A broad variety of real life applications are defined by biosensors\(^{(39)}\). In essence, future uses are medicinal and nonclinical\(^{(40)}\). The use of biosensors to track toxins\(^{(41)}\), micro-organisms, microbes, viruses\(^{(42)}\) and chemical and biological defense against terrorism is of more recent concern. In agricultural and environmental applications\(^{(43)}\), it is also common. A few days now, applications based on nanotechnology\(^{(44)}\) are also being created.
The Immobilization Techniques (Mechanism)

The biological part has to be correctly attached to the transducer to make a biosensor. Immobilization is called this method. On a solid support, the biorecognition components are typically immobilized. A membrane, rubber, copolymer, or semiconductor material are typically the solid supports. A biorecognition component, either by a physical methodology (such as adsorption) or by chemical association, is immobilized on the solid support. In sure ways, the issue of biorecognition is cursed with controlled porosity within the volume of the matrix (solid support), wherever solid support typically provides property against associate analyte of a precise size in step with its pore dimension (44).

Conclusion

A significant variety of drugs for effective treatment are administered to people diagnosed with hypertension, raising the risk of adverse effects and drug reactions. But Gupta et al. (37-44) have documented some electro analytical approaches.

UV and HPLC strategies and optical biosensors for the determination of ACE inhibitor in active ingredients, pharmaceutical formulations and biological specimens square measure investigated alone or in conjunction with different medication during this study.

In general, pricey instrumentation, provision for the utilization and disposal of solvents, labour-intensive sample preparation procedure and private skilled chromatographical techniques were needed for HPLC strategies.

Furthermore, several of the examined HPLC approaches have the ability to apply drug combination, multi-drug pharmacokinetics trials and association studies to clinical science.

Thus, optical biosensors are one of the most interesting alternatives in this situation, since they play an important role in drug quantification and are one of the most specific, sensitive, low-cost and
easy-to-use options available at the moment. This has provided a niche for them as new clinical instruments that allow more precision medicine to be controlled therapeutically by minimizing symptoms or secondary effects as much as possible and contributing to safer patient treatment and prescription.

In addition, the use of biosensors offers doctors a benefit when making the most precise choices for real-time dosing at the bedside of a patient, since biosensors are compact instruments that are easy to use and miniaturized. When formulating medicines, they have stricter and more effective regulation. There is an immediate need for more research based on the quantification of blood medications and including all pharmacological classes to ensure that a greater range of medications have personalized doses in accordance with the premise of usage.

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**Conflict of Interest** there has been no conflict of interest of any kind with the authors of this work

**Ethical standard**: The study was formally approved the research plan by the ethical committee board at the Babylon health directorate.

**Informed consent** was taken from all the participant patients before being enrolled in the study

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